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(54) **ACYLATED GLP-1 COMPOUNDS**

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(52) **U.S. Cl.**

USPC **514/7.2**; 514/11.7; 530/308

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Protracted GLP-1 compounds and therapeutic uses thereof

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ACYLATED GLP-1 COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of copending U.S. application Ser. No. 11/908,834, filed Sep. 17, 2007, which is a 35 U.S.C. §371 national stage application of International Patent Application PCT/EP2006/060855 (published as WO 2006/097537), filed Mar. 20, 2006, which claimed priority of European Patent Application 05102171.5, filed Mar. 18, 2005; this application further claims priority under 35 U.S.C. §119 of U.S. Provisional Application 60/664,497, filed Mar. 23, 2005.

FIELD OF THE INVENTION

This invention relates to the field of therapeutic peptides, i.e. to new protracted GLP-1 compounds.

BACKGROUND OF THE INVENTION

A range of different approaches have been used for modifying the structure of glucagon-like peptide 1 (GLP-1) compounds in order to provide a longer duration of action in vivo. WO 96/29342 discloses peptide hormone derivatives wherein the parent peptide hormone has been modified by introducing a lipophilic substituent in the C-terminal amino acid residue or in the N-terminal amino acid residue.

WO 98/08871 discloses GLP-1 derivatives wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.

WO 99/43708 discloses GLP-1(7-35) and GLP-1(7-36) derivatives which have a lipophilic substituent attached to the C-terminal amino acid residue.

WO 00/34331 discloses acylated GLP-1 analogs.

WO 00/69911 discloses activated insulinotropic peptides to be injected into patients where they are supposed to react with blood components to form conjugates and thereby allegedly providing longer duration of action in vivo.

WO 02/46227 discloses GLP-1 and exendin-4 analogs fused to human serum albumin in order to extend in vivo half-life.

Many diabetes patients particularly in the type 2 diabetes segment are subject to so-called "needle-phobia", i.e. a substantial fear of injecting themselves. In the type 2 diabetes segment most patients are treated with oral hypoglycaemic agents, and since GLP-1 compounds are expected to be the first injectable product these patients will be administered, the fear of injections may become a serious obstacle for the widespread use of the clinically very promising GLP-1 compounds. Thus, there is a need to develop new GLP-1 compounds which can be administered less than once daily, e.g. once every second or third day preferably once weekly, while retaining an acceptable clinical profile.

SUMMARY OF THE INVENTION

The invention provides a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

invention and the use of compounds according to the present invention for preparing medicaments for treating disease.

The invention provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B—U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

DESCRIPTION OF THE INVENTION

In the present specification, the following terms have the indicated meaning:

The term "polypeptide" and "peptide" as used herein means a compound composed of at least five constituent amino acids connected by peptide bonds. The constituent amino acids may be from the group of the amino acids encoded by the genetic code and they may be natural amino acids which are not encoded by the genetic code, as well as synthetic amino acids. Natural amino acids which are not encoded by the genetic code are e.g., γ-carboxyglutamate, ornithine, phosphoserine, D-alanine and D-glutamine. Synthetic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), β-alanine, 3-aminomethyl benzoic acid, anthranilic acid.

The 22 proteogenic amino acids are:
Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Cystine, Glutamine, Glutamic acid, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine.

Thus a non-proteogenic amino acid is a moiety which can be incorporated into a peptide via peptide bonds but is not a proteogenic amino acid. Examples are γ-carboxyglutamate, ornithine, phosphoserine, the D-amino acids such as D-alanine and D-glutamine, Synthetic non-proteogenic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α-aminoisobutyric acid), Abu (α-aminobutyric acid), Tle (tert-butylglycine), 3-aminomethyl benzoic acid, anthranilic acid, des-amino-Histidine, the beta analogs of amino acids such as β-alanine etc. D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, N^{α}-acetyl-histidine, α-fluoromethyl-histidine, α-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

The term "analogue" as used herein referring to a polypeptide means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and/or wherein one or more amino acid residues have been deleted from the peptide and or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide. A simple system is often used to describe analogues: For example [Arg^{34}]GLP-1(7-37)Lys designates a GLP-1(7-37) analogue wherein the naturally occurring lysine at position 34 has been substituted

for which the optical isomer is not stated is to be understood to mean the L-isomer. In embodiments of the invention a maximum of 17 amino acids have been modified. In embodiments of the invention a maximum of 15 amino acids have been modified. In embodiments of the invention a maximum of 10 amino acids have been modified. In embodiments of the invention a maximum of 8 amino acids have been modified. In embodiments of the invention a maximum of 7 amino acids have been modified. In embodiments of the invention a maximum of 6 amino acids have been modified. In embodiments of the invention a maximum of 5 amino acids have been modified. In embodiments of the invention a maximum of 4 amino acids have been modified. In embodiments of the invention a maximum of 3 amino acids have been modified. In embodiments of the invention a maximum of 2 amino acids have been modified. In embodiments of the invention 1 amino acid has been modified.

The term "derivative" as used herein in relation to a peptide means a chemically modified peptide or an analogue thereof, wherein at least one substituent is not present in the unmodified peptide or an analogue thereof, i.e. a peptide which has been covalently modified. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters and the like. An example of a derivative of GLP-1(7-37) is $N^{\epsilon 26}$-((4S)-4-(hexadecanoylamino)-carboxy-butanoyl)[Arg^{34}, Lys^{26}] GLP-1-(7-37).

The term "GLP-1 peptide" as used herein means GLP-1(7-37) (SEQ ID No 1), a GLP-1(7-37) analogue, a GLP-1(7-37) derivative or a derivative of a GLP-1(7-37) analogue. In one embodiment the GLP-1 peptide is an insulinotropic agent.

The term "insulinotropic agent" as used herein means a compound which is an agonist of the human GLP-1 receptor, i.e. a compound which stimulates the formation of cAMP in a suitable medium containing the human GLP-1 receptor (one such medium disclosed below). The potency of an insulinotropic agent is determined by calculating the EC_{50} value from the dose-response curve as described below.

Baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor (BHK-467-12A) were grown in DMEM media with the addition of 100 IU/mL penicillin, 100 µg/mL streptomycin, 5% fetal calf serum and 0.5 mg/mL Geneticin G-418 (Life Technologies). The cells were washed twice in phosphate buffered saline and harvested with Versene. Plasma membranes were prepared from the cells by homogenisation with an Ultraturrax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was centrifuged at 48,000×g for 15 min at 4° C. The pellet was suspended by homogenization in buffer 2 (20 mM HEPES-Na, 0.1 mM EDTA, pH 7.4), then centrifuged at 48,000×g for 15 min at 4° C. The washing procedure was repeated one more time. The final pellet was suspended in buffer 2 and used immediately for assays or stored at −80° C.

The functional receptor assay was carried out by measuring cyclic AMP (cAMP) as a response to stimulation by the insulinotropic agent. cAMP formed was quantified by the AlphaScreen™ cAMP Kit (Perkin Elmer Life Sciences). Incubations were carried out in half-area 96-well microtiter plates in a total volume of 50 µL buffer 3 (50 mM Tris-HCl, 5 mM HEPES, 10 mM $MgCl_2$, pH 7.4) and with the following additions: 1 mM ATP, 1 µM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01% Tween-20, 0.1% BSA, 6 µg membrane preparation, 15 µg/mL acceptor beads, 20 µg/mL donor beads preincubated with 6 nM biotinyl-cAMP. Compounds to be tested for agonist activity were dissolved and diluted in buffer 3. GTP was freshly prepared for each experi-

the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC_{50} values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, Calif.).

The term "DPP-IV protected" as used herein referring to a polypeptide means a polypeptide which has been chemically modified in order to render said compound resistant to the plasma peptidase dipeptidyl aminopeptidase-4 (DPP-IV). The DPP-IV enzyme in plasma is known to be involved in the degradation of several peptide hormones, e.g. GLP-1, GLP-2, Exendin-4 etc. Thus, a considerable effort is being made to develop analogues and derivatives of the polypeptides susceptible to DPP-IV mediated hydrolysis in order to reduce the rate of degradation by DPP-IV. In one embodiment a DPP-IV protected peptide is more resistant to DPP-IV than GLP-1(7-37) or Exendin-4(1-39).

Resistance of a peptide to degradation by dipeptidyl aminopeptidase IV is determined by the following degradation assay:

Aliquots of the peptide (5 nmol) are incubated at 37° C. with 1 µL of purified dipeptidyl aminopeptidase IV corresponding to an enzymatic activity of 5 mU for 10-180 minutes in 100 µL of 0.1 M triethylamine-HCl buffer, pH 7.4. Enzymatic reactions are terminated by the addition of 5 µL of 10% trifluoroacetic acid, and the peptide degradation products are separated and quantified using HPLC analysis. One method for performing this analysis is: The mixtures are applied onto a Vydac C18 widepore (30 nm pores, 5 µm particles) 250×4.6 mm column and eluted at a flow rate of 1 ml/min with linear stepwise gradients of acetonitrile in 0.1% trifluoroacetic acid (0% acetonitrile for 3 min, 0-24% acetonitrile for 17 min, 24-48% acetonitrile for 1 min) according to Siegel et al., Regul. Pept. 1999; 79:93-102 and Mentlein et al. Eur. J. Biochem. 1993; 214:829-35. Peptides and their degradation products may be monitored by their absorbance at 220 nm (peptide bonds) or 280 nm (aromatic amino acids), and are quantified by integration of their peak areas related to those of standards. The rate of hydrolysis of a peptide by dipeptidyl aminopeptidase IV is estimated at incubation times which result in less than 10% of the peptide being hydrolysed.

The term "C_{1-6}-alkyl" as used herein means a saturated, branched, straight or cyclic hydrocarbon group having from 1 to 6 carbon atoms. Representative examples include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, tert-pentyl, n-hexyl, isohexyl, cyclohexane and the like. The term "pharmaceutically acceptable" as used herein means suited for normal pharmaceutical applications, i.e. giving rise to no adverse events in patients etc.

The term "excipient" as used herein means the chemical compounds which are normally added to pharmaceutical compositions, e.g. buffers, tonicity agents, preservatives and the like.

The term "effective amount" as used herein means a dosage which is sufficient to be effective for the treatment of the patient compared with no treatment.

The term "pharmaceutical composition" as used herein means a product comprising an active compound or a salt thereof together with pharmaceutical excipients such as buffer, preservative, and optionally a tonicity modifier and/or a stabilizer. Thus a pharmaceutical composition is also known in the art as a pharmaceutical formulation.

The term "treatment of a disease" as used herein means the management and care of a patient having developed the dis-

the administration of the active compounds to eliminate or control the disease, condition or disorder as well as to alleviate the symptoms or complications associated with the disease, condition or disorder.

In another aspect the present invention relates to an acylated GLP-1 analogue that can bind to albumin and the GLP-1 receptor simultaneously.

In another aspect the present invention relates to an acylated GLP-1 analogue that bind to the GLP-1 receptor with an affinity below 100 nM, preferable below 30 nM in the presence of 2% albumin.

In another aspect the present invention relates to an acylated GLP-1 analogue which affinity to the GLP-1 receptor is only partly decreased when comparing the affinity in the presence of very low concentration (e.g. 0.005% to 0.2%) of human albumin to the affinity in the presence of 2% human albumin. The shift in binding affinity under these conditions is less than 50 fold, preferable below 30 fold and more preferable below 10 fold.

The term "albumin binding moiety" as used herein means a residue which binds non-covalently to human serum albumin. The albumin binding residue attached to the therapeutic polypeptide typically has an affinity below 10 μM to human serum albumin and preferably below 1 μM. A range of albumin binding residues are known among linear and branched lipohophillic moieties containing 4-40 carbon atoms having a distal acidic group.

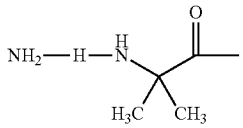
The term "hydrophilic linker" as used herein means a spacer that separates a peptide and an albumin binding residue with a chemical moiety which comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

The term "acidic groups" as used herein means organic chemical groups which are fully or partly negatively charged at physiological pH. The pKa value of such groups is below 7, preferable below 5. This includes but is not limited to carboxylic acids, sulphonic acids, phosphoric acids or heterocyclic ring systems which are fully or partly negatively charged at physiological pH.

In the below structural formula II the moiety U is a diradical may be attached to the terminal groups B and the aminogroup of the lysine amino acid in the peptide in two different ways. In embodiments of the invention the U in formula II is attached with the group B attached at the end of the alkyl chain and the peptide at the other end.

In the formulas below the terminal bonds from the attached groups are to be regarded as attachment bonds and not ending in methylene groups unless stated.

In the formulas below



means the H_2N-His-Aib-N-terminal of the GLP-1 analogue.

In an embodiment the invention provides a GLP-1 analog acylated with a lipophillic albumin binding moiety containing at least two free acidic chemical groups attached via a non natural amino acid linker to the lysine residue in position 26.

In an embodiment, the term free acidic chemical groups is to be understood as having the same meaning as "acidic groups" as used herein.

In an embodiment the invention provides an acylated GLP-1 analog where said GLP-1 analog is stabilised against DPP-IV by modification of at least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), and where said acylation is a diacid attached to the lysine residue in position 26 optionally via a non natural amino acid hydrophilic linker.

In an embodiment of the invention a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

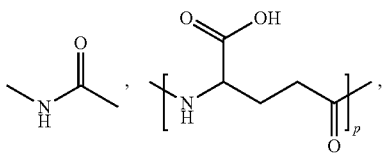
An embodiment provides a GLP-1 analog according to the above embodiment, wherein the moiety attached in position 26 comprises a hydrophilic linker.

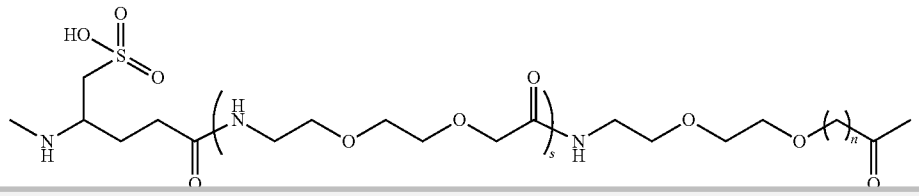
An embodiment provides a GLP-1 analog according to the above embodiments, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the moiety attached in position 26 comprises an albumin binding moiety separated from the peptide by the hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiment, wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the acylated moiety is B—U', where U' is selected from





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