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(54) Title: EXENDIN ANALOGS

(57) Abstract: Stable compositions of Exendin-4 and Exendin agonists that include at least one modified amino acid residue at positions Q13, M14, D28 of the Exendin-4 molecule. Use of the compositions for treatment of a variety of disease states, including diabetes.

EXENDIN ANALOGS

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of the filing dates of United States Provisional Patent Application No. 60/896,584 filed 23 March 2007 under the title EXENDIN ANALOGS and United States Provisional Patent Application No. 60/915,489 filed May 2, 2007 under the title EXENDIN ANALOGS. The content of the above patent applications is hereby expressly incorporated by reference into the detailed description hereof.

FIELD OF THE INVENTION

[002] The present invention relates to new peptide analog compositions. These compositions have improved potency over Exendin-4 and have improved stability under a variety of conditions, including improved radiation stability and improved aqueous stability. The compositions are useful for treatment of diabetes type 1 or type 2, insulin resistance syndrome, impaired glucose tolerance (IGT), obesity, eating disorders, hyperglycemia, metabolic disorders, and gastric disease.

BACKGROUND

[003] Single and triple letter designations for the amino acids are used interchangeably. For example, it will be appreciated that Lys and K refer to lysine, Asp and D refer to aspartic acid, Glu and E refer to glutamic acid, etc. A complete description of the amino acid designations can be found in [Alberts, B. et al. in Molecular Biology of the Cell, 2.sup.nd Ed. Garland Publishing, Inc. (New York) (1989)].

Exendins and Exendin analogs

[004] Exendin-4 is part of a family of peptides known as incretins, having multiple primary and secondary activities on a growing number of activities related to metabolic

conditions, including food intake, insulin secretion, glucose utilization, gastric motility and digestion, and satiety. As such, Exendin is a very useful therapeutic in type 2 diabetes and in weight loss, among other indications.

[005] Exendins are peptides that were first isolated from the salivary secretions of the Gila-monster, a lizard found in Arizona, and the Mexican Beaded Lizard. Exendin-3 is present in the salivary secretions of Heloderma horridum, and exendin-4 is present in the salivary secretions of Heloderma suspectum (Eng, J., et al., J. Biol. Chem., 265:20259-62, 1990; Eng., J., et al., J. Biol. Chem., 267:7402-05, 1992). The exendins have some sequence similarity to several members of the glucagon-like peptide family, with the highest homology, 53%, being to GLP-1[7-36]NH.sub.2 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993). GLP-1[7-36]NH.sub.2, also known as proglucagon[78-107] and most commonly as "GLP-1," has an insulinotropic effect, stimulating insulin secretion from pancreatic .beta.-cells; GLP-1 also inhibits glucagon secretion from pancreatic .alpha.-cells (Orskov, et al., Diabetes, 42:658-61, 1993; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). GLP-1 is reported to inhibit gastric emptying (Williams B, et al., J Clin Encocrinol Metab 81 (1): 327-32, 1996; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993), and gastric acid secretion. (Schjoldager B T, et al., Dig Dis Sci 34 (5): 703-8, 1989; O'Halloran D J, et al., J Endocrinol 126 (1): 169-73, 1990; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993). GLP-1[7-37], which has an additional glycine residue at its carboxy terminus, also stimulates insulin secretion in humans (Orskov, et al., Diabetes, 42:658-61, 1993). A transmembrane G-protein adenylate-cyclase-coupled receptor believed to be responsible for the insulinotropic effect of GLP-1 is reported to have been cloned from a .beta.-cell line (Thorens, Proc. Natl. Acad. Sci. USA 89:8641-45 (1992)).

[006] Exendin-4 potently binds at GLP-1 receptors on insulin-secreting .beta.TC1 cells, at dispersed acinar cells from guinea pig pancreas, and at parietal cells from stomach; the peptide is also said to stimulate somatostatin release and inhibit gastrin release in isolated stomachs (Goke, et al., J. Biol. Chem. 268:19650-55, 1993; Schepp, et al., Eur. J. Pharmacol., 69:183-91, 1994; Eissele, et al., Life Sci., 55:629-34, 1994). Exendin-3 and exendin-4 were reported to stimulate cAMP production in, and amylase release from, pancreatic acinar cells (Malhotra, R., et al., Regulatory Peptides,41:149-56, 1992;

Raufman, et al., J. Biol. Chem. 267:21432-37, 1992; Singh, et al., Regul. Pept. 53:47-59, 1994). The use of exendin-3 and exendin-4 as insulinotrophic agents for the treatment of diabetes mellitus and the prevention of hyperglycemia has been proposed (Eng, U.S. Pat. No. 5,424,286).

[007] Central GLP-1 receptors are present in both the rodent (Jin et al 1988, Shughrue et al 1996) and human (Wei and Mojsov 1995, Satoh et al 2000) brains. The chemoarchitecture of the distribution appears to be largely confined to the hypothalamus, thalamus, brainstem, lateral septum, the subfornical organ and the area postrema, all circumventricular areas where generally large numbers of peptide receptors are located. However, specific binding sites for GLP-1 have also been detected throughout the caudate-putamen, cerebral cortex and cerebellum (Campos et al. 1994, Calvo et al. 1995, Goke et al. 1995), albeit at low densities.

[008] C-terminally truncated exendin peptides such as exendin-4[9-39], a carboxyamidated molecule, and fragments 3-39 through 9-39 have been reported to be potent and selective antagonists of GLP-1 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993; Raufman, J. P., et al., J. Biol. Chem. 266:2897-902, 1991; Schepp, W., et al., Eur. J. Pharm. 269:183-91, 1994; Montrose-Rafizadeh, et al., Diabetes, 45(Suppl. 2):152A, 1996). Exendin-4[9-39] is said to block endogenous GLP-1 in vivo, resulting in reduced insulin secretion. Wang, et al., J. Clin. Invest., 95:417-21, 1995; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). The receptor apparently responsible for the insulinotropic effect of GLP-1 has reportedly been cloned from rat pancreatic islet cell (Thorens, B., Proc. Natl. Acad. Sci. USA 89:8641-8645, 1992). Exendins and exendin-4[9-39] are said to bind to the cloned GLP-1 receptor (rat pancreatic .beta.-cell GLP-1 receptor (Fehmann H C, et al., Peptides 15 (3): 453-6, 1994) and human GLP-1 receptor (Thorens B, et al., Diabetes 42 (11): 1678-82, 1993). In cells transfected with the cloned GLP-1 receptor, exendin-4 is reportedly an agonist, i.e., it increases cAMP, while exendin[9-39] is identified as an antagonist, i.e., it blocks the stimulatory actions of exendin-4 and GLP-1.

[009] Exendin-4[9-39] is also reported to act as an antagonist of the full length exendins, inhibiting stimulation of pancreatic acinar cells by exendin-3 and exendin-4 (Raufman, et

al., J. Biol. Chem. 266:2897-902, 1991; Raufman, et al., J. Biol. Chem., 266:21432-37, 1992). It is also reported that exendin[9-39] inhibits the stimulation of plasma insulin levels by exendin-4, and inhibits the somatostatin release-stimulating and gastrin release-inhibiting activities of exendin-4 and GLP-1 (Kolligs, F., et al., Diabetes, 44:16-19, 1995; Eissele, et al., Life Sciences, 55:629-34, 1994).

[010] Methods for regulating gastrointestinal motility using exendin agonists are described and claimed in U.S. application Ser. No. 08/908,867. Methods of reducing food intake using exendin agonists are described and claimed in U.S. application Ser. No. 09/003,869. Exendins have also been found to have inotropic and diuretic effects in International Application No. PCT/US99/02554. Additionally, exendins have been found to suppress glucagon secretion in U.S. Provisional Application No. 60/132,017.

[011] Exendin [9-39] has been used to investigate the physiological relevance of central GLP-1 in control of food intake (Turton, M. D. et al. Nature 379:69-72, 1996). GLP-1 administered by intracerebroventricular injection inhibits food intake in rats. This satiety-inducing effect of GLP-1 delivered ICV is reported to be inhibited by ICV injection of exendin [9-39] (Turton, supra). However, it has been reported that GLP-1 does not inhibit food intake in mice when administered by peripheral injection (Turton, M. D., Nature 379:69-72, 1996; Bhavsar, S. P., Soc. Neurosci. Abstr. 21:460 (188.8), 1995).

[012] Peripheral neuropathy is commonly associated with diabetes. Twenty to thirty percent of all diabetes subjects eventually develop peripheral neuropathy. Furthermore, there are reports of increased risk of Alzheimer's disease with heart disease, stroke, hypertension, and diabetes (Moceri et al., 2000; Ott et al., 1999). Thus, diabetes is a disease that is also associated with neurodegenerative diseases.

[013] N-terminal fragments of Exendin-4 have recently been demonstrated to have effects in cognition and cerebral health [US publications 20020115605, 20040014660, 20040092432].

[014] Exendin analogs have been described. Exendin agonist compounds include exendin acids, for example exendin-3 acid and exendin-4 acid. Exendin agonist compounds include those described in International Application No. PCT/US98/16387, U.S. Provisional Patent Application Serial No. 60/055,404, International Application No. PCT/US98/24210, U.S. Provisional Patent Application Serial No. 60/065,442, International Application No. PCT/US98/24273 and United States U.S. Provisional Patent Application Serial No. 60/066,029. Additional exendin agonist compounds are those described and claimed in U.S. Provisional Application Serial No. 60/132,018 These disclosures define the specific analogs, notably analogs resulting from Alanine screening of the native peptide (Ala substitution for each AA in the structure), including positions Q13, M14 and N28.

[015] Exendin solution formulation is acid (pH<5) (see Prescribing Information for BYETTA™). At acid pH, the chemical structure is more readily maintained, particularly with respect to reactions such as deamidation and chain hydrolysis. As a consequence of the pH and repeated injections, as with a chronic disease condition, local site irritation often results. Thus, there is a need for a peptide that can be formulated at neutral pH and is stable, while maintaining potency.

[016] US Patent Application 20060194719 demonstrates that Exendin-4 degradation products resulting from oxidation at M14 and W25 and from hydrolytic degradation reactions at Q13, N28, as well as combinations of each, can lead to useful analogs with potency. No new analogs were demonstrated. Similarly, Hargrove et.al.[Regulatory Peptides, 2007] presents a novel L14 analog of Exendin-4 with increased oxidative stability, but lesser potency than Exendin-4.

[017] Thus it would be desirable to have stable Exendin analogs than previously known that maintain therapeutic potency and have stable structures. Such compositions would be highly practical, since they would be less susceptible to degradation during handling and storage, particularly when ideal conditions cannot be maintained. In addition, they would find favour in manufacturing of more complex formulations as found in drug delivery systems, where the compositions are suspended, extruded, sheared, and otherwise handled

under conditions that could lead to the formation of degradation products of normal peptides and proteins. Finally, certain drug delivery devices require exceptionally stability in-vivo, such as in a delivery pump or a hydrogel implant. In these devices, the compositions are maintained in an aqueous or humid environment for extended periods of time, from days to weeks and even months.

[018] Changes in the primary structure that affect stability can also lead to profound changes in the peptide's potency, as they can affect the peptide's binding strength to the receptor, selectivity relative to multiple receptors, receptor activation, etc, all of which have a direct impact on the activity. The structure of an insulin secretalog VPAC2 was modified to provide a peptide analog with excellent stability in both solid and solution states; analogs were obtained with varying degrees of activity [James P. Whelan, et al. "Engineering Novel VPAC2-Selective Agonists with Improved Stability and Glucose-Lowering Activity in Vivo" J Pharm Exp Ther, published on-line November 16, 2006]. Vasointestinal peptide (VIP) analogs have been improved in a similar manner after extensive synthesis of analog compounds [D Bolin, Biopolymers, 1995, 37(2), 57].

[019] Needed in the art are polypeptides that are of therapeutic value in the treatment of diabetes and the treatment of degenerative disorders such as Alzheimer's and Parkinson's diseases, as well as the peripheral neuropathy associated with type 2 diabetes mellitus.

SUMMARY OF THE INVENTION

[020] The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following description.

[021] The present invention provides new peptide analog compositions with increased stability under a variety of conditions, including improved radiation stability, improved processing stability, improved storage stability and improved aqueous stability.

[022] In anther aspect of the invention, these compositions have similar or improved potency over Exendin-4.

[023] The invention provides for formulation of the novel analogs in solution or in controlled-release systems, such as liposomes, microspheres or implants.

[024] The invention also provides a method for treating diabetes type 1 or type 2, insulin resistance syndrome, impaired glucose tolerance (IGT), obesity, eating disorders, hyperglycemia, metabolic disorders, and gastric disease. The method includes administering a therapeutically effective amount of at least one of the stabilized Exendin-4 compounds or related molecules disclosed herein.

[025] The invention also provides a method for treating disease states associated with elevated blood glucose levels. The method includes administering a therapeutically effective amount of at least one of the stabilized Exendin-4 compounds or related molecules disclosed herein.

[026] Also provided is a method for regulation of blood glucose levels. The method includes administering a therapeutically effective amount of at least one of at least one of the stabilized Exendin-4 compounds or related molecules disclosed herein.

[027] The invention also provides a method for regulation of gastric emptying. The method includes administering a therapeutically effective amount of at least one of the stabilized Exendin-4 compounds or related molecules provided herein.

[028] The present invention also provides a method of stimulating insulin release in a mammal. The method includes administering an effective insulinotropic amount of at least one of the stabilized Exendin-4 compounds disclosed herein.

[029] Additionally provided is a method of lowering blood glucose level in a mammal. The method includes administering an amount of at least one of the stabilized Exendin-4

compounds or related molecules described herein in an amount effective to lower blood glucose level in said mammal.

[030] The invention also provides a method of lowering plasma lipid level in a mammal. The method includes administering an amount of at least one of the stabilized Exendin-4 compounds described herein in an amount effective to lower plasma lipid level in said mammal.

[031] Also provided is a method of reducing mortality and morbidity after myocardial infarction in a mammal. The method includes administering an amount of at least one of the stabilized Exendin-4 compounds disclosed herein in an amount effective to reduce mortality and morbidity after myocardial infarction.

[032] Also provided is a method of stimulating insulin release in a mammal. The method includes administering an effective insulinotropic amount of at least one of the stabilized Exendin-4 compounds provided herein.

[033] Preferably, the mammal featured in each of the foregoing methods is a primate, preferably a human patient in need of treatment.

[034] Also provided is an exendin-4(1-39) analog having:

[035] (a) A substitution of Met14 with an substituent selected from the group consisting of: R-alkylglycine, S-alkylglycine, H2N-CHR^1-CO-, C2-C20, and a Hse(OR^2) derivative, wherein R^1 is a linear or branched alkyl and R^2 is a short chain linear or branched C1-C6 alkyl; and

[036] (b) A substitution of Asn28 with a polar amino acid residue selected from the group consisting of S, T, Q, R, K, E, G, P, hydroxyproline, and beta-Alanine.

[037] A further aspect of the present invention is an exendin-4(1-39) analog as described above, also having a substitution of Q13 with a polar amino acid residue selected from the group consisting of S, T, Q, Y, R, K, E, G, P, hydroxyproline and beta-alanine.

[038] In another embodiment, the Met14 is substituted with octylglycine.

[039] In a further embodiment, the Met14 is substituted with O-methyl-homoserine.

[040] In yet a further embodiment the exendin-4(1-39) analog has an acylated N-terminal amino group.

[041] In yet a further embodiment of the present invention, the exendin-4(1-39) analog has as a C-terminal carboxylic acid a substituent selected from the group consisting of a free acid, a pharmaceutically accepted salt form and an amide $N(R^3)_2$, wherein R^3 is the same or different and selected from the group consisting of H, a linear C1-6 alkyl group, and a branched C1-6 alkyl group.

[042] In a further embodiment, the exendin-4(1-39) analog has an amino acid sequence as described in SEQ ID No. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

[043] Another aspect of the present invention is a pharmaceutically acceptable salt form of the exendin analogs as herein described.

[044] In a further embodiment is provided a pharmaceutical composition comprising a exendin analog as herein described, and a pharmaceutically acceptable excipient.

[045] In one embodiment, the pharmaceutical composition is in a form selected from the group consisting of an injectable solution, an injectable suspension, a depot formulation, a microsphere formulation, a liposome formulation, and a hydrogel implant.

[046] A further aspect of the present invention is a method of treating or regulating a disorder selected from the group consisting of diabetes type 1, diabetes type 2, insulin resistance syndrome, stimulation of insulin secretion, impaired glucose tolerance, elevated glucose levels (hyperglycemia), polycystic ovarian syndrome, a metabolic disorder, an eating disorder, a weight disorder, a gastric disease, regulation of gastric emptying, cerebral health, and associated neuropathies, comprising administration of a pharmaceutical composition comprising an exendin analog as herein described.

[047] In a further embodiment, the administration may be in combination with the administration of a second therapeutically relevant compound, such as, for example, insulin, EFG, an anti-inflammatory drug, a specific phosphodiesterase-4 (PDE-4) inhibitor, or a non-specific phosphodiesterase inhibitor. The administration may be a single treatment or repeated treatments.

[048] In yet a further embodiment, the composition is administered via a continuous infusion, wherein the continuous infusion is selected from the group consisting of subcutaneous, intravenous, or intraperitonial.

[049] In yet a further embodiment, the composition is administered via an implant, a pump or a depot for continuous delivery.

[050] Another aspect of the present invention is the use of a composition as herein described in treating or regulating a disorder selected from the group consisting of diabetes type 1, diabetes type 2, insulin resistance syndrome, stimulation of insulin secretion, impaired glucose tolerance, elevated glucose levels (hyperglycemia), polycystic ovarian syndrome, a metabolic disorder, an eating disorder, a weight disorder, a gastric disease, regulation of gastric emptying, cerebral health, and associated neuropathies.

[051] In one embodiment, the use further comprises the use of a second therapeutically relevant compound, such as, for example, insulin, EFG, an anti-inflammatory drug, a specific phosphodiesterase-4 (PDE-4) inhibitor, or a non-specific phosphodiesterase inhibitor.

[052] In a further embodiment, the use of the composition comprises administration of a single treatment or repeated treatments.

[053] In a further embodiment, the use of the composition comprises administration via a continuous infusion, wherein the continuous infusion is selected from the group consisting of subcutaneous, intravenous, or intraperitonial.

[054] In a further embodiment, the use of the composition comprises administration via an implant, a pump or a depot for continuous delivery.

[055] A further aspect of the present invention is the use of a exendin(1-39) analog as herein described in the preparation of a medicament for the treatment or regulation of a disorder selected from the group consisting of diabetes type 1, diabetes type 2, insulin resistance syndrome, stimulation of insulin secretion, impaired glucose tolerance, elevated glucose levels (hyperglycemia), polycystic ovarian syndrome, a metabolic disorder, an eating disorder, a weight disorder, a gastric disease, regulation of gastric emptying, cerebral health, and associated neuropathies.

DETAILED DESCRIPTION OF THE INVENTION

[056] As discussed, the invention provides for particularly stable compositions of Exendin-4 and Exendin agonists that include at least one modified amino acid residue at positions Q13, M14, D28 of the Exendin-4 molecule. In one embodiment, modifications include the acid, amide or substituted amide forms of Exendin-4 with substitution of M14 for an alkylglycine (R- or S-alkylglycine, H2N-CHR-CO- wherein R=C2-C20) and substitution of D28 for a polar amino acid group, for example, S, T, Q, R, Y, K, E, or G.

[057] Also provided are compositions wherein the above modifications are included with additional substitution of Q13 for a polar amino acid group, for example, S, T, Q, R, Y, K, E, G.

[058] Preferred compositions are those that retain at least 70% of the activity of the original Exendin-4 compound, more preferably at least 80%, 90% or activity greater than the original Exendin-4 compound. Methods for testing the biological activity of a variety of Exendin-4 (1-39) compounds and related molecules are routine, and have been disclosed, for example, in WO 01/04156, EP application 99610043.4 and U.S. provisional application 60/143,591, the disclosures of which are incorporated herein by reference.

[059] The polypeptides of the invention can be prepared using any of a number of chemical polypeptide synthesis techniques well known to those of ordinary skill in the art including solution methods and solid phase methods. Solid phase synthesis in which the C-terminal amino acid of the polypeptide sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is one synthetic method for preparing the polypeptides. Techniques for solid phase synthesis are described by Merrifield et al., J. Am. Chem. Soc. 85:2149-2156 (1963). Many automated systems for performing solid phase peptide synthesis are commercially available.

[060] Solid phase synthesis is started from the carboxy-terminal end (i.e., the C-terminus) of the polypeptide by coupling a protected amino acid via its carboxyl group to a suitable solid support. The solid support used is not a critical feature provided that it is capable of binding to the carboxyl group while remaining substantially inert to the reagents utilized in the peptide synthesis procedure. For example, a starting material can be prepared by attaching an amino-protected amino acid via a benzyl ester linkage to a chloromethylated resin or a hydroxymethyl resin or via an amide bond to a benzhydrylamine (BHA) resin or p-methylbenzhydrylamine (MBHA) resin. Materials suitable for use as solid supports are well known to those of skill in the art and include, but are not limited to, the following: halomethyl resins, such as chloromethyl resin or bromomethyl resin; hydroxymethyl resins; phenol resins, such as 4-(a-[2,4-dimethoxyphenyl]-Fmoc-aminomethyl)phenoxy resin; tert-alkyloxycarbonyl-hydrazidated resins; and the like. Such resins are commercially available and their methods of preparation are known to those of ordinary skill in the art.

[061] The acid form of the peptides may be prepared by the solid phase peptide synthesis procedure using a benzyl ester resin as a solid support. The corresponding amides may be produced by using benzhydrylamine or methylbenzhydrylamine resin as the solid support. Those skilled in the art will recognize that when the BHA or MBHA resin is used, treatment with anhydrous hydrofluoric acid to cleave the peptide from the solid support produces a peptide having a terminal amide group.

[062] The .alpha.-amino group of each amino acid used in the synthesis should be protected during the coupling reaction to prevent side reactions involving the reactive .alpha.-amino function. Certain amino acids also contain reactive side-chain functional groups (e.g. sulthydryl, amino, carboxyl, hydroxyl, etc.) which must also be protected with appropriate protecting groups to prevent chemical reactions from occurring at those sites during the peptide synthesis. Protecting groups are well known to those of skill in the art. See, for example, The Peptides: Analysis, Synthesis, Biology, Vol. 3: Protection of Functional Groups in Peptide Synthesis (Gross and Meienhofer (eds.), Academic Press, N.Y. (1981)).

[063] A properly selected .alpha.-amino protecting group will render the .alpha.-amino function inert during the coupling reaction, will be readily removable after coupling under conditions that will not remove side chain protecting groups, will not alter the structure of the peptide fragment, and will prevent racemization upon activation immediately prior to coupling. Similarly, side-chain protecting groups must be chosen to render the side chain functional group inert during the synthesis, must be stable under the conditions used to remove the .alpha.-amino protecting group, and must be removable after completion of the peptide synthesis under conditions that will not alter the structure of the peptide.

[064] Coupling of the amino acids may be accomplished by a variety of techniques known to those of skill in the art. Typical approaches involve either the conversion of the amino acid to a derivative that will render the carboxyl group more susceptible to reaction with the free N-terminal amino group of the peptide fragment, or use of a suitable coupling agent such as, for example, N,N'-dicyclohexylcarbodimide (DCC) or N,N'-diisopropylcarbodiimide (DIPCDI). Frequently, hydroxybenzotriazole (HOBt) is employed as a catalyst in these coupling reactions.

[065] Generally, synthesis of the peptide is commenced by first coupling the C-terminal amino acid, which is protected at the N-amino position by a protecting group such as fluorenylmethyloxycarbonyl (Fmoc), to a solid support. Prior to coupling of Fmoc-Asn, the Fmoc residue has to be removed from the polymer. Fmoc-Asn can, for example, be coupled to the 4-(a-[2,4-dimethoxyphenyl]-Fmoc-amino-methyl)phenoxy resin using N,N'-

dicyclohexylcarbodimide (DCC) and hydroxybenzotriazole (HOBt) at about 25.degree. C. for about two hours with stirring. Following the coupling of the Fmoc-protected amino acid to the resin support, the .alpha.-amino protecting group is removed using 20% piperidine in DMF at room temperature.

[066] After removal of the .alpha.-amino protecting group, the remaining Fmoc-protected amino acids are coupled stepwise in the desired order. Appropriately protected amino acids are commercially available from a number of suppliers (e.g., Novartis (Switzerland) or Bachem (Torrance, Calif.)). As an alternative to the stepwise addition of individual amino acids, appropriately protected peptide fragments consisting of more than one amino acid may also be coupled to the "growing" peptide. Selection of an appropriate coupling reagent, as explained above, is well known to those of skill in the art.

[067] Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in excess and the coupling is carried out in a medium of dimethylformamide (DMF), methylene chloride (CH.sub.2Cl.sub.2), or mixtures thereof. If coupling is incomplete, the coupling reaction may be repeated before deprotection of the N-amino group and addition of the next amino acid. Coupling efficiency may be monitored by a number of means well known to those of skill in the art. A preferred method of monitoring coupling efficiency is by the ninhydrin reaction. Peptide synthesis reactions may be performed automatically using a number of commercially available peptide synthesizers such as the Biosearch 9500.TM. synthesizer (Biosearch, San Raphael, Calif.).

[068] The peptide can be cleaved and the protecting groups removed by stirring the insoluble carrier or solid support in anhydrous, liquid hydrogen fluoride (HF) in the presence of anisole and dimethylsulfide at about 0.degree. C. for about 20 to 90 minutes, preferably 60 minutes; by bubbling hydrogen bromide (HBr) continuously through a 1 mg/10 mL suspension of the resin in trifluoroacetic acid (TFA) for 60 to 360 minutes at about room temperature, depending on the protecting groups selected; or by incubating the solid support inside the reaction column used for the solid phase synthesis with 90% trifluoroacetic acid, 5% water and 5% triethylsilane for about 30 to 60 minutes. Other deprotection methods well known to those of skill in the art may also be used.

[069] The peptides can be isolated and purified from the reaction mixture by means of peptide purification well known to those of skill in the art. For example, the peptides may be purified using known chromatographic procedures such as reverse phase HPLC, gel permeation, ion exchange, size exclusion, affinity, partition, or countercurrent distribution.

[070] The polypeptides of the invention can also be prepared by other means including, for example, recombinant techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Sambrook et al. (1989) Molecular Cloning--A Laboratory Manual (2 nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook).

[071] The invention further provides a method of treating a subject with diabetes, comprising administering to the subject the polypeptide of the invention in an amount that has an insulinotropic effect. By "diabetes" is meant diabetes mellitus. The method of the present invention is considered to be useful in the treatment of a subject having type 2 diabetes. The method of the present invention could be of use in other forms of diabetes (including, for example, type 1 diabetes) when the polypeptide promotes non-insulin producing cells to produce insulin.

[072] The polypeptides of the present invention may have uses in the nervous system [US pub 20040242853]. The polypeptides are neurotrophic (i.e. promoting proliferation, differentiation or neurite outgrowth) or neuroprotective (i.e. rescuing neuron cells or reducing neuronal cell death). Thus, the invention further relates to a method of reducing neuronal death, comprising contacting one or more neurons with a polypeptide of the present invention. Neuronal death may occur, for example, with mechanical injury (e.g., trauma or surgery), toxic injury, neurodegenerative disease, apoptosis, and peripheral neuropathy. One skilled in the art would recognize that rescuing neurons (i.e., promoting viability of cells that show signs of cell death) and reducing neuronal death (i.e., promoting viability of cells that do not show signs of cell death) may be desired. For example, treatment with a compound that reduced neuronal death would be useful in treating an explant or culture of neuronal cells, prior to subsequent transplantation. Also, such

treatment could be used to rescue neurons and reduce neuronal death following a stroke, brain or spinal cord injury, nerve injury, or neurotoxic injury. Furthermore, rescuing neurons or reducing neuronal death would be useful in the treatment of neurodegenerative condition or disease diseases, including, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis, and peripheral neuropathy.

[073] The invention also relates to a method of promoting neuronal differentiation or proliferation, comprising contacting one or more neurons or neuronal precursor cells with a polypeptide of the present invention. Differentiation involves a transition from a cell state in which the cell lacks neuronal characteristics (e.g., lacks characteristics such as a distinct nucleolus, neuronal processes, extensive rough endoplasmic reticulum, expression of neuronal markers) to a cell state characterized by a neuronal phenotype. By neuronal proliferation is meant that stem cells or cells of neuronal lineage divide and/or differentiate into neurons. The effect of either differentiation or proliferation is an increase in the number of neurons. By "an increase in the number of neurons" is meant an addition of neurons to the total number of all neurons present. Thus, the rate of neuronal cell death may exceed the rate of differentiation or proliferation, but the addition of new neurons is still considered to be an increase over the total neurons and such an increase in number, even in the absence of an increase in the total number of living neurons, could still have therapeutic advantages.

[074] The present invention also relates to a method of reducing formation or accumulation of amyloid .beta. protein, comprising contacting one or more neurons with a polypeptide of the present invention. Such a method could be useful in lowering levels of amyloid protein or in preventing the deposition of amyloid protein, which is observed in senile plaques in a subject with Alzheimer's Disease. The method of the present invention could reduce formation or accumulation of amyloid .beta. protein by acting at various points in the processing of .beta.-amyloid precursor protein. For example, the polypeptide may decrease synthesis of .beta.-amyloid precursor protein, promote cleavage of .beta.-amyloid precursor protein within the amyloid .beta. protein region, increase secretion of

soluble .beta.-amyloid precursor protein, decrease secretion of amyloid .beta. protein, or increase degradation of amyloid .beta. protein.

[075] The present invention also relates to a method of treating a subject with a neurodegenerative condition or of reducing one or more symptoms of a neurodegenerative condition in a subject, comprising administering to the subject a therapeutically effective amount of a polypeptide of the present invention. More specifically, the treatment could be directed to neurodegenerative conditions selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, stroke, multiple sclerosis, brain injury, spinal cord injury, and peripheral neuropathy.

[076] Also provided by the present invention is a pharmaceutical composition comprised of a polypeptide of the invention in combination with a pharmaceutically acceptable carrier. One skilled in the art would recognize how to monitor the effectiveness of the treatment and how to adjust the treatment accordingly. For example, blood glucose levels could be monitored with normoglycemia being the optimal effect of treatment. If blood glucose levels are higher than preferred levels, then the amount of polypeptide administered should be increased, and, if blood glucose levels are lower than preferred levels, then the amount of polypeptide administered would be decreased.

[077] The dosages of the polypeptides to be used in the in vivo method of the invention preferably range from about 0.1 pmoles/kg/minute to about 100 nmoles/kg/minute for continuous administration and from about 0.01 nmoles/kg to about 400 nmoles/kg for bolus injection. Preferably, the dosage of the polypeptide in in vivo methods range from about 0.01 nmoles/kg/min to about 10 nmoles/kg/min. The exact amount required will vary from polypeptide to polypeptide and subject to subject, depending on the species, age, and general condition of the subject, the severity of disease that is being treated, the particular polypeptide used, its mode of administration, and the like. Thus, it is not possible to specify an exact "insulinotropic amount" or an amount useful in treating neuronal disease or injury. However, an appropriate amount may be determined by one of ordinary skill in the art using only routine experimentation.

[078] A wide variety of pharmaceutically acceptable formulations are known in the field. Such formulations may be in a form adapted to oral, parenteral (including subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), epidural, direct brain and intraperitoneal (i.p.)), rectal, intratracheal, intranasal, dermal, vaginal, buccal, ocularly, or pulmonary administration, preferably in a form adapted to subcutaneous or oral administration, and such compositions may be prepared in a manner well-known to the field. See generally described in "Remington's Pharmaceutical Sciences", 17. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, Pa., U.S.A., 1985 and more recent editions and in the monographs in the "Drugs and the Pharmaceutical Sciences" series, Marcel Dekker. The compositions may appear in conventional forms, for example, capsules, tablets, aerosols, topical application forms, liquid or semiliquid forms, such as solutions, suspensions, dispersions, emulsions, micelles or liposomes. Preferred are liquid compositions suitable for s.c. administration. The compositions of the present invention are administered subcutaneously. In an alternative preferred method, the compositions of the present invention are administered orally, and in such cases one preferred administration form is a tablet or capsule.

[079] If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

[080] For nasal administration, the preparation may contain a compound of the present invention, preferably a conjugate, dissolved or suspended in a liquid carrier, in particular, an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents, e.g., propylene glycol, surfactants such as bile acid salts or polyoxyethylene higher alcohol ethers, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabines. A stabilized Exendin-4 (1-39) compound or related molecule of the invention may also be in a form suited for local or systemic injection or infusion and may, as such, be formulated with sterile water or an isotonic saline or glucose solution. The compositions may be sterilized by conventional sterilization techniques, which are well known in the art. The resulting aqueous solutions may be packaged for use or filtered under aseptic conditions and

lyophilized, the lyophilized preparation being combined with the sterile aqueous solution prior to administration. Preferably, the formulation to be used for intravenous, subcutaneous and oral dosing will be a solution of the active compound in buffer. The preparation may be produced immediately before use from active drug substance and sterile buffer solution. One preferred method of sterilization may be by sterile filtration of a solution made immediately prior to use. The compound or related molecule may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents and the like, for instance sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc.

[081] The stabilized Exendin-4 (1-39) compounds and related molecules of the invention find use in a wide spectrum of applications. Some are described in WO 99/40788 (relating to the inotropic and diuretic effects of Exendin and GLP-1); and WO 98/39022 (relating to a method of sedating a mammalian subject having increased activation of the central or peripheral nervous system comprising administering Exendin or GLP-1 or an agonist of Exendin or GLP-1 to the subject to produce a sedative or anxiolytic effect on the subject); and WO 93/18786 (relating to the treatment of diabetes using GLP-1(7-37) or GLP-1(7-36)amide in a regimen which additionally comprises treatment with an oral hypoglycaemic agent, such as sulfonylurea, producing a strong synergistic effect); and WO 98/19698 (relating to the use of GLP-1 analogs for the regulation of obesity); WO 98/08531 (relating to the use of GLP-1 or analogs in a method of reducing mortality and morbidity after myocardial infarction); WO 98/08873 (relating to the use of GLP-1 or analogs in a method of attenuating post-surgical catabolic changes and hormonal responses to stress). Besides, the compounds of the invention are suitable in a combination therapy with other antidiabetic agents, such as insulin, metformin, sulfonyl ureas and thiazolidinediones, or in combination therapy with other antiobesity agents, such as leptin, dexphenfluramine, amphetamin etc, or in combination with anti-inflammatory agents to synergistically treat underlying and associated low-level inflammation aggravating the diabetes situation [Biochem Biophys Res Commun. 2006 Jun 9;344(3):1017-22].

[082] Other formulations are within the scope of the present invention. Such formulations include, but are not limited to, formulations that include at least one of the

stabilized Exendin-4 (1-39) compounds disclosed herein combined with liposomes, microspheres and liquid stabilizers. Depot formulations that include at least one of the stabilized Exendin-4 (1-39) compounds are also envisioned. See U.S. Pat. Nos. 5,407,609 and 5,654,008 for additional information.

[083] Other formulations also included within the scope of the present invention, include hydrogel implants, as described US5292515, US6969480. These hollow hydrogel implants serve as rate limiting diffusion barriers for long-term sustained drug release. Release can be made to occur for periods well beyond 3 months and even up to 2 years [Drugs R D. 2005;6(1):53-5 and N Engl J Med. 1999 May 6;340(18):1439]. Other implants include the alzet minipump [Alza Corp], used primarily in animal research and the Duros osmotic pump implant [Alza Corp]. Hydrogel implants are placed in the subcutaneous region either by a minor surgical procedure or using a trocar device [US D492,995] which have for effect to separate the dermal layer from the muscle layer, and allow for insertion of the implant into this subcutaneous space. Stability of a peptide must be exemplary when these devices are used for extended periods of time.

[084] The stabilized Exendin-4 (1-39) compounds of the invention can be made by conventional peptide synthetic routes including use of the Merrifield synthesis. Modified amino acid residues in accord with the invention can be purchased from commercial suppliers (eg., modified Gln and Asp) or they can be readily made using standard techniques (eg., oxidation of Met). See Merrifield, B. (1985) in Science 232: 341.

[085] Suitable methods for detecting the stabilized Exendin-4 (1-39) compositions and related molecules disclosed herein are known in the field and include, but are not limited to, reverse phase high performance liquid chromatography (RP-HPLC), and liquid chromatography/mass spectrometry (LC-MS). Additionally suitable techniques include conventional amino acid sequencing, peptide mapping, MS/MS and fluorescence.

[086] It should be understood that the compositions and compounds of the invention might also be in the amide form (NR.sub.2, where each R is identical or different and

selected from a group consisting of H or an linear or branched alkyl group consisting of 1-6 carbons) or in the free acid (OH) form or in the form of a salt thereof.

[087] In methods in which one or more of the stabilized Exendin-4 (1-39) compounds and related molecules are used therapuetically, such use will typically involve administration of one or more of the pharmaceutically acceptable compositions disclosed herein. Such a composition can be combined with a suitable amount of vehicle and/or stabilizer. One such approach involves administering the composition (eg. as a depot formulation, liquid formulation, with microspheres or liposomes, i.v) to provide a dosage of about 0.1 pg/kg, to 1.000 mg/kg body weight. The amount of the composition to use will depend on recognized parameters including age, severity of the disease, total body weight, sex and other factors.

[088] "Salts" include pharmaceutically acceptable salts, such as acid addition salts and basic salts. Examples of acid addition salts are hydrochloride salts, sodium salts, hydrobromide salts, etc. Examples of basic salts are salts where the cation is selected from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium, and ammonium ions .sup.+N(R.sup.3).sub.3(R.sup.4), where R.sup.3 and R.sup.4 independently designates optionally substituted C.sub.1-6alkyl, optionally substituted C.sub.2-6alkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Other examples of pharmaceutically acceptable salts are; e.g., those described in "Remington's Pharmaceutical Sciences" 17. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, Pa., U.S.A., 1985 and more recent editions, and in Encyclopedia of Pharmaceutical Technology.

[089] The following Examples are illustrative and not limiting as to the scope of the present invention.

[090] EXAMPLES

Example 1

[091] Exendin-4 amide (SEQ ID1) (EXENATIDE™, American Peptide Corp) was placed in a pH7.2 phosphate buffer and maintained at 37C for several months. Periodically, the solution was analyzed by HPLC (Shimadzu, RP18, mobile phase gradient 20-80%B over 30 min, A=H2O/0.1TFA, B=ACN /0.1TFA). Degradation products from hydrolytic reactions were identified by +ESI-LCMS. The degradation products included deamidation at each of the 3 sites Q19, N34 and the N-terminal amide at S45. 50% Deamidation was observed as soon as 2 weeks from the onset of the experiment.

Example 2

[092] Exenatide powder was placed in a pH4 acetate buffer and maintained at 37C for several months. Periodically, the solution was analyzed by HPLC (Shimadzu, RP18, mobile phase gradient 20-80%B over 30 min, A=H2O/0.1TFA, B=ACN /0.1TFA). Degradation products from hydrolytic reactions were identified by +ESI-LCMS. The degradation products included deamidation at each of the 3 sites Q19, N34 and the N-terminal amide at S45. Deamidation was slow, progressing by <10% per month.

Example 3

[093] An exendin-4 acid analog having the sequence of SEQ ID No. 2 (i.e. having the following modifications: E13, Z14, D28, with Z= octylglycine) was prepared by standard solid phase peptide techniques.

Example 4

[094] An exendin-4 acid analog having the sequence of SEQ ID No. 3 (i.e. having the following modifications: Y13, Z14, Q28, with Z=octylglycine) was prepared by standard solid phase peptide techniques.

Example 5

[095] An exendin-4 acid analog having the sequence of SEQ ID No. 4 (i.e. having the following modifications: R13, Z14, K28, where Z=octylglycine) was prepared by standard solid phase peptide techniques.

Example 6

[096] An exendin-4 acid analog having the sequence of SEQ ID No. 5 (i.e. having the following modifications: R13, Z14, R28, where Z=octylglycine) was prepared by standard solid phase peptide techniques.

Example 7

[097] An exendin-4 acid analog having the sequence of SEQ ID No. 6 (i.e. having the following modifications: S13, Z14, S28, where Z= octylglycine) was prepared by standard solid phase peptide techniques.

Example 8

[098] An exendin-4 acid analog having the sequence of SEQ ID No. 7 (i.e. having the following modifications: Y13, Z14, Y28, where Z= octylglycine) was prepared by standard solid phase peptide techniques.

Example 9

[099] An exendin-4 acid analog having the sequence of SEQ ID No. 8 (i.e. having the following modifications: Y13, Hse14, E28, where Hse= O-methyl-homoserine) is prepared by standard solid phase peptide techniques.

Example 10

[0100] An exendin-4 acid analog having the sequence of SEQ ID No. 9 (i.e. having the following modifications: Y13, Hse14, b-Ala28, where Hse= O-methyl-homoserine and b-Ala = beta-alanine) is prepared by standard solid phase peptide techniques.

Example 11

[0101] An exendin-4 acid analog having the sequence of SEQ ID No. 10 (i.e. having the following modifications: E13, Hse14, b-Ala28, where Hse= O-methyl-homoserine and b-Ala = beta-alanine) is prepared by standard solid phase peptide techniques.

Example 12

[0102] An exendin-4 acid analog having the sequence of SEQ ID No. 11 (i.e. having the following modifications: S13, Hse14, b-Ala28, where Hse= O-methyl-homoserine and b-Ala = beta-alanine) is prepared by standard solid phase peptide techniques.

Example 13

[0103] An exendin-4 acid analog having the sequence of SEQ ID No. 12 (i.e. having the following modifications: E13, Hse14, E28, where Hse= O-methyl-homoserine) is prepared by standard solid phase peptide techniques.

Example 14

[0104] An exendin-4 acid analog having the sequence of SEQ ID No. 13 (i.e. having the following modifications: S13, Hse14, E28, where Hse= O-methyl-homoserine) is prepared by standard solid phase peptide techniques.

Example 15

[0105] An exendin-4 acid analog having the sequence of SEQ ID No. 14 (i.e. having the following modifications: Y13, Hse14, S28, where Hse=O-methyl-homoserine) is prepared by standard solid phase peptide techniques.

Example 16

[0106] An exendin-4 acid analog having the sequence of SEQ ID No. 15 (i.e. having the following modifications: E13, Hse14, S28, where Hse = O-methyl-homoserine) is prepared by standard solid phase peptide techniques.

Example 17

[0107] The compositions of examples 3-16 are placed in aqueous pH 7 buffer and incubated at 40°C for 3 months. Periodically, an aliquot is removed and analyzed by HPLC. <5% degradation is observed after the test period for all of the peptides tested.

Example 18

[0108] The time-dependent duration of insulinotropic action is evaluated by quantifying plasma insulin and glucose levels in Zucker rats following intraperitoneal (i.p.) peptide administration. Specifically, after overnight fasting, diabetic male rats, approximately 400 g weight, are anesthetized with 50 mg/kg pentobarbital and a catheter was tied into their right femoral artery for blood collection. Thereafter, a bolus of exendin-4 (i.e. SEQ ID NO. 1) or a peptide prepared as described in Examples 3-16 and having SEQ ID No. 2-15, respectively (0.4 nmol/kg) is administered intraperitonally. Blood is taken prior to peptide administration, as well as at 30 and 60 min, and at 2, 4, 6 and 24 h, and drawn into heparinized tubes containing EDTA and aprotinin for insulin determination. A separate blood sample is also taken to measure glucose.

[0109] Plasma is separated, removed and immediately frozen to -70.degree. C. Thereafter insulin levels are quantified by using a rat insulin ELISA kit (Crystal Chem Inc., Chicago, Ill.) and plasma glucose is quantified by the glucose oxidase method.

[0110] The peptides of Examples 3-16 show insulinotropic action roughly equivalent to, or greater than, the insulinotropic action of exendin-4. Many of the peptides show insulinotropic action greater than exendin-4.

Example 19

[0111] Example 18 is repeated, this time using peptides and exendin-4 that have been incubated at 40°C for two months prior to interperitoneal administration. The peptides of Examples 3-16 show insulinotropic action roughly equivalent to the insulinotropic action obtained for the same peptide in Example 18 (i.e. the non-stored peptide). Exendin-4 shows significantly less insulinotropic action than the exendin-4 in Example 18. The peptides of examples 3-16 show significantly greater isulinotropic action than exendin-4.

SEQUENCE ID NUMBERS

SEQ ID1: HGEGT FTSDL SKQME EEAVR LFIEW LKNGG PSSGA PPPS-NH2

SEQ ID2: HGEGT FTSDL SKEZE EEAVR LFIEW LKDGG PSSGA PPPS-OH

SEQ ID3: HGEGT FTSDL SKYZE EEAVR LFIEW LKQGG PSSGA PPPS-OH

SEQ ID4: HGEGT FTSDL SKRZE EEAVR LFIEW LKKGG PSSGA PPPS-OH

SEQ ID5: HGEGT FTSDL SKRZE EEAVR LFIEW LKRGG PSSGA PPPS-OH

SEQ ID6: HGEGT FTSDL SKSZE EEAVR LFIEW LKSGG PSSGA PPPS-OH

SEQ ID7: HGEGT FTSDL SKYZE EEAVR LFIEW LKYGG PSSGA PPPS-OH

SEQ ID8: HGEGT FTSDL SKYXE EEAVR LFIEW LKEGG PSSGA PPPS-OH (X=Hse(OMe))

SEQ ID9: HGEGT FTSDL SKYXE EEAVR LFIEW LKBGG PSSGA PPPS-OH (B=beta-Ala)

SEQ ID10: HGEGT FTSDL SKEXE EEAVR LFIEW LKBGG PSSGA PPPS-OH (B=beta-Ala)

SEQ ID11: HGEGT FTSDL SKSXE EEAVR LFIEW LKBGG PSSGA PPPS-OH (B=beta-Ala)

SEQ ID12: HGEGT FTSDL SKEXE EEAVR LFIEW LKEGG PSSGA PPPS-OH (B=beta-Ala)

SEQ ID13: HGEGT FTSDL SKSXE EEAVR LFIEW LKEGG PSSGA PPPS-OH (B=beta-Ala)

SEQ ID14: HGEGT FTSDL SKYXE EEAVR LFIEW LKSGG PSSGA PPPS-OH (B=beta-Ala)

SEQ ID15: HGEGT FTSDL SKEXE EEAVR LFIEW LKSGG PSSGA PPPS-OH (B=beta-Ala)

CLAIMS

1. An exendin-4(1-39) analog having:

(a) A substitution of Met14 with an substituent selected from the group consisting of: R-alkylglycine, S-alkylglycine, H2N-CHR^1-CO-, C2-C20, and a Hse(OR^2) derivative, wherein R^1 is a linear or branched alkyl and R^2 is a short chain linear or branched C1-C6 alkyl; and

(b) A substitution of Asn28 with a polar amino acid residue selected from the group consisting of S, T, Q, R, K, E, G, P, hydroxyproline, and beta-Alanine.

2. An exendin-4(1-39) analog of claim 1 also having a substitution of Q13 with a polar amino acid residue selected from the group consisting of S, T, Q, Y, R, K, E, G, P, hydroxyproline and beta-alanine.

3. An exendin-4(1-39) analog of claim 1 or 2 wherein Met14 is substituted with octylglycine.

4. An exendin-4(1-39) analog of claim 1 or 2 wherein Met14 is substituted with O-methyl-homoserine.

5. An exendin-4(1-39) analog of any one of claims 1-4, having an acylated N-terminal amino group.

6. An exendin-4(1-39) analog of any one of claims 1-5, having as a C-terminal carboxylic acid a substituent selected from the group consisting of a free acid, a pharmaceutically accepted salt form and an amide N(R^3)$_2$, wherein R^3 is the same or different and selected from the group consisting of H, a linear C1-6 alkyl group, and a branched C1-6 alkyl group.

7. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 2.

8. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 3.

9. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 4.

10. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 5.

11. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 6.

12. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 7.

13. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 8.

14. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 9.

15. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 10.

16. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 11.

17. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 12.

18. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 13.

19. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 14.

20. An exendin-4(1-39) analog of claim 1 having a sequence of SEQ ID No. 15.

21. A pharmaceutically acceptable salt form of the exendin analog of any one of claims 1-20.

22. A pharmaceutical composition comprising the exendin analog of any one of claims 1-21, and a pharmaceutically acceptable excipient.

23. The composition of claim 22, further characterized as being in a form selected from the group consisting of an injectable solution, an injectable suspension, a depot formulation, a microsphere formulation, a liposome formulation, and a hydrogel implant.

24. A method of treating or regulating a disorder selected from the group consisting of diabetes type 1, diabetes type 2, insulin resistance syndrome, stimulation of insulin secretion, impaired glucose tolerance, elevated glucose levels (hyperglycemia), polycystic ovarian syndrome, a metabolic disorder, an eating disorder, a weight disorder, a gastric disease, regulation of gastric emptying, cerebral health, and associated neuropathies, comprising administration of a composition of any one of claims 21 or 22.

25. The method of claim 23 further comprising administration of a therapeutically relevant compound.

26. The method of claim 24 wherein the therapeutically relevant compound is selected from the group consisting of insulin, EFG, an anti-inflammatory drug, a specific phosphodiesterase-4 (PDE-4) inhibitor, and a non-specific phosphodiesterase inhibitor.

27. The method of any one of claims 24-26 wherein the composition is administered in a regime selected from the group consisting of a single treatment and repeated treatments.

28. The method of any one of claims 24-26 wherein the composition is administered via a continuous infusion, wherein the continuous infusion is selected from the group consisting of subcutaneous, intravenous, or intraperitonial.

29. The method of any one of claims 24-26 wherein the composition is administered via an implant, a pump or a depot for continuous delivery.

30. Use of a composition of any one of claims 22 or 23 in treating or regulating a disorder selected from the group consisting of diabetes type 1, diabetes type 2, insulin resistance syndrome, stimulation of insulin secretion, impaired glucose tolerance, elevated glucose levels (hyperglycemia), polycystic ovarian syndrome, a metabolic disorder, an eating

disorder, a weight disorder, a gastric disease, regulation of gastric emptying, cerebral health, and associated neuropathies.

31. The use of claim 30 further comprising use of a second therapeutically relevant compound.

32. The use of claim 31 wherein the second therapeutically relevant compound is selected from the group consisting of insulin, EFG, an anti-inflammatory drug, a specific phosphodiesterase-4 (PDE-4) inhibitor, and a non-specific phosphodiesterase inhibitor.

33. The use of any one of claims 30-32 wherein the composition is administered in a regime selected from the group consisting of a single treatment and repeated treatments.

34. The use of any one of claims 30-32 wherein the composition is administered via a continuous infusion, wherein the continuous infusion is selected from the group consisting of subcutaneous, intravenous, or intraperitonial.

35. The use of any one of claims 30-32 wherein the composition is administered via an implant, a pump or a depot for continuous delivery.

36. Use of a exendin(1-39) analog of any one of claims 1-20 in the preparation of a medicament for the treatment or regulation of a disorder selected from the group consisting of diabetes type 1, diabetes type 2, insulin resistance syndrome, stimulation of insulin secretion, impaired glucose tolerance, elevated glucose levels (hyperglycemia), polycystic ovarian syndrome, a metabolic disorder, an eating disorder, a weight disorder, a gastric disease, regulation of gastric emptying, cerebral health, and associated neuropathies.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000537

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 14/575*** (2006.01) , ***A61K 38/26*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 14/575 (2006.01) , ***A61K 38/26*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Scopus, PubMed, Delphion, Medline, CAPlus, Sequence Search (Uniprot, RefSeq, NCBI GenPept, IPI, ENSEMBLE, NCBI IGBlast, PDB, DrugBank) for SEQ ID NO: 2-15
Key words: exendin-4*, exendin*, analog*, derivative, agonist, Met14, M14, N28, Asn28, substitution, position, diabetes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/035623 A2 (ZEALAND PHARMA A/S), 29 April 2004 *see abstract, whole document	1-36
X	CA 2603630 A1 (WUXI GRANDCHAMP PHARMACEUTICAL TECHNOLOGY CO., LTD., CN), 20 July 2006 *see whole document; page 9, lines 6-8	1
Y		2-36
Y	US 6858576 B1 (AMYLIN PHARMACEUTICALS, INC.), 22 February 2005 *see abstract; SEQ ID NO:18, 19; Table II	1-36

[ ] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 May 2008 (7-05-2008)	11 July 2008 (11-07-2008)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Colleen MacFarlane 819- 997-4614

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/000537

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 24-29
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 24-29 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under PCT Rule 39.1 (iv). Regardless, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1-23.

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

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