GLUCAGON/GLP-1 RECEPTOR CO-AGONISTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Provisional Patent Application No. 60/890,087 filed on February 15, 2007 and United States Provisional Patent Application No. 60/938,565 filed May 17, 2007. The subject matter disclosed in these provisional applications is hereby expressly incorporated by reference into the present application.

BACKGROUND

Pre-proglucagon is a 158 amino acid precursor polypeptide that is processed in different tissues to form a number of different proglucagon-derived peptides, including glucagon, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and oxyntomodulin (OXM), that are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake. Glucagon is a 29-amino acid peptide that corresponds to amino acids 33 through 61 of pre-proglucagon, while GLP-1 is produced as a 37-amino acid peptide that corresponds to amino acids 72 through 108 of pre-proglucagon. GLP-1(7-36) amide or GLP-1(7-37)acid are biologically potent forms of GLP-1, that demonstrate essentially equivalent activity at the GLP-1 receptor.

Hypoglycemia occurs when blood glucose levels drops too low to provide enough energy for the body's activities. In adults or children older than 10 years, hypoglycemia is uncommon except as a side effect of diabetes treatment, but it can result from other medications or diseases, hormone or enzyme deficiencies, or tumors. When blood glucose begins to fall, glucagon, a hormone produced by the pancreas, signals the liver to break down glycogen and release glucose, causing blood glucose levels to rise toward a normal level. Thus, glucagon's general role in glucose regulation is to counteract the action of insulin and maintain blood glucose levels. However for diabetics, this glucagon response to hypoglycemia may be impaired, making it harder for glucose levels to return to the normal range.

Hypoglycemia is a life threatening event that requires immediate medical attention. The administration of glucagon is an established medication for treating acute hypoglycemia and it can restore normal levels of glucose within minutes of

administration. When glucagon is used in the acute medical treatment of hypoglycemia, a crystalline form of glucagon is solubilized with a dilute acid buffer and the solution is injected intramuscularly. While this treatment is effective, the methodology is cumbersome and dangerous for someone that is semi-conscious. Accordingly, there is a need for a glucagon analog that maintains or exceeds the biological performance of the parent molecule but is sufficiently soluble and stable, under relevant physiological conditions, that it can be pre-formulated as a solution, ready for injection.

Additionally, diabetics are encouraged to maintain near normal blood glucose levels to delay or prevent microvascular complications. Achievement of this goal usually requires intensive insulin therapy. In striving to achieve this goal, physicians have encountered a substantial increase in the frequency and severity of hypoglycemia in their diabetic patients. Accordingly, improved pharmaceuticals and methodologies are needed for treating diabetes that are less likely to induce hypoglycemia than current insulin therapies.

GLP-1 has different biological activities compared to glucagon. Its actions include stimulation of insulin synthesis and secretion, inhibition of glucagon secretion, and inhibition of food intake. GLP-1 has been shown to reduce hyperglycemia (elevated glucose levels) in diabetics. Exendin-4, a peptide from lizard venom that shares about 50% amino acid identity with GLP-1, activates the GLP-1 receptor and likewise has been shown to reduce hyperglycemia in diabetics.

There is also evidence that GLP-1 and exendin-4 may reduce food intake and promote weight loss, an effect that would be beneficial not only for diabetics but also for patients suffering from obesity. Patients with obesity have a higher risk of diabetes, hypertension, hyperlipidemia, cardiovascular disease, and musculoskeletal diseases.

Accordingly, there remains a need for alternative and preferably improved methods for treating diabetes and obesity.

SUMMARY

As described herein, high potency glucagon agonists analogs are provided that also exhibit increased activity at the glucagon receptor, and in further embodiments exhibit enhanced biophysical stability and/or aqueous solubility. In addition, in

accordance with another aspect of the invention, glucagon agonist analogs are provided that have lost native glucagon's selectivity for the glucagon receptor verses the GLP-1 receptor, and thus represent co-agonists of those two receptors. Selected amino acid modifications within the glucagon analogs can control the relative activity of the analog at the GLP-1 receptor verses the glucagon receptor. Thus, yet another aspect of the invention provides glucagon co-agonist analogs that have higher activity at the glucagon receptor versus the GLP-1 receptor, glucagon co-agonist analogs that have approximately equivalent activity at both receptors, and glucagon co-agonist analogs that have higher activity at the GLP-1 receptor versus the glucagon receptor. The latter category of co-agonist can be engineered to exhibit little or no activity at the glucagon receptor, and yet retain ability to activate the GLP-1 receptor with the same or better potency than native GLP-1. Any of these analogs may also include modifications that confer enhanced biophysical stability and/or aqueous solubility.

Glucagon analogs that demonstrate co-agonism at the glucagon and GLP-1 receptors are advantageous for several applications. First of all the use of glucagon to treat hypoglycemia may overcompensate for low blood glucose levels and result in excess blood glucose levels. If a glucagon/GLP-1 receptor co-agonist is administered, the additional GLP-1 stimulation may buffer the glucagon agonist effect to prevent excessive glucose blood levels resulting from treatment of hypoglycemia.

In addition as described herein, glucagon co-agonist analogs of the invention may be used to control hyperglycemia, or to induce weight loss or prevent weight gain, when administered alone or in combination with other anti-diabetic or anti-obesity treatments. Another compound that induces weight loss is oxyntomodulin, a naturally occurring digestive hormone found in the small intestine (see Diabetes 2005; 54:2390-2395). Oxyntomodulin is a 37 amino acid peptide that contains the 29 amino acid sequence of glucagon (i.e. SEQ ID NO: 1) followed by an 8 amino acid carboxy terminal extension of SEQ ID NO: 27 (KRNRNNIA). While the present invention contemplates that glucagon analogs described herein may optionally be joined to this 8 amino acid carboxy terminal extension (SEQ ID NO: 27), the invention in some embodiments also specifically contemplates analogs and uses of analogs lacking the 8 contiguous carboxy amino acids of SEQ ID NO: 27.

The compounds can be customized by amino acid modifications to regulate the GLP-1 activity of the peptide, and thus the glucagon analogs of the present can be

tailored to treat a particular condition or disease. More particularly, glucagon analogs are provided herein wherein each analog displays a characteristic relative level of activity at the respective glucagon and GLP-1 receptors. For example, modifications can be made to each peptide to produce a glucagon peptide having anywhere from at least about 10% (including at least about 20%, 30%, 40%, 50%, 60%, 75%, 100%, 125%, 150%, 175%) to about 200% or higher activity at the GLP-1 receptor relative to native GLP-1 and anywhere from at least about 10% (including about 20%, 30%, 40%, 50%, 60%, 75%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%) to about 500% or higher activity at the glucagon receptor relative to native glucagon. The amino acid sequence of native glucagon is SEQ ID NO: 1, the amino acid sequence of GLP-1(7-36)amide is SEQ ID NO: 52, and the amino acid sequence of GLP-1(7-37)acid is SEQ ID NO: 50. In exemplary embodiments, a glucagon peptide may exhibit at least 10% of the activity of native glucagon at the glucagon receptor and at least 50% of the activity of native GLP-1 at the GLP-1 receptor, or at least 40% of the activity of native glucagon at the glucagon receptor and at least 40% of the activity of native GLP-1 at the GLP-1 receptor, or at least 60% of the activity of native glucagon at the glucagon receptor and at least 60% of the activity of native GLP-1 at the GLP-1 receptor.

Selectivity of a glucagon peptide for the glucagon receptor versus the GLP-1 receptor can be described as the relative ratio of glucagon/GLP-1 activity (the peptide's activity at the glucagon receptor relative to native glucagon, divided by the peptide's activity at the GLP-1 receptor relative to native GLP-1). For example, a glucagon peptide that exhibits 60% of the activity of native glucagon at the glucagon receptor and 60% of the activity of native GLP-1 at the GLP-1 receptor has a 1:1 ratio of glucagon/GLP-1 activity. Exemplary ratios of glucagon/GLP-1 activity include about 1:1, 1.5:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1, or about 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, or 1:1.5. As an example, a glucagon/GLP-1 activity ratio of 10:1 indicates a 10-fold selectivity for the glucagon receptor versus the GLP-1 receptor. Similarly, a GLP-1/glucagon activity ratio of 10:1 indicates a 10-fold selectivity for the GLP-1 receptor versus the glucagon receptor.

In accordance with one embodiment, analogs of glucagon are provided that have enhanced potency and optionally improved solubility and stability. In one embodiment, enhanced glucagon potency is provided by an amino acid modification

at position 16 of native glucagon (SEQ ID NO: 1). By way of nonlimiting example, such enhanced potency can be provided by substituting the naturally occurring serine at position 16 with glutamic acid or with another negatively charged amino acid having a side chain with a length of 4 atoms, or alternatively with any one of glutamine, homoglutamic acid, or homocysteic acid, or a charged amino acid having a side chain containing at least one heteroatom, (e.g. N, O, S, P) and with a side chain length of about 4 (or 3-5) atoms. In one embodiment the enhanced potency glucagon agonist comprises a peptide of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 or a glucagon agonist analog of SEQ ID NO: 5. In accordance with one embodiment a glucagon analog protein having enhanced potency at the glucagon receptor relative to wild type glucagon is provided wherein the peptide comprises the sequence of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 or SEQ ID NO: 10, wherein the glucagon peptide retains its selectivity for the glucagon receptor relative to the GLP-1 receptors.

Glucagon receptor activity can be reduced by an amino acid modification at position 3, e.g. substitution of the naturally occurring glutamine at position 3 with any amino acid. Substitution at this position with an acidic, basic, or a hydrophobic amino acid (glutamic acid, ornithine, norleucine) has been shown to substantially reduce or destroy glucagon receptor activity. In some embodiments the analogs have about 10% or less of the activity of native glucagon at the glucagon receptor, e.g. about 1-10%, or about 0.1-10%, or greater than about 0.1% but less than about 10%, while exhibiting at least 20% of the activity of GLP-1 at the GLP-1 receptor. For example, exemplary analogs described herein have about 0.5%, about 1% or about 7% of the activity of native glucagon, while exhibiting at least 20% of the activity of GLP-1 at the GLP-1 receptor.

In another embodiment analogs of glucagon are provided that have enhanced or retained potency at the glucagon receptor relative to the native glucagon peptide, but also have greatly enhanced activity at the GLP-1 receptor. Glucagon normally has about 1% of the activity of native-GLP-1 at the GLP-1 receptor, while GLP-1 normally has less than about 0.01% of the activity of native glucagon at the glucagon receptor. Enhanced activity at the GLP-1 receptor is provided by replacing the carboxylic acid of the C-terminal amino acid with a charge-neutral group, such as an amide or ester. In one embodiment, these glucagon analogs comprise a sequence of

SEQ ID NO: 20 wherein the carboxy terminal amino acid has an amide group in place of the carboxylic acid group found on the native amino acid. These glucagon analogs have strong activity at both the glucagon and GLP-1 receptors and thus act as co-agonists at both receptors. In accordance with one embodiment a glucagon and GLP-1 receptor co-agonist is provided wherein the peptide comprises the sequence of SEQ ID NO: 20, wherein the amino acid at position 28 is Asn or Lys and the amino acid at position 29 is Thr-amide.

Enhanced activity at the GLP-1 receptor is also provided by stabilizing the alpha-helix structure in the C-terminal portion of glucagon (around amino acids 12-29), through formation of an intramolecular bridge between the side chains of two amino acids that are separated by three intervening amino acids. In exemplary embodiments, the bridge or linker is about 8 (or about 7-9) atoms in length and forms between side chains of amino acids at positions 12 and 16, or at positions 16 and 20, or at positions 20 and 24, or at positions 24 and 28. The side chains of these amino acids can be linked to one another through hydrogen-bonding or ionic interactions, such as the formation of salt bridges, or by covalent bonds. In accordance with one embodiment a glucagon agonist is provided comprising a glucagon peptide of SEQ ID NO: 20, wherein a lactam ring is formed between the side chains of a lysine residue, located at position 12, 20 or 28, and a glutamic acid residue, located at position 16 or 24, wherein the two amino acids of the glucagon peptide whose side chains participate in forming the lactam ring are spaced from one another by three intervening amino acids. In accordance with one embodiment the lactam bearing glucagon analog comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18. In one embodiment the carboxy terminal amino acid of the lactam bearing peptide comprises an amide group or an ester group in place of the terminal carboxylic acid. In one embodiment a glucagon peptide of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18 further comprises an additional amino acid covalently bound to the carboxy terminus of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18. In a further embodiment a glucagon peptide is provided comprising a sequence selected from the group consisting of SEQ ID NO:

66, SEQ ID NO: 67, SEQ ID NO: 68 and SEQ ID NO: 69 further comprises an additional amino acid covalently bound to the carboxy terminus of SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 and SEQ ID NO: 69. In one embodiment the amino acid at position 28 is asparagine or lysine and the amino acid at position 29 is threonine.

Enhanced activity at the GLP-1 receptor is also provided by an amino acid modification at position 20. In one embodiment, the glutamine at position 20 is replaced with another hydrophilic amino acid having a side chain that is either charged or has an ability to hydrogen-bond, and is at least about 5 (or about 4-6) atoms in length, for example, lysine, citrulline, arginine, or ornithine.

Any of the modifications described above which increase or decrease glucagon receptor activity and which increase GLP-1 receptor activity can be applied individually or in combination. Combinations of the modifications that increase GLP-1 receptor activity generally provide higher GLP-1 activity than any of such modifications taken alone. For example, the invention provides glucagon analogs that comprise modifications at position 16, at position 20, and at the C-terminal carboxylic acid group, optionally with a covalent bond between the amino acids at positions 16 and 20; glucagon analogs that comprise modifications at position 16 and at the C-terminal carboxylic acid group; glucagon analogs that comprise modifications at positions 16 and 20, optionally with a covalent bond between the amino acids at positions 16 and 20; and glucagon analogs that comprise modifications at position 20 and at the C-terminal carboxylic acid group; optionally with the proviso that the amino acid at position 12 is not Arg; or optionally with the proviso that the amino acid at position 9 is not Glu.

Other modifications at position 1 or 2, as described herein, can increase the peptide's resistance to dipeptidyl peptidase IV (DPP IV) cleavage. For example, the amino acid at position 2 may be substituted with D-serine, alanine, D-alanine, valine, glycine, N-methyl serine, N-methyl alanine, or amino isobutyric acid. Alternatively, or in addition, the amino acid at position 1 may be substituted with D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-histidine, N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, or alpha, alpha-dimethyl imidiazole acetic acid (DMIA). It was observed that modifications at position 2 (e.g. AIB at position 2) and in some cases modifications at position 1 may reduce glucagon

activity, sometimes significantly; surprisingly, this reduction in glucagon activity can be restored by a covalent bond between amino acids at positions 12 and 16, 16 and 20, or 20 and 24, e.g. a lactam bridge between a glutamic acid at position 16 and a lysine at position 20.

In yet further exemplary embodiments, any of the foregoing compounds can be further modified to improve stability by modifying the amino acid at position 15 of SEQ ID NO: 1 to reduce degradation of the peptide over time, especially in acidic or alkaline buffers.

In another embodiment the solubility of the glucagon peptides disclosed herein are enhanced by the covalent linkage of a hydrophilic moiety to the peptide. In one embodiment the hydrophilic moiety is a polyethylene glycol (PEG) chain, optionally linked to the peptide at one or more of positions 16, 17, 21, 24, 29, or the C-terminus. In some embodiments, the native amino acid at that position is substituted with an amino acid having a side chain suitable for crosslinking with hydrophilic moieties, to facilitate linkage of the hydrophilic moiety to the peptide. In other embodiments, an amino acid modified to comprise a hydrophilic group is added to the peptide at the C-terminus. In one embodiment the peptide co-agonist comprises a sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19 wherein the side chain of an amino acid residue at one of position 16, 17, 21 or 24 of said glucagon peptide further comprises a polyethylene glycol chain, having a molecular weight selected from the range of about 500 to about 40,000 Daltons. In one embodiment the polyethylene glycol chain has a molecular weight selected from the range of about 500 to about 5,000 Daltons. In another embodiment the polyethylene glycol chain has a molecular weight of about 10,000 to about 20,000 Daltons. In yet other exemplary embodiments the polyethylene glycol chain has a molecular weight of about 20,000 to about 40,000 Daltons.

In another embodiment the solubility of any of the preceding glucagon analogs can be improved by amino acid substitutions and/or additions that introduce a charged amino acid into the C-terminal portion of the peptide, preferably at a position C-terminal to position 27 of SEQ ID NO: 1. Optionally, one, two or three charged amino acids may be introduced within the C-terminal portion, preferably C-terminal to position 27. In accordance with one embodiment the native amino acid(s) at

positions 28 and/or 29 are substituted with a charged amino acids, and/or in a further embodiment one to three charged amino acids are also added to the C-terminus of the peptide. In exemplary embodiments, one, two or all of the charged amino acids are negatively charged. Additional modifications, e.g. conservative substitutions, may be made to the glucagon peptide that still allow it to retain glucagon activity. In one embodiment an analog of the peptide of SEQ ID NO: 20 is provided wherein the analog differs from SEQ ID NO: 20 by 1 to 2 amino acid substitutions at positions 17-26, and in one embodiment the analog differs from the peptide of SEQ ID NO: 20 by an amino acid substitution at position 20.

In accordance with one embodiment the glucagon peptides disclosed herein are modified by the addition of a second peptide to the carboxy terminus of the glucagon peptide, for example, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28. In one embodiment a glucagon peptide having a peptide sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, and SEQ ID NO: 69 is covalently bound through a peptide bond to a second peptide, wherein the second peptide comprises a sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28. In a further embodiment, in glucagon peptides which comprise the C-terminal extension, the threonine at position 29 of the native glucagon peptide is replaced with a glycine. A glucagon analog having a glycine substitution for threonine at position 29 and comprising the carboxy terminal extension of SEQ ID NO: 26 is four times as potent at the GLP-1 receptor as native glucagon modified to comprise the carboxy terminal extension of SEQ ID NO: 26. Potency at the GLP-1 receptor can be further enhanced by an alanine substitution for the native arginine at position 18.

Thus, as disclosed herein high potency glucagon analogs or glucagon co-agonist analogs are provided that also exhibit improved solubility and/or stability. An exemplary high potency glucagon analog exhibits at least about 200% of the activity of native glucagon at the glucagon receptor, and optionally is soluble at a concentration of at least 1 mg/mL at a pH between 6 and 8 (e.g. pH 7), and optionally retains at least 95% of the original peptide (e.g. 5% or less of the original peptide is degraded or cleaved) after 24 hours at 25°C. As another example, an exemplary

glucagon co-agonist analog exhibits greater than about 40% or greater than about 60% activity at both the glucagon and the GLP-1 receptors (at a ratio between about 1:3 and 3:1, or between about 1:2 and 2:1), is optionally soluble at a concentration of at least 1 mg/mL at a pH between 6 and 8 (e.g. pH 7), and optionally retains at least 95% of the original peptide after 24 hours at 25°C. Another exemplary glucagon co-agonist analog exhibits about 175% or more of the activity of native glucagon at the glucagon receptor and about 20% or less of the activity of native GLP-1 at the GLP-1 receptor, is optionally soluble at a concentration of at least 1 mg/mL at a pH between 6 and 8 (e.g. pH 7), and optionally retains at least 95% of the original peptide after 24 hours at 25°C. Yet another exemplary glucagon co-agonist analog exhibits about 10% or less of the activity of native glucagon at the glucagon receptor and at least about 20% of the activity of native GLP-1 at the GLP-1 receptor, is optionally soluble at a concentration of at least 1 mg/mL at a pH between 6 and 8 (e.g. pH 7), and optionally retains at least 95% of the original peptide after 24 hours at 25°C. Yet another exemplary glucagon co-agonist analog exhibits about 10% or less but above 0.1% , 0.5% or 1% of the activity of native glucagon at the glucagon receptor and at least about 50%, 60%, 70%, 80%, 90% or 100% or more of the activity of native GLP-1 at the GLP-1 receptor, is optionally soluble at a concentration of at least 1 mg/mL at a pH between 6 and 8 (e.g. pH 7), and optionally retains at least 95% of the original peptide after 24 hours at 25°C. In some embodiments, such glucagon analogs retain at least 22, 23, 24, 25, 26, 27 or 28 of the naturally occurring amino acids at the corresponding positions in native glucagon (e.g. have 1-7, 1-5 or 1-3 modifications relative to naturally occurring glucagon).

Any one of the following peptides is excluded from the compounds of the invention, although further modifications thereto exhibiting the desired co-agonist activity, pharmaceutical compositions, kits, and treatment methods using such compounds may be included in the invention: The peptide of SEQ ID NO: 1 with an [Arg12] substitution and with a C-terminal amide; The peptide of SEQ ID NO: 1with [Arg12,Lys20] substitutions and with a C-terminal amide; The peptide of SEQ ID NO: 1with [Arg12,Lys24] substitutions and with a C-terminal amide; The peptide of SEQ ID NO: 1with [Arg12,Lys29] substitutions and with a C-terminal amide; The peptide of SEQ ID NO: 1with a [Glu9] substitution; The peptide of SEQ ID NO: 1missing His1, with [Glu9, Glu16, Lys29] substitutions and C-terminal amide; The

peptide of SEQ ID NO: 1with [Glu9, Glu16, Lys29] substitutions and with a C-terminal amide; The peptide of SEQ ID NO: 1with [Lys13, Glu17] substitutions linked via lactam bridge and with a C-terminal amide; The peptide of SEQ ID NO: 1with [Lys17, Glu21] substitutions linked via lactam bridge and with a C-terminal amide; The peptide of SEQ ID NO: 1missing His1, with [Glu20, Lys24] substitutions linked via lactam bridge.

In accordance with one embodiment a pharmaceutical composition is provided comprising any of the novel glucagon peptides disclosed herein, preferably sterile and preferably at a purity level of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, and a pharmaceutically acceptable diluent, carrier or excipient. Such compositions may contain a glucagon peptide at a concentration of at least 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 11 mg/ml, 12 mg/ml, 13 mg/ml, 14 mg/ml, 15 mg/ml, 16 mg/ml, 17 mg/ml, 18 mg/ml, 19 mg/ml, 20 mg/ml, 21 mg/ml, 22 mg/ml, 23 mg/ml, 24 mg/ml, 25 mg/ml or higher. In one embodiment the pharmaceutical compositions comprise aqueous solutions that are sterilized and optionally stored within various containers. The compounds of the present invention can be used in accordance with one embodiment to prepare pre-formulated solutions ready for injection. In other embodiments the pharmaceutical compositions comprise a lyophilized powder. The pharmaceutical compositions can be further packaged as part of a kit that includes a disposable device for administering the composition to a patient. The containers or kits may be labeled for storage at ambient room temperature or at refrigerated temperature.

In accordance with one embodiment a method of rapidly increasing glucose level or treating hypoglycemia using a pre-formulated aqueous composition of glucagon peptides of the invention is provided. The method comprises the step of administering an effective amount of an aqueous solution comprising a novel modified glucagon peptide of the present disclosure. In one embodiment the glucagon peptide is pegylated at position 21 or 24 of the glucagon peptide and the PEG chain has a molecular weight of about 500 to about 5,000 Daltons. In one embodiment the modified glucagon solution is prepackaged in a device that is used to administer the composition to the patient suffering from hypoglycemia.

In accordance with one embodiment an improved method of regulating blood glucose levels in insulin dependent patients is provided. The method comprises the steps of administering insulin in an amount therapeutically effective for the control of diabetes and administering a novel modified glucagon peptide of the present disclosure in an amount therapeutically effective for the prevention of hypoglycemia, wherein said administering steps are conducted within twelve hours of each other. In one embodiment the glucagon peptide and the insulin are co-administered as a single composition, wherein the glucagon peptide is pegylated with a PEG chain having a molecular weight selected from the range of about 5,000 to about 40,000 Daltons

In another embodiment a method is provided for inducing the temporary paralysis of the intestinal tract. The method comprises the step of administering one or more of the glucagon peptides disclosed herein to a patient.

In yet another embodiment a method of treating hyperglycemia, or a method of reducing weight gain or inducing weight loss is provided, which involves administering an effective amount of an aqueous solution comprising a glucagon peptide of the invention. In one embodiment either method comprises administering an effective amount of a composition comprising a glucagon agonist selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19. In another embodiment, the method comprises administering an effective amount of a composition comprising a glucagon agonist, wherein the glucagon agonist comprising a glucagon peptide selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, and SEQ ID NO: 69, wherein amino acid 29 of the glucagon peptide is bound to a second peptide through a peptide bond, and said second peptide comprises the sequence of SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28. In further embodiments, methods of treating diabetes involving co-administering a conventional dose or a reduced dose of insulin and a glucagon peptide of the invention are provided. Methods of treating diabetes with a glucagon peptide of the invention, without co-administering insulin are also provided.

In yet another aspect, the invention provides novel methods for treating hyperglycemia and novel methods for decreasing appetite or promoting body weight

loss that involve administration of a glucagon/GLP-1 co-agonist molecule (including pharmaceutically acceptable salts thereof) that activates both the glucagon receptor and the GLP-1 receptor. Agonism, i.e., activation, of both the glucagon and GLP-1 receptors provides an unexpected improvement compared to GLP-1 agonism alone in treating hyperglycemia. Thus, the addition of glucagon agonism provides an unexpected additive or synergistic effect, or other unexpected clinical benefit(s). Administration with a conventional dose of insulin, a reduced dose of insulin, or without insulin is contemplated according to such methods. Agonism of the glucagon receptor also has an unexpected beneficial effect compared to GLP-1 agonism alone in promoting weight loss or preventing weight gain.

Exemplary glucagon/GLP-1 co-agonist molecules include glucagon peptides of the invention, GLP-1 analogs that activate both GLP-1 and glucagon receptors, fusions of glucagon and GLP-1, or fusions of glucagon analogs and GLP-1 analogs, or chemically modified derivatives thereof. Alternatively, a compound that activates the glucagon receptor can be co-administered with a compound that activates the GLP-1 receptor (such as a GLP-1 analog, an exendin-4 analog, or derivatives thereof). The invention also contemplates co-administration of a glucagon agonist analog with a GLP-1 agonist analog.

Such methods for treating hyperglycemia and/or for decreasing appetite or promoting body weight loss include administration of a glucagon analog with a modification at position 12 (e.g. Arg12), optionally in combination with modifications at position 16 and/or 20. The methods of the invention also include administration of glucagon analogs comprising an intramolecular bridge between the side chains of two amino acids within the region of amino acids 12 and 29 that are separated by three intervening amino acids, e.g. positions 12 and 16, positions 13 and 17 (e.g,. Lys13 Glu17 or Glu13 Lys17), positions 16 and 20, positions 17 and 21 (e.g. Lys17 Glu 21 or Glu17 Lys 21), positions 20 and 24, or positions 24 and 28, with the optional proviso that the amino acid at position 9 is not Glu, and optionally including a C-terminal amide or ester.

In accordance with one embodiment excluded from such glucagon/GLP-1 co-agonist molecules are any glucagon analogs or GLP-1 analogs in the prior art known to be useful in such a method. In another embodiment peptides described in U.S. Patent No. 6,864,069 as acting as both a GLP-1 agonist and a glucagon antagonist for

treating diabetes are also excluded as glucagon/GLP-1 co-agonist molecules. In another embodiment, excluded is the use of glucagon antagonists to treat diabetes, such as the antagonists described in Unson et al., *J. Biol. Chem.*, 264:789-794 (1989), Ahn et al., *J. Med. Chem.*, 44:3109-3116 (2001), and Sapse et al., *Mol. Med.*, 8(5):251-262 (2002). In a further embodiment oxyntomodulin or a glucagon analog that contains the 8 C-terminal amino acids of oxyntomodulin (SEQ ID NO: 27) are also excluded as glucagon/GLP-1 co-agonist molecules.

Such methods for treating hyperglycemia are expected to be useful for a variety of types of hyperglycemia, including diabetes, diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes, either insulin-dependent or non-insulin-dependent, and reducing complications of diabetes including nephropathy, retinopathy and vascular disease. Such methods for reducing appetite or promoting loss of body weight are expected to be useful in reducing body weight, preventing weight gain, or treating obesity of various causes, including drug-induced obesity, and reducing complications associated with obesity including vascular disease (coronary artery disease, stroke, peripheral vascular disease, ischemia reperfusion, etc.), hypertension, onset of diabetes type II, hyperlipidemia and musculoskeletal diseases.

All therapeutic methods, pharmaceutical compositions, kits and other similar embodiments described herein contemplate that the use of the term glucagon analogs includes all pharmaceutically acceptable salts or esters thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a bar graph representing the stability of Glucagon Cys^{21}maleimidoPEG$_{5K}$ at 37°C incubated for 24, 48, 72, 96, 144 and 166 hours, respectively.

Fig. 2 represents data generated from HPLC analysis of Glucagon Cys^{21}maleimidoPEG$_{5K}$ at pH 5 incubated at 37°C for 24, 72 or 144 hours, respectively.

Fig. 3 represents data showing receptor mediated cAMP induction by glucagon analogs. More particularly, Fig. 3A compares induction of the glucagon receptor by glucagon analogs E16, K20 ●, E15, E16 ▲, E16, K20 ▼, E15, E16 ◀, E16 ▶ and Gluc-NH_2 ■

Fig. 4A and 4B represents data showing receptor mediated cAMP induction by glucagon analogs. More particularly, Fig. 4A compares induction of the glucagon receptor by glucagon analogs Gluc-NH_2 ●, E16Gluc-NH_2 ▲, E3, E16 Gluc-NH_2 ▼, Orn3, E16 Gluc-NH_2 ◀ and Nle3, E16 Gluc-NH_2, ▶ relative to native glucagon ■, whereas Fig. 4B compares induction of the GLP-1 receptor by glucagon analogs Gluc-NH_2 ●, E16 Gluc-NH_2 ▲, E3, E16Gluc-NH_2 ▼, Orn3, E16 Gluc-NH_2 ◀ and Nle3, E16 Gluc-NH_2, ▶ relative to native GLP-1 ■.

Fig. 5A and 5B represents data showing receptor mediated cAMP induction by glucagon analogs. More particularly, Fig. 5A compares induction of the glucagon receptor by glucagon analogs (E16, K20 Gluc-NH_2 ●(5nM, stock solution), E15, E16 Gluc-NH_2 ▲(5nM, stock solution), E16, K20 Gluc-NH_2 ▼(10nM, stock solution), E15, E16 Gluc-NH_2 ◀ (10nM, stock solution) and E16 Gluc-NH_2 ▶) relative to glucagon-NH_2 (■), whereas Fig. 5B compares induction of the GLP-1 receptor by glucagon analogs (E16, K20 Gluc-NH_2 ●, E15, E16 Gluc-NH_2 ▲, and E16 Gluc-NH_2, ▶) relative to GLP-1 (■) and glucagon-NH_2 (□).

Fig. 6A and 6B represents data showing receptor mediated cAMP induction by glucagon analogs. More particularly, Fig. 6A compares induction of the glucagon receptor by glucagon analogs (Gluc-NH_2 ●, K12E16-NH_2 lactam ▲, E16K20-NH_2 lactam ▼, K20E24-NH_2 lactam ◀ and E24K28-NH_2 lactam ▶) relative to glucagon (■), whereas Fig. 6B compares induction of the GLP-1 receptor by glucagon analogs (Gluc-NH_2 ●, K12E16-NH_2 lactam ▲, E16K20-NH_2 lactam ▼, K20E24-NH_2 lactam ◀ and E24K28-NH_2 lactam ▶) relative to GLP-1 (■).

Fig. 7A and 7B represents data showing receptor mediated cAMP induction by glucagon analogs. More particularly, Fig. 7A compares induction of the glucagon receptor by glucagon analogs (Gluc-NH_2 ●, E16 Gluc-NH_2, ▲, K12, E16 Gluc-NH_2 lactam ▼, E16, K20 Gluc-NH_2 ◀ and E16, K20 Gluc-NH_2 lactam ▶) relative to glucagon (■), whereas Fig. 7B compares induction of the GLP-1 receptor by glucagon analogs (Gluc-NH_2 ●, E16 Gluc-NH_2, ▲, K12, E16 Gluc-NH_2 lactam ▼, E16, K20 Gluc-NH_2 ◀ and E16, K20 Gluc-NH_2 lactam ▶) relative to GLP-1 (■).

Figs. 8A-8F represent data showing receptor mediated cAMP induction by glucagon analogs at the glucagon receptor (Figs. 8A, 8C and 8E) or the GLP-1 receptor (Figs. 8B, 8C and 8F) wherein hE = homoglutamic acid and hC = homocysteic acid.

Fig. 9A and 9B: represent data showing receptor mediated cAMP induction by GLP (17-26) glucagon analogs, wherein amino acid positions 17-26 of native glucagon (SEQ ID NO: 1) have been substituted with the amino acids of positions 17-26 of native GLP-1 (SEQ ID NO: 50). More particularly, Fig. 9A compares induction of the glucagon receptor by the designated GLP (17-26) glucagon analogs, and Fig. 9B compares induction of the GLP-1 receptor by the designated GLP (17-26) glucagon analogs.

Figs. 10A-E: are graphs providing *in vivo* data demonstrating the ability of the glucagon peptides of the present invention to induce weight loss in mice injected subcutaneously with the indicated amounts of the respective compounds. Sequence Identifiers for the glucagon peptide listed in Figs 10A -10E are as follows, for Fig. 10A: Chimera 2 Aib2 C24 40K PEG (SEQ ID NO: 486), Aib2 C24 Chimera 2 40K lactam (SEQ ID NO: 504) and Aib2 E16 K20 Gluc-NH2 Lac 40K (SEQ ID NO: 528); Fig. 10B: Aib2 C24 Chi 2 lactam 40K (SEQ ID NO: 504), DMIA1 C24 Chi 2 Lactam 40K (SEQ ID NO: 505), Chimera 2 DMIA1 C24 40K (SEQ ID NO: 519), and Chimera 2 Aib2 C24 40K (SEQ ID NO: 486), wherein the number at the end of the sequence designates the dosage used, either 70 or 350 nmol/kg; Fig. 10C: AIB2 w/ lactam C24 40K (SEQ ID NO: 504), AIB2 E16 K20 w/ lactam C24 40K (SEQ ID NO: 528), DMIA1 E16 K20 w/ lactam C24 40K (SEQ ID NO: 510), DMIA1 E16 K20 w/ lactam CEX 40K (SEQ ID NO: 513) and DMIA1 E16 K20 w/o lactam CEX 40K (SEQ ID NO: 529); Fig. 10D: AIB2 w lactam C24 40K (SEQ ID NO: 504), AIB2 E16 K20 w lactam C24 40K (SEQ ID NO: 528), DMIA1 E16 K20 w lactam C24 40K (SEQ ID NO: 510) and DMIA1 E16 K20 w lactam/Cex C24 40K (SEQ ID NO: 513), wherein the number at the end of the sequence designates the dosage used, either 14 or 70 nmol/kg/wk; Fig. 10E: AIB2 w/o lactam C24 40K (SEQ ID NO: 486), Chi 2 AIB2 C24 CEX 40K (SEQ ID NO: 533), AIB2 E16 A18 K20 C24 40K (SEQ ID NO: 492), AIB2 w/o lactam CEX G29 C40 40K (SEQ ID NO: 488), AIB2 w/o lactam CEX C40 C41-2 (SEQ ID NO: 532), AIB2 w/o lactam CEX C24 C40- 2 (SEQ ID NO: 531) and AIB2 w/o lactam C24 60K (SEQ ID NO: 498), wherein the designation 40K or 60K represents the molecular weight of the polyethylene chain attached to the glucagon peptide.

DETAILED DESCRIPTION

DEFINITIONS

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein the term "pharmaceutically acceptable salt" refers to salts of compounds that retain the biological activity of the parent compound, and which are not biologically or otherwise undesirable. Many of the compounds disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

As used herein, the term "treating" includes prophylaxis of the specific disorder or condition, or alleviation of the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms. For example, as used herein the term "treating diabetes" will refer in general to altering glucose

blood levels in the direction of normal levels and may include increasing or decreasing blood glucose levels depending on a given situation.

As used herein an "effective" amount or a "therapeutically effective amount" of a glucagon peptide refers to a nontoxic but sufficient amount of the peptide to provide the desired effect. For example one desired effect would be the prevention or treatment of hypoglycemia, as measured, for example, by an increase in blood glucose level. An alternative desired effect for the co-agonist analogs of the present disclosure would include treating hyperglycemia, e.g., as measured by a change in blood glucose level closer to normal, or inducing weight loss/preventing weight gain, e.g., as measured by reduction in body weight, or preventing or reducing an increase in body weight, or normalizing body fat distribution. The amount that is "effective" will vary from subject to subject, depending on the age and general condition of the individual, mode of administration, and the like. Thus, it is not always possible to specify an exact "effective amount." However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous.

As used herein, the term "purified" and like terms relate to the isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment. As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. The term "purified polypeptide" is used herein to describe a polypeptide which has been separated from other compounds including, but not limited to nucleic acid molecules, lipids and carbohydrates.

The term "isolated" requires that the referenced material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated.

As used herein, the term "peptide" encompasses a sequence of 3 or more amino acids and typically less than 50 amino acids, wherein the amino acids are naturally occurring or non-naturally occurring amino acids. Non-naturally occurring

amino acids refer to amino acids that do not naturally occur in vivo but which, nevertheless, can be incorporated into the peptide structures described herein.

As used herein, the terms "polypeptide" and "protein" are terms that are used interchangeably to refer to a polymer of amino acids, without regard to the length of the polymer. Typically, polypeptides and proteins have a polymer length that is greater than that of "peptides."

A "glucagon peptide" as used herein includes any peptide comprising, either the amino acid sequence of SEQ ID NO: 1, or any analog of the amino acid sequence of SEQ ID NO: 1, including amino acid substitutions, additions, deletions or post translational modifications (e.g., methylation, acylation, ubiquitination, intramolecular covalent bonding such as lactam bridge formation, PEGylation, and the like) of the peptide, wherein the analog stimulates glucagon or GLP-1 receptor activity, e.g., as measured by cAMP production using the assay described in Example 14.

The term "glucagon agonist" refers to a complex comprising a glucagon peptide that stimulates glucagon receptor activity, e.g., as measured by cAMP production using the assay described in Example 14.

As used herein a "glucagon agonist analog" is a glucagon peptide comprising a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15, or an analog of such a sequence that has been modified to include one or more conservative amino acid substitutions at one or more of positions 2, 5, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20, 21, 24, 27, 28 or 29.

As used herein an amino acid "modification" refers to a substitution, addition or deletion of an amino acid, and includes substitution with or addition of any of the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids. Throughout the application, all references to a particular amino acid position by number (e.g. position 28) refer to the amino acid at that position in native glucagon (SEQ ID NO:1) or the corresponding amino acid position in any analogs thereof. For example, a reference herein to "position 28" would mean the corresponding position 27 for a glucagon analog in which the first amino acid of SEQ ID NO: 1 has been deleted. Similarly, a reference herein to "position 28" would mean the corresponding position 29 for a glucagon analog in which one amino acid has been added before the N-terminus of SEQ ID NO: 1.

Commercial sources of atypical amino acids include Sigma-Aldrich (Milwaukee, WI), ChemPep Inc. (Miami, FL), and Genzyme Pharmaceuticals (Cambridge, MA). Atypical amino acids may be purchased from commercial suppliers, synthesized de novo, or chemically modified or derivatized from other amino acids.

As used herein a "glucagon co-agonist" is a glucagon peptide that exhibits activity at the glucagon receptor of at least about 10% to about 500% or more relative to native glucagon and also exhibits activity at the GLP-1 receptor of about at least 10% to about 200% or more relative to native GLP-1.

As used herein a "glucagon/GLP-1 co-agonist molecule" is a molecule that exhibits activity at the glucagon receptor of at least about 10% relative to native glucagon and also exhibits activity at the GLP-1 receptor of at least about 10% relative to native GLP-1.

As used herein the term "native glucagon" refers to a peptide consisting of the sequence of SEQ ID NO: 1, and the term "native GLP-1" is a generic term that designates GLP-1(7-36)amide (consisting of the sequence of SEQ ID NO: 52), GLP-1(7-37)acid (consisting of the sequence of SEQ ID NO: 50) or a mixture of those two compounds. As used herein, a general reference to "glucagon" or "GLP-1" in the absence of any further designation is intended to mean native glucagon or native GLP-1, respectively.

As used herein an amino acid "substitution" refers to the replacement of one amino acid residue by a different amino acid residue.

As used herein, the term "conservative amino acid substitution" is defined herein as exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:
Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides and esters:
Asp, Asn, Glu, Gln, cysteic acid and homocysteic acid;

III. Polar, positively charged residues:
His, Arg, Lys; Ornithine (Orn)

IV. Large, aliphatic, nonpolar residues:
Met, Leu, Ile, Val, Cys, Norleucine (Nle), homocysteine

V. Large, aromatic residues:

Phe, Tyr, Trp, acetyl phenylalanine

As used herein the general term "polyethylene glycol chain" or "PEG chain", refers to mixtures of condensation polymers of ethylene oxide and water, in a branched or straight chain, represented by the general formula $H(OCH_2CH_2)_nOH$, wherein n is at least 9. Absent any further characterization, the term is intended to include polymers of ethylene glycol with an average total molecular weight selected from the range of 500 to 40,000 Daltons. "polyethylene glycol chain" or "PEG chain" is used in combination with a numeric suffix to indicate the approximate average molecular weight thereof. For example, PEG-5,000 refers to polyethylene glycol chain having a total molecular weight average of about 5,000.

As used herein the term "pegylated" and like terms refers to a compound that has been modified from its native state by linking a polyethylene glycol chain to the compound. A "pegylated glucagon peptide" is a glucagon peptide that has a PEG chain covalently bound to the glucagon peptide.

As used herein a general reference to a peptide is intended to encompass peptides that have modified amino and carboxy termini. For example, an amino acid chain comprising an amide group in place of the terminal carboxylic acid is intended to be encompassed by an amino acid sequence designating the standard amino acids.

As used herein a "linker" is a bond, molecule or group of molecules that binds two separate entities to one another. Linkers may provide for optimal spacing of the two entities or may further supply a labile linkage that allows the two entities to be separated from each other. Labile linkages include photocleavable groups, acid-labile moieties, base-labile moieties and enzyme-cleavable groups.

As used herein a "dimer" is a complex comprising two subunits covalently bound to one another via a linker. The term dimer, when used absent any qualifying language, encompasses both homodimers and heterodimers. A homodimer comprises two identical subunits, whereas a heterodimer comprises two subunits that differ, although the two subunits are substantially similar to one another.

As used herein the term "charged amino acid" refers to an amino acid that comprises a side chain that is negatively charged (i.e., de-protonated) or positively charged (i.e., protonated) in aqueous solution at physiological pH. For example negatively charged amino acids include aspartic acid, glutamic acid, cysteic acid,

homocysteic acid, and homoglutamic acid, whereas positively charged amino acids include arginine, lysine and histidine. Charged amino acids include the charged amino acids among the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids.

As used herein the term "acidic amino acid" refers to an amino acid that comprises a second acidic moiety, including for example, a carboxylic acid or sulfonic acid group.

EMBODIMENTS

The invention provides glucagon peptides with increased or decreased activity at the glucagon receptor, or GLP-1 receptor, or both. The invention also provides glucagon peptides with altered selectivity for the glucagon receptor versus the GLP-1 receptor.

Increased activity at the glucagon receptor is provided by an amino acid modification at position 16 of native glucagon (SEQ ID NO: 1) as described herein.

Reduced activity at the glucagon receptor is provided, e.g., by an amino acid modification at position 3 as described herein.

Increased activity at the GLP-1 receptor is provided by replacing the carboxylic acid of the C-terminal amino acid with a charge-neutral group, such as an amide or ester.

Increased activity at the GLP-1 receptor is provided by modifications that permit formation of an intramolecular bridge between the side chains of two amino acids that are separated by three intervening amino acids, for example, positions 12 and 16, or 16 and 20, or 20 and 24, as described herein.

Increased activity at the GLP-1 receptor is provided by an amino acid modification at position 20 as described herein.

Increased activity at the GLP-1 receptor is provided in glucagon analogs comprising the C-terminal extension of SEQ ID NO: 26. GLP-1 activity in such analogs comprising SEQ ID NO: 26 can be further increased by modifying the amino acid at position 18, 28 or 29, or at position 18 and 29, as described herein.

Restoration of glucagon activity which has been reduced by amino acid modifications at positions 1 and 2 is provided by a covalent bond between the side

chains of two amino acids that are separated by three intervening amino acids, for example, positions 12 and 16, or 16 and 20, or 20 and 24, as described herein.

A further modest increase in GLP-1 potency is provided by modifying the amino acid at position 10 to be Trp.

Any of the modifications described above which increase or decrease glucagon receptor activity and which increase GLP-1 receptor activity can be applied individually or in combination. Any of the modifications described above can also be combined with other modifications that confer other desirable properties, such as increased solubility and/or stability and/or duration of action. Alternatively, any of the modifications described above can be combined with other modifications that do not substantially affect solubility or stability or activity. Exemplary modifications include but are not limited to:

(A) Improving solubility, for example, by introducing one, two, three or more charged amino acid(s) to the C-terminal portion of native glucagon, preferably at a position C-terminal to position 27. Such a charged amino acid can be introduced by substituting a native amino acid with a charged amino acid, e.g. at positions 28 or 29, or alternatively by adding a charged amino acid, e.g. after position 27, 28 or 29. In exemplary embodiments, one, two, three or all of the charged amino acids are negatively charged. In other embodiments, one, two, three or all of the charged amino acids are positively charged. Such modifications increase solubility, e.g. provide at least 2-fold, 5-fold, 10-fold, 15-fold, 25-fold, 30-fold or greater solubility relative to native glucagon at a given pH between about 5.5 and 8, e.g., pH 7, when measured after 24 hours at 25°C.

(B) Increasing solubility and duration of action or half-life in circulation by addition of a hydrophilic moiety such as a polyethylene glycol chain, as described herein, e.g. at position 16, 17, 20, 21, 24 or 29, or at the C-terminus of the peptide.

(C) Increasing stability by modification of the aspartic acid at position 15, for example, by deletion or substitution with glutamic acid, homoglutamic acid, cysteic acid or homocysteic acid. Such modifications can reduce degradation or cleavage at a pH within the range of 5.5 to 8, for example, retaining at least 75%, 80%, 90%, 95%, 96%, 97%, 98% or 99% of the original peptide after 24 hours at 25°C.

(D) Increasing stability by modification of the amino acid at position 27, for example, by substitution with methionine, leucine or norleucine. Such modifications can reduce oxidative degradation.

(E) Increasing resistance to dipeptidyl peptidase IV (DPP IV) cleavage by modification of the amino acid at position 1 or 2 as described herein.

(F) Conservative substitutions, additions or deletions that do not affect activity, for example, conservative substitutions at one or more of positions 2, 5, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 or 29; or deletion of amino acid 29 optionally combined with a C-terminal amide or ester in place of the C-terminal carboxylic acid group;

(G) Adding C-terminal extensions as described herein;

(H) Homodimerization or heterodimerization as described herein.

In exemplary embodiments, the glucagon peptide may comprise a total of 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid modifications relative to the native glucagon sequence. .

One embodiment disclosed herein is directed to a glucagon agonist that has been modified relative to the wild type peptide of His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser- Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr (SEQ ID NO: 1) to enhance the peptide's potency at the glucagon receptor. Surprisingly, applicants have discovered that the normally occurring serine at position 16 of native glucagon (SEQ ID NO: 1) can be substituted with select acidic amino acids to enhance the potency of glucagon, in terms of its ability to stimulate cAMP synthesis in a validated *in vitro* model assay (see Example 14). More particularly, this substitution enhances the potency of the analog at least 2-fold, 4-fold, 5-fold, and up to 10-fold greater at the glucagon receptor. This substitution also enhances the analog's activity at the GLP-1 receptor at least 5-fold, 10-fold, or 15-fold relative to native glucagon, but selectivity is maintained for the glucagon receptor over the GLP-1 receptor.

In accordance with one embodiment the serine residue at position 16 of native glucagon is substituted with an amino acid selected from the group consisting of glutamic acid, glutamine, homoglutamic acid, homocysteic acid, threonine or glycine. In accordance with one embodiment the serine residue at position 16 of native glucagon is substituted with an amino acid selected from the group consisting of

glutamic acid, glutamine, homoglutamic acid and homocysteic acid, and in one embodiment the serine residue is substituted with glutamic acid. In one embodiment the glucagon peptide having enhanced specificity for the glucagon receptor comprises the peptide of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 or a glucagon agonist analog thereof, wherein the carboxy terminal amino acid retains its native carboxylic acid group. In accordance with one embodiment a glucagon agonist comprising the sequence of NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-COOH (SEQ ID NO: 10) is provided, wherein the peptide exhibits approximately fivefold enhanced potency at the glucagon receptor, relative to native glucagon as measured by the *in vitro* cAMP assay of Example 14.

The glucagon peptides of the present invention can be further modified to improve the peptide's solubility and stability in aqueous solutions at physiological pH, while retaining the high biological activity relative to native glucagon. In accordance with one embodiment, introduction of hydrophilic groups at positions 17, 21, and 24 of the peptide of SEQ ID NO: 9 or SEQ ID NO: 10 are anticipated to improve the solubility and stability of the high potency glucagon analog in solutions having a physiological pH. Introduction of such groups also increases duration of action, e.g. as measured by a prolonged half-life in circulation. Suitable hydrophilic moieties include any water soluble polymers known in the art, including PEG, homo- or co-polymers of PEG, a monomethyl-substituted polymer of PEG (mPEG), or polyoxyethylene glycerol (POG). In accordance with one embodiment the hydrophilic group comprises a polyethylene (PEG) chain. More particularly, in one embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 6 or SEQ ID NO: 7 wherein a PEG chain is covalently linked to the side chains of amino acids present at positions 21 and 24 of the glucagon peptide and the carboxy terminal amino acid of the peptide has the carboxylic acid group.

The present disclosure also encompasses other conjugates in which glucagon peptides of the invention are linked, optionally via covalent bonding and optionally via a linker, to a conjugate. Linkage can be accomplished by covalent chemical bonds, physical forces such electrostatic, hydrogen, ionic, van der Waals, or hydrophobic or hydrophilic interactions. A variety of non-covalent coupling systems may be used, including biotin-avidin, ligand/receptor, enzyme/substrate, nucleic

acid/nucleic acid binding protein, lipid/lipid binding protein, cellular adhesion molecule partners; or any binding partners or fragments thereof which have affinity for each other.

Exemplary conjugates include but are not limited to a heterologous peptide or polypeptide (including for example, a plasma protein), a targeting agent, an immunoglobulin or portion thereof (e.g. variable region, CDR, or Fc region), a diagnostic label such as a radioisotope, fluorophore or enzymatic label, a polymer including water soluble polymers, or other therapeutic or diagnostic agents. In one embodiment a conjugate is provided comprising a glucagon peptide of the present invention and a plasma protein, wherein the plasma protein is selected form the group consisting of albumin, transferin, fibrinogen and glubulins. In one embodiment the plasma protein moiety of the conjugate is albumin or transferin. In some embodiments, the linker comprises a chain of atoms from 1 to about 60, or 1 to 30 atoms or longer, 2 to 5 atoms, 2 to 10 atoms, 5 to 10 atoms, or 10 to 20 atoms long. In some embodiments, the chain atoms are all carbon atoms. In some embodiments, the chain atoms in the backbone of the linker are selected from the group consisting of C, O, N, and S. Chain atoms and linkers may be selected according to their expected solubility (hydrophilicity) so as to provide a more soluble conjugate. In some embodiments, the linker provides a functional group that is subject to cleavage by an enzyme or other catalyst or hydrolytic conditions found in the target tissue or organ or cell. In some embodiments, the length of the linker is long enough to reduce the potential for steric hindrance. If the linker is a covalent bond or a peptidyl bond and the conjugate is a polypeptide, the entire conjugate can be a fusion protein. Such peptidyl linkers may be any length. Exemplary linkers are from about 1 to 50 amino acids in length, 5 to 50, 3 to 5, 5 to 10, 5 to 15, or 10 to 30 amino acids in length. Such fusion proteins may alternatively be produced by recombinant genetic engineering methods known to one of ordinary skill in the art.

The present disclosure also encompasses glucagon fusion peptides or proteins wherein a second peptide or polypeptide has been fused to a terminus, e.g., the carboxy terminus of the glucagon peptide. More particularly, the fusion glucagon peptide may comprise a glucagon agonist of SEQ ID NO: 55, SEQ ID NO: 9 or SEQ ID NO: 10 further comprising an amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS), SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 (KRNR) linked

to amino acid 29 of the glucagon peptide. In one embodiment the amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS), SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 (KRNR) is bound to amino acid 29 of the glucagon peptide through a peptide bond. Applicants have discovered that in glucagon fusion peptides comprising the C-terminal extension peptide of Exendin-4 (e.g., SEQ ID NO: 26 or SEQ ID NO: 29), substitution of the native threonine residue at position 29 with glycine dramatically increases GLP-1 receptor activity. This amino acid substitution can be used in conjunction with other modifications disclosed herein to enhance the affinity of the glucagon analogs for the GLP-1 receptor. For example, the T29G substitution can be combined with the S16E and N20K amino acid substitutions, optionally with a lactam bridge between amino acids 16 and 20, and optionally with addition of a PEG chain as described herein. In one embodiment a glucagon/GLP-1 receptor co-agonist is provided, comprising the sequence of SEQ ID NO: 64. In one embodiment the glucagon peptide portion of the glucagon fusion peptide is selected from the group consisting of SEQ ID NO: 55, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5 wherein a PEG chain, when present at positions 17, 21, 24, or the C-terminal amino acid, or at both 21 and 24, is selected from the range of 500 to 40,000 Daltons. More particularly, in one embodiment the glucagon peptide segment is selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 63, wherein the PEG chain is selected from the range of 500 to 5,000. In one embodiment the glucagon peptide is a fusion peptide comprising the sequence of SEQ ID NO: 55 and SEQ ID NO: 65 wherein the peptide of SEQ ID NO: 65 is linked to the carboxy terminus of SEQ ID NO: 55.

In accordance with one embodiment, an additional chemical modification of the glucagon peptide of SEQ ID NO: 10 bestows increased GLP-1 receptor potency to a point where the relative activity at the glucagon and GLP-1 receptors is virtually equivalent. Accordingly, in one embodiment a glucagon/GLP-1 receptor co-agonist is provided wherein the terminal amino acid of the glucagon peptides of the present invention have an amide group in place of the carboxylic acid group that is present on the native amino acid. The relative activity of the glucagon analog at the respective glucagon and GLP-1 receptors can be adjusted by further modifications to the glucagon peptide to produce analogs demonstrating about 40% to about 500% or more of the activity of native glucagon at the glucagon receptor and about 20% to

about 200% or more of the activity of native GLP-1 at the GLP-1 receptor, e.g. 50-fold, 100-fold or more increase relative to the normal activity of glucagon at the GLP-1 receptor

In a further embodiment glucagon analogs are provided that exhibit glucagon/GLP-1 receptor co-agonist activity wherein an intramolecular bridge is formed between two amino acid side chains to stabilize the three dimensional structure of the carboxy terminus of the peptide. More particularly, the side chains of the amino acid pairs 12 and 16, 16 and 20 , 20 and 24 or 24 and 28 are linked to one another and thus stabilize the glucagon alpha helix. The two side chains can be linked to one another through hydrogen-bonding, ionic interactions, such as the formation of salt bridges, or by covalent bonds. In some embodiments, the size of the ring or linker is about 8 atoms, or about 7-9 atoms.

Examples of amino acid pairings that are capable of covalently bonding to form a seven-atom linking bridge include Orn-Glu (lactam ring); Lys-Asp (lactam); or Homoser-Homoglu (lactone). Examples of amino acid pairings that may form an eight-atom linker include Lys-Glu (lactam); Homolys-Asp (lactam); Orn-Homoglu (lactam); 4-aminoPhe-Asp (lactam); or Tyr-Asp (lactone). Examples of amino acid pairings that may form a nine-atom linker include Homolys-Glu (lactam); Lys-Homoglu (lactam); 4-aminoPhe-Glu (lactam); or Tyr-Glu (lactone). Any of the side chains on these amino acids may additionally be substituted with additional chemical groups, so long as the three-dimensional structure of the alpha-helix is not disrupted. One of ordinary skill in the art can envision alternative pairings or alternative amino acid analogs, including chemically modifiedderivatives, that would create a stabilizing structure of similar size and desired effect. For example, a homocysteine-homocysteine disulfide bridge is 6 atoms in length and may be further modified to provide the desired effect. Even without covalent linkage, the amino acid pairings described above or similar pairings that one of ordinary skill in the art can envision may also provide added stability to the alpha-helix through non-covalent bonds, for example, through formation of salt bridges or hydrogen-bonding interactions.

Further exemplary embodiments include the following pairings, optionally with a lactam bridge: Glu at position 12 with Lys at position 16; native Lys at position 12 with Glu at position 16; Glu at position 16 with Lys at position 20; Lys at position 16 with Glu at position 20; Glu at position 20 with Lys at position 24; Lys at position

20 with Glu at position 24; Glu at position 24 with Lys at position 28; Lys at position 24 with Glu at position 28.

In accordance with one embodiment a glucagon analog is provided that exhibits glucagon/GLP-1 receptor co-agonist activity wherein the analog comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 47, 48 and 49. In one embodiment the side chains are covalently bound to one another, and in one embodiment the two amino acids are bound to one another to form a lactam ring. The size of the lactam ring can vary depending on the length of the amino acid side chains, and in one embodiment the lactam is formed by linking the side chains of a lysine amino acid to a glutamic acid side chain.

The order of the amide bond in the lactam ring can be reversed (e.g., a lactam ring can be formed between the side chains of a Lys12 and a Glu16 or alternatively between a Glu 12 and a Lys16). In accordance with one embodiment a glucagon analog of SEQ ID NO: 45 is provided wherein at least one lactam ring is formed between the side chains of an amino acid pair selected from the group consisting of amino acid pairs 12 and 16, 16 and 20 , 20 and 24 or 24 and 28. In one embodiment a glucagon/GLP-1 receptor co-agonist is provided wherein the co-agonist comprises a glucagon peptide analog of SEQ ID NO: 20 wherein the peptide comprises an intramolecular lactam bridge formed between amino acid positions 12 and 16 or between amino acid positions 16 and 20. In one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising the sequence of SEQ ID NO: 20, wherein an intramolecular lactam bridge is formed between amino acid positions 12 and 16, between amino acid positions 16 and 20, or between amino acid positions 20 and 24 and the amino acid at position 29 is glycine, wherein the sequence of SEQ ID NO: 29 is linked to the C-terminal amino acid of SEQ ID NO: 20. In a further embodiment the amino acid at position 28 is aspartic acid.

The solubility of the glucagon peptide of SEQ ID NO: 20 can be further improved, for example, by introducing one, two, three or more charged amino acid(s) to the C-terminal portion of glucagon peptide of SEQ ID NO: 20, preferably at a position C-terminal to position 27. Such a charged amino acid can be introduced by substituting a native amino acid with a charged amino acid, e.g. at positions 28 or 29, or alternatively by adding a charged amino acid, e.g. after position 27, 28 or 29. In exemplary embodiments, one, two, three or all of the charged amino acids are

negatively charged. Alternatively, solubility can also be enhanced by covalently linking hydrophilic moieties, such as polyethylene glycol, to the peptide.

In accordance with one embodiment, a glucagon analog is provided comprising the sequence of SEQ ID NO: 55, wherein said analog differs from SEQ ID NO: 55 by 1 to 3 amino acids, selected from positions 1, 2, 3, 5, 7, 10, 11, 13, 14, 17, 18, 19, 21, 24, 27, 28, and 29, wherein said glucagon peptide exhibits at least 20% of the activity of native GLP-1 at the GLP-1 receptor.

In accordance with one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising the sequence:

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Xaa-Xaa-Arg-Arg-Ala-Xaa-Asp-Phe-Val-Xaa-Trp-Leu-Met-Xaa-Xaa-R (SEQ ID NO: 33) wherein the Xaa at position 15 is selected from the group of amino acids consisting of Asp, Glu, cysteic acid, homoglutamic acid and homocysteic acid, Xaa at position 16 is selected from the group of amino acids consisting of Ser, Glu, Gln, homoglutamic acid and homocysteic acid, the Xaa at position 20 is Gln or Lys, the Xaa at position 24 is Gln or Glu, the Xaa at position 28 is Asn, Lys or an acidic amino acid, the Xaa at position 29 is Thr, Gly or an acidic amino acid, and R is COOH or $CONH_2$, with the proviso that when position 16 is serine, position 20 is Lys, or alternatively when position 16 is serine the position 24 is Glu and either position 20 or position 28 is Lys. In one embodiment the glucagon/GLP-1 receptor co-agonist comprises the sequence of SEQ ID NO: 33 wherein the amino acid at position 28 is aspartic acid and the amino acid at position 29 is glutamic acid. In another embodiment the amino acid at position 28 is the native asparagine, the amino acid at position 29 is glycine and the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 65 is covalently linked to the carboxy terminus of SEQ ID NO: 33.

In one embodiment a co-agonist is provided comprising the sequence of SEQ ID NO: 33 wherein an additional acidic amino acid added to the carboxy terminus of the peptide. In a further embodiment the carboxy terminal amino acid of the glucagon analog has an amide in place of the carboxylic acid group of the natural amino acid. In one embodiment the glucagon analog comprises a sequence selected from the group consisting of SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44.

In accordance with one embodiment a glucagon peptide analog of SEQ ID NO: 33 is provided, wherein said analog differs from SEQ ID NO: 33 by 1 to 3 amino acids, selected from positions 1, 2, 3, 5, 7, 10, 11, 13, 14, 17, 18, 19, 21 and 27, with the proviso that when the amino acid at position 16 is serine, either position 20 is lysine, or a lactam bridge is formed between the amino acid at position 24 and either the amino acid at position 20 or position 28. In accordance with one embodiment the analog differs from SEQ ID NO: 33 by 1 to 3 amino acids selected from positions 1, 2, 3, 21 and 27. In one embodiment the glucagon peptide analog of SEQ ID NO: 33 differs from that sequence by 1 to 2 amino acids, or in one embodiment by a single amino acid, selected form positions 1, 2, 3, 5, 7, 10, 11, 13, 14, 17, 18, 19, 21 and 27, with the proviso that when the amino acid at position 16 is serine, either position 20 is lysine, or a lactam bridge is formed between the amino acid at position 24 and either the amino acid at position 20 or position 28.

In accordance with another embodiment a relatively selective GLP-1 receptor agonist is provided comprising the sequence NH2-His-Ser-Xaa-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Xaa-Xaa-Arg-Arg-Ala-Xaa-Asp-Phe-Val-Xaa-Trp-Leu-Met-Xaa-Xaa-R (SEQ ID NO: 53) wherein the Xaa at position 3 is selected from the group of amino acids consisting of Glu, Orn or Nle, the Xaa at position 15 is selected from the group of amino acids consisting of Asp, Glu, cysteic acid, homoglutamic acid and homocysteic acid, Xaa at position 16 is selected from the group of amino acids consisting of Ser, Glu, Gln, homoglutamic acid and homocysteic acid, the Xaa at position 20 is Gln or Lys, the Xaa at position 24 is Gln or Glu, the Xaa at position 28 is Asn, Lys or an acidic amino acid, the Xaa at position 29 is Thr, Gly or an acidic amino acid, and R is COOH, $CONH_2$, SEQ ID NO: 26 or SEQ ID NO: 29, with the proviso that when position 16 is serine, position 20 is Lys, or alternatively when position 16 is serine the position 24 is Glu and either position 20 or position 28 is Lys. In one embodiment the amino acid at position 3 is glutamic acid. In one embodiment the acidic amino acid substituted at position 28 and/or 29 is aspartic acid or glutamic acid. In one embodiment the glucagon peptide, including a co-agonist peptide, comprises the sequence of SEQ ID NO: 33 further comprising an additional acidic amino acid added to the carboxy terminus of the peptide. In a further embodiment the carboxy terminal amino acid of the glucagon analog has an amide in place of the carboxylic acid group of the natural amino acid.

In accordance with one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising a modified glucagon peptide selected from the group consisting of:

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Xaa-Xaa-Arg-Arg-Ala-Xaa-Asp-Phe-Val-Xaa-Trp-Leu-Met-Xaa-Xaa-R (SEQ ID NO: 34), wherein the Xaa at position 15 is selected from the group of amino acids consisting of Asp, Glu, cysteic acid, homoglutamic acid and homocysteic acid, Xaa at position 16 is selected from the group of amino acids consisting of Ser, Glu, Gln, homoglutamic acid and homocysteic acid, the Xaa at position 20 is Gln or Lys, the Xaa at position 24 is Gln or Glu and the Xaa at position 28 is Asn, Asp or Lys, R is COOH or $CONH_2$, the Xaa at position 29 is Thr or Gly, and R is COOH, $CONH_2$, SEQ ID NO: 26 or SEQ ID NO: 29, with the proviso that when position 16 is serine, position 20 is Lys, or alternatively when position 16 is serine the position 24 is Glu and either position 20 or position 28 is Lys. In one embodiment R is $CONH_2$, the Xaa at position 15 is Asp, the Xaa at position 16 is selected from the group of amino acids consisting of Glu, Gln, homoglutamic acid and homocysteic acid, the Xaas at positions 20 and 24 are each Gln the Xaa at position 28 is Asn or Asp and the Xaa at position 29 is Thr. In one embodiment the Xaas at positions 15 and 16 are each Glu, the Xaas at positions 20 and 24 are each Gln, the Xaa at position 28 is Asn or Asp, the Xaa at position 29 is Thr and R is $CONH_2$.

It has been reported that certain positions of the native glucagon peptide can be modified while retaining at least some of the activity of the parent peptide. Accordingly, applicants anticipate that one or more of the amino acids located at positions at positions 2, 5, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20, 21, 24, 27, 28 or 29 of the peptide of SEQ ID NO: 11 can be substituted with an amino acid different from that present in the native glucagon peptide, and still retain activity at the glucagon receptor. In one embodiment the methionine residue present at position 27 of the native peptide is changed to leucine or norleucine to prevent oxidative degradation of the peptide. In another embodiment the amino acid at position 20 is substituted with Lys, Arg, Orn or Citrullene and/or position 21 is substituted with Glu, homoglutamic acid or homocysteic acid.

In one embodiment a glucagon analog of SEQ ID NO: 20 is provided wherein 1 to 6 amino acids, selected from positions 1, 2, 5, 7, 10, 11, 13, 14, 17, 18, 19, 21, 27,

28 or 29 of the analog differ from the corresponding amino acid of SEQ ID NO: 1, with the proviso that when the amino acid at position 16 is serine, position 20 is Lys, or alternatively when position 16 is serine the position 24 is Glu and either position 20 or position 28 is Lys. In accordance with another embodiment a glucagon analog of SEQ ID NO: 20 is provided wherein 1 to 3 amino acids selected from positions 1, 2, 5, 7, 10, 11, 13, 14, 17, 18, 19, 20, 21, 27, 28 or 29 of the analog differ from the corresponding amino acid of SEQ ID NO: 1. In another embodiment, a glucagon analog of SEQ ID NO: 8, SEQ ID NO: 9 or SEQ ID NO: 11 is provided wherein 1 to 2 amino acids selected from positions 1, 2, 5, 7, 10, 11, 13, 14, 17, 18, 19, 20 or 21 of the analog differ from the corresponding amino acid of SEQ ID NO: 1, and in a further embodiment the one to two differing amino acids represent conservative amino acid substitutions relative to the amino acid present in the native glucagon sequence (SEQ ID NO: 1). In one embodiment a glucagon peptide of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15 is provided wherein the glucagon peptide further comprises one, two or three amino acid substitutions at positions selected from positions 2, 5, 7, 10, 11, 13, 14, 17, 18, 19, 20, 21, 27 or 29. In one embodiment the substitutions at positions 2, 5, 7, 10, 11, 13, 14, 16, 17, 18, 19, 20, 21, 27 or 29 are conservative amino acid substitutions.

In accordance with one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising a variant of the sequence of SEQ ID NO 33, wherein 1 to 10 amino acids selected from positions 16, 17, 18, 20, 21, 23, 24, 27, 28 and 29, respectively, of the variant differ from the corresponding amino acid of SEQ ID NO: 1. In accordance with one embodiment a variant of the sequence of SEQ ID NO 33 is provided wherein the variant differs from SEQ ID NO: 33 by one or more amino acid substitutions selected from the group consisting of Gln17, Ala18, Glu21, Ile23, Ala24, Val27 and Gly29. In accordance with one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising variants of the sequence of SEQ ID NO 33, wherein 1 to 2 amino acids selected from positions 17-26 of the variant differ from the corresponding amino acid of SEQ ID NO: 1. In accordance with one embodiment a variant of the sequence of SEQ ID NO 33 is provided wherein the variant differs from SEQ ID NO: 33 by an amino acid substitution selected from the group consisting of Gln17, Ala18, Glu21, Ile23 and Ala24. In accordance with one embodiment a variant of the sequence of SEQ ID NO 33 is provided wherein the

variant differs from SEQ ID NO: 33 by an amino acid substitution at position 18 wherein the substituted amino acid is selected from the group consisting of Ala, Ser, Thr, Pro and Gly. In accordance with one embodiment a variant of the sequence of SEQ ID NO 33 is provided wherein the variant differs from SEQ ID NO: 33 by an amino acid substitution of Ala at position 18. Such variations are encompassed by SEQ ID NO: 55. In another embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising variants of the sequence of SEQ ID NO 33, wherein 1 to 2 amino acids selected from positions 17-22 of the variant differ from the corresponding amino acid of SEQ ID NO: 1, and in a further embodiment a variant of SEQ ID NO 33 is provided wherein the variant differs from SEQ ID NO: 33 by 1or 2 amino acid substitutions at positions 20 and 21. In accordance with one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising the sequence: NH2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Xaa-Xaa-Arg-Arg-Ala-Xaa-Xaa-Phe-Val-Xaa-Trp-Leu-Met-Xaa-Xaa-R (SEQ ID NO: 51), wherein the

Xaa at position 15 is Asp, Glu, cysteic acid, homoglutamic acid or homocysteic acid, the Xaa at position 16 is Ser, Glu, Gln, homoglutamic acid or homocysteic acid, the Xaa at position 20 is Gln, Lys, Arg, Orn or citrulline, the Xaa at position 21 is Asp, Glu, homoglutamic acid or homocysteic acid, the Xaa at position 24 is Gln or Glu, the Xaa at position 28 is Asn, Lys or an acidic amino acid, the Xaa at position 29 is Thr or an acid amino acid and R is COOH or $CONH_2$. In one embodiment R is $CONH_2$. In accordance with one embodiment a glucagon/GLP-1 receptor co-agonist is provided comprising a variant of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 47, SEQ ID NO: 48 or SEQ ID NO: 49, wherein the variant differs from said sequence by an amino acid substitution at position 20. In one embodiment the amino acid substitution is selected form the group consisting of Lys, Arg, Orn or citrulline for position 20.

In one embodiment a glucagon agonist is provided comprising an analog peptide of SEQ ID NO: 34 wherein the analog differs from SEQ ID NO: 34 by having an amino acid other than serine at position 2. In one embodiment the serine residue is substituted with aminoisobutyric acid or alanine, and in one embodiment the serine residue is substituted with aminoisobutyric acid. Such modifications suppresses cleavage by dipeptidyl peptidase IV while retaining the inherent potency of the parent

compound (e.g. at least 75, 80, 85, 90, 95% or more of the potentcy of the parent compound). In one embodiment the solubility of the analog is increased, for example, by introducing one, two, three or more charged amino acid(s) to the C-terminal portion of native glucagon, preferably at a position C-terminal to position 27. In exemplary embodiments, one, two, three or all of the charged amino acids are negatively charged. In another embodiment the analog further comprises an acidic amino acid substituted for the native amino acid at position 28 or 29 or an acidic amino acid added to the carboxy terminus of the peptide of SEQ ID NO: 34.

In one embodiment the glucagon analogs disclosed herein are further modified at position 1 or 2 to reduce susceptibility to cleavage by dipeptidyl peptidase IV. In one embodiment a glucagon analog of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15 is provided wherein the analog differs from the parent molecule by a substitution at position 2 and exhibits reduced susceptibility (i.e. resistance) to cleavage by dipeptidyl peptidase IV. More particularly, in one embodiment position 2 of the analog peptide is substituted with an amino acid selected from the group consisting of d-serine, alanine, valine, amino n-butyric acid, glycine, N-methyl serine and aminoisobutyric acid. In one embodiment position 2 of the analog peptide is substituted with an amino acid selected from the group consisting of d-serine, alanine, glycine, N-methyl serine and aminoisobutyric acid. In another embodiment position 2 of the analog peptide is substituted with an amino acid selected from the group consisting of d-serine glycine, N-methyl serine and aminoisobutyric acid. In one embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

In one embodiment a glucagon analog of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15 is provided wherein the analog differs from the parent molecule by a substitution at position 1 and exhibits reduced susceptibility (i.e. resistance) to cleavage by dipeptidyl peptidase IV. More particularly, position 1 of the analog peptide is substituted with an amino acid selected from the group consisting of d-histidine, alpha, alpha-dimethyl imidiazole acetic acid (DMIA), N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, desaminohistidine, hydroxyl-histidine, acetyl-histidine and homo-histidine. In another embodiment a glucagon agonist is provided comprising an analog peptide of SEQ ID NO: 34 wherein the analog differs from SEQ ID NO: 34 by having an amino

acid other than histidine at position 1. In one embodiment the solubility of the analog is increased, for example, by introducing one, two, three or more charged amino acid(s) to the C-terminal portion of native glucagon, preferably at a position C-terminal to position 27. In exemplary embodiments, one, two, three or all of the charged amino acids are negatively charged. In another embodiment the analog further comprises an acidic amino acid substituted for the native amino acid at position 28 or 29 or an acidic amino acid added to the carboxy terminus of the peptide of SEQ ID NO: 34. In one embodiment the acidic amino acid is aspartic acid or glutamic acid.

In one embodiment the glucagon/GLP-1 receptor co-agonist comprises a sequence of SEQ ID NO: 20 further comprising an additional carboxy terminal extension of one amino acid or a peptide selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28. In the embodiment wherein a single amino acid is added to the carboxy terminus of SEQ ID NO: 20, the amino acid is typically selected from one of the 20 common amino acids, and in one embodiment the additional carboxy terminus amino acid has an amide group in place of the carboxylic acid of the native amino acid. In one embodiment the additional amino acid is selected from the group consisting of glutamic acid, aspartic acid and glycine.

In an alternative embodiment a glucagon/GLP-1 receptor co-agonist is provided wherein the peptide comprises at least one lactam ring formed between the side chain of a glutamic acid residue and a lysine residue, wherein the glutamic acid residue and a lysine residue are separated by three amino acids. In one embodiment the carboxy terminal amino acid of the lactam bearing glucagon peptide has an amide group in place of the carboxylic acid of the native amino acid. More particularly, in one embodiment a glucagon and GLP-1 co-agonist is provided comprising a modified glucagon peptide selected from the group consisting of:

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu- Met-Xaa-Xaa-R (SEQ ID NO: 66)

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Lys-Asp-Phe-Val-Gln-Trp-Leu- Met-Xaa-Xaa-R (SEQ ID NO: 67)

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Lys-Asp-Phe-Val-Glu-Trp-Leu- Met-Xaa-Xaa-R (SEQ ID NO: 68)

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Glu-Trp-Leu- Met-Lys-Xaa-R (SEQ ID NO: 69)

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Lys-Asp-Phe-Val-Glu-Trp-Leu- Met-Asn-Thr-R (SEQ ID NO: 16)

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Gln-Asp-Phe-Val-Glu-Trp-Leu- Met-Lys-Thr-R (SEQ ID NO: 17)

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Lys-Asp-Phe-Val-Glu-Trp-Leu- Met-Lys-Thr-R (SEQ ID NO: 18)

wherein Xaa at position 28 = Asp, or Asn, the Xaa at position 29 is Thr or Gly, R is selected from the group consisting of COOH, $CONH_2$, glutamic acid, aspartic acid, glycine, SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, and a lactam bridge is formed between Lys at position 12 and Glu at position 16 for SEQ ID NO: 66, between Glu at position 16 and Lys at position 20 for SEQ ID NO: 67, between Lys at position 20 and Glu at position 24 for SEQ ID NO: 68, between Glu at position 24 and Lys at position 28 for SEQ ID NO: 69, between Lys at position 12 and Glu at position 16 and between Lys at position 20 and Glu at position 24 for SEQ ID NO: 16, between Lys at position 12 and Glu at position 16 and between Glu at position 24 and Lys at position 28 for SEQ ID NO: 17 and between Glu at position 16 and Lys at position 20 and between Glu at position 24 and Lys at position 28 for SEQ ID NO: 18. In one embodiment R is selected from the group consisting of COOH, $CONH_2$, glutamic acid, aspartic acid, glycine, the amino acid at position 28 is Asn, and the amino acid at position 29 is threonine. In one embodiment R is $CONH_2$, the amino acid at position 28 is Asn and the amino acid at position 29 is threonine. In another embodiment R is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 29 and SEQ ID NO: 65 and the amino acid at position 29 is glycine.

In a further embodiment the glucagon/GLP-1 receptor co-agonist is selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, wherein the peptide further comprises an additional carboxy terminal extension of one amino acid or a peptide selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28. In one embodiment the terminal extension comprises the sequence of SEQ ID NO: 26, SEQ ID NO: 29 or SEQ ID NO: 65 and the glucagon peptide comprises the sequence of SEQ ID NO: 55. In one embodiment the

glucagon/GLP-1 receptor co-agonist comprises the sequence of SEQ ID NO: 33 wherein the amino acid at position 16 is glutamic acid, the amino acid at position 20 is lysine, the amino acid at position 28 is asparagine and the amino acid sequence of SEQ ID No: 26 or SEQ ID NO: 29 is linked to the carboxy terminus of SEQ ID NO: 33.

In the embodiment wherein a single amino acid is added to the carboxy terminus of SEQ ID NO: 20, the amino acid is typically selected from one of the 20 common amino acids, and in one embodiment the amino acid has an amide group in place of the carboxylic acid of the native amino acid. In one embodiment the additional amino acid is selected from the group consisting of glutamic acid and aspartic acid and glycine. In the embodiments wherein the glucagon agonist analog further comprises a carboxy terminal extension, the carboxy terminal amino acid of the extension, in one embodiment, ends in an amide group or an ester group rather than a carboxylic acid.

In another embodiment the glucagon/GLP-1 receptor co-agonist comprises the sequence: NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-Xaa-$CONH_2$ (SEQ ID NO: 19), wherein the Xaa at position 30 represents any amino acid. In one embodiment Xaa is selected from one of the 20 common amino acids, and in one embodiment the amino acid is glutamic acid, aspartic acid or glycine. The solubility of this peptide can be further improved by covalently linking a PEG chain to the side chain of amino acid at position 17, 21, 24 or 30 of SEQ ID NO: 19. In a further embodiment the peptide comprises an additional carboxy terminal extension of a peptide selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28. In accordance with one embodiment the glucagon/GLP-1 receptor co-agonist comprises the sequence of SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32.

Additional site specific modifications internal to the glucagon sequence of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 64 can be made to yield a set of glucagon agonists that possess variable degrees of GLP-1 agonism. Accordingly, peptides that possess virtually identical *in vitro* potency at each receptor have been prepared and characterized. Similarly, peptides

with tenfold selectively enhanced potency at each of the two receptors have been identified and characterized. As noted above substitution of the serine residue at position 16 with glutamic acid enhances the potency of native glucagon at both the Glucagon and GLP-1 receptors, but maintains approximately a tenfold selectivity for the glucagon receptor. In addition by substituting the native glutamine at position 3 with glutamic acid (SEQ ID NO: 22) generates a glucagon analog that exhibits approximately a tenfold selectivity for the GLP-1 receptor.

The solubility of the glucagon/GLP-1 co-agonist peptides can be further enhanced in aqueous solutions at physiological pH, while retaining the high biological activity relative to native glucagon by the introduction of hydrophilic groups at positions 16, 17, 21, and 24 of the peptide, or by the addition of a single modified amino acid (i.e. an amino acid modified to comprise a hydrophilic group) at the carboxy terminus of the glucagon/GLP-1 co-agonist peptide. In accordance with one embodiment the hydrophilic group comprises a polyethylene (PEG) chain. More particularly, in one embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18 wherein a PEG chain is covalently linked to the side chain of an amino acids at position 16, 17, 21, 24, 29 or the C-terminal amino acid of the glucagon peptide, with the proviso that when the peptide comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17, 21 or 24, when the peptide comprises SEQ ID NO: 14 or SEQ ID NO: 15 the polyethylene glycol chain is covalently bound to an amino acid residue at position 16, 17 or 21, and when the peptide comprises SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17 or 21.

In one embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13, wherein a PEG chain is covalently linked to the side chain of an amino acids at position 17, 21, 24, or the C-terminal amino acid of the glucagon peptide, and the carboxy terminal amino acid of the peptide has an amide group in place of the carboxylic acid group of the native amino acid. In one embodiment the glucagon/GLP-1 receptor co-agonist peptide comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13,

SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, wherein a PEG chain is covalently linked to the side chain of an amino acid at position 17, 21 or 24 of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 19, or at position 16, 17 or 21 of SEQ ID NO: 14 and SEQ ID NO: 15 or at position 17 or 21 of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18 of the glucagon peptide. In another embodiment the glucagon/GLP-1 receptor co-agonist peptide comprises the sequence of SEQ ID NO: 11 or SEQ ID NO: 19, wherein a PEG chain is covalently linked to the side chain of an amino acids at position 17, 21 or 24 or the C-terminal amino acid of the glucagon peptide.

In accordance with one embodiment, and subject to the proviso limitations described in the preceding paragraphs, the glucagon co-agonist peptide is modified to contain one or more amino acid substitution at positions 16, 17, 21, 24, or 29 or the C-terminal amino acid, wherein the native amino acid is substituted with an amino acid having a side chain suitable for crosslinking with hydrophilic moieties, including for example, PEG. The native peptide can be substituted with a naturally occurring amino acid or a synthetic (non-naturally occurring) amino acid. Synthetic or non-naturally occurring amino acids refer to amino acids that do not naturally occur *in vivo* but which, nevertheless, can be incorporated into the peptide structures described herein. Alternatively, the amino acid having a side chain suitable for crosslinking with hydrophilic moieties, including for example, PEG, can be added to the carboxy terminus of any of the glucagon analogs disclosed herein. In accordance with one embodiment an amino acid substitution is made in the glucagon/GLP-1 receptor co-agonist peptide at a position selected from the group consisting of 16, 17, 21, 24, or 29 replacing the native amino acid with an amino acid selected from the group consisting of lysine, cysteine, ornithine, homocysteine and acetyl phenylalanine, wherein the substituting amino acid further comprises a PEG chain covalently bound to the side chain of the amino acid. In one embodiment a glucagon peptide selected form the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19 is further modified to comprise a PEG chain is covalently linked to the side chain of an amino acid at position 17 or 21 of the glucagon peptide. In one embodiment the pegylated glucagon/GLP-1 receptor co-agonist further comprises the sequence of SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 29.

In another embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 55 or SEQ ID NO: 56, further comprising a C-terminal extension of SEQ ID NO: 26, SEQ ID NO: 29 or SEQ ID NO: 65 linked to the C-terminal amino acid of SEQ ID NO: 55 or SEQ ID NO: 56, and optionally further comprising a PEG chain covalently linked to the side chain of an amino acids at position 17, 18, 21, 24 or 29 or the C-terminal amino acid of the peptide. In another embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 55 or SEQ ID NO: 56, wherein a PEG chain is covalently linked to the side chain of an amino acids at position 21 or 24 of the glucagon peptide and the peptide further comprises a C-terminal extension of SEQ ID NO: 26, or SEQ ID NO: 29.

In another embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 55, or SEQ ID NO: 33 or SEQ ID NO: 34, wherein an additional amino acid is added to the carboxy terminus of SEQ ID NO: 33 or SEQ ID NO: 34, and a PEG chain is covalently linked to the side chain of the added amino acid. In a further embodiment, the pegylated glucagon analog further comprises a C-terminal extension of SEQ ID NO: 26 or SEQ ID NO: 29 linked to the C-terminal amino acid of SEQ ID NO: 33 or SEQ ID NO: 34. In another embodiment the glucagon peptide comprises the sequence of SEQ ID NO: 19, wherein a PEG chain is covalently linked to the side chain of the amino acid at position 30 of the glucagon peptide and the peptide further comprises a C-terminal extension of SEQ ID NO: 26 or SEQ ID NO: 29 linked to the C-terminal amino acid of SEQ ID NO: 19.

The polyethylene glycol chain may be in the form of a straight chain or it may be branched. In accordance with one embodiment the polyethylene glycol chain has an average molecular weight selected from the range of about 500 to about 10,000 Daltons. In one embodiment the polyethylene glycol chain has an average molecular weight selected from the range of about 1,000 to about 5,000 Daltons. In an alternative embodiment the polyethylene glycol chain has an average molecular weight selected from the range of about 10,000 to about 20,000 Daltons. In accordance with one embodiment the pegylated glucagon peptide comprises two or more polyethylene chains covalently bound to the glucagon peptide wherein the total molecular weight of the glucagon chains is about 1,000 to about 5,000 Daltons. In one embodiment the pegylated glucagon agonist comprises a peptide consisting of SEQ ID NO: 5 or a glucagon agonist analog of SEQ ID NO: 5, wherein a PEG chain

is covalently linked to the amino acid residue at position 21 and at position 24, and wherein the combined molecular weight of the two PEG chains is about 1,000 to about 5,000 Daltons.

As described in detail in the Examples, the glucagon agonists of the present invention have enhanced biophysical stability and aqueous solubility while demonstrating enhanced bioactivity relative to the native peptide. Accordingly, the glucagon agonists of the present invention are believed to be suitable for any use that has previously been described for the native glucagon peptide. Accordingly, the modified glucagon peptides described herein can be used to treat hypoglycemia or to increase blood glucose level, to induce temporary paralysis of the gut for radiological uses, or treat other metabolic diseases that result from low blood levels of glucagon. The glucagon peptides described herein also are expected to be used to reduce or maintain body weight, or to treat hyperglycemia, or to reduce blood glucose level, or to normalize blood glucose level.

The glucagon peptides of the invention may be administered alone or in combination with other anti-diabetic or anti-obesity agents. Anti-diabetic agents known in the art or under investigation include insulin, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicron); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPARγ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as miglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; or FBPase (fructose 1,6-bisphosphatase) inhibitors.

Anti-obesity agents known in the art or under investigation include appetite suppressants, including phenethylamine type stimulants, phentermine (optionally with fenfluramine or dexfenfluramine), diethylpropion (Tenuate®), phendimetrazine (Prelu-2®, Bontril®), benzphetamine (Didrex®), sibutramine (Meridia®, Reductil®); rimonabant (Acomplia®), other cannabinoid receptor antagonists; oxyntomodulin;

fluoxetine hydrochloride (Prozac); Qnexa (topiramate and phentermine), Excalia (bupropion and zonisamide) or Contrave (bupropion and naltrexone); or lipase inhibitors, similar to xenical (Orlistat) or Cetilistat (also known as ATL-962), or GT 389-255.

One aspect of the present disclosure is directed to a pre-formulated aqueous solution of the presently disclosed glucagon agonist for use in treating hypoglycemia. The improved stability and solubility of the agonist compositions described herein allow for the preparation of pre-formulated aqueous solutions of glucagon for rapid administration and treatment of hypoglycemia. In one embodiment a solution comprising a pegylated glucagon agonist is provided for administration to a patient suffering from hypoglycemia, wherein the total molecular weight of the PEG chains linked to the pegylated glucagon agonist is between about 500 to about 5,000 Daltons. In one embodiment the pegylated glucagon agonist comprises a peptide selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, and glucagon agonist analogs of SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, or a pegylated lactam derivative of glucagon comprising the sequence of SEQ ID NO: 20, wherein the side chain of an amino acid residue of said glucagon peptide is covalently bound to the polyethylene glycol chain.

The method of treating hypoglycemia in accordance with the present invention comprises the steps of administering the presently disclosed glucagon agonists to a patient using any standard route of administration, including parenterally, such as intravenously, intraperitoneally, subcutaneously or intramuscularly, intrathecally, transdermally, rectally, orally, nasally or by inhalation. In one embodiment the composition is administered subcutaneously or intramuscularly. In one embodiment, the composition is administered parenterally and the glucagon composition is prepackaged in a syringe.

Surprisingly, applicants have discovered that pegylated glucagon peptides can be prepared that retain the parent peptide's bioactivity and specificity. However, increasing the length of the PEG chain, or attaching multiple PEG chains to the peptide, such that the total molecular weight of the linked PEG is greater than 5,000 Daltons, begins to delay the time action of the modified glucagon. In accordance with one embodiment, a glucagon peptide of SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, or a glucagon agonist analog thereof, or a pegylated lactam derivative of

glucagon comprising the sequence of SEQ ID NO: 20 is provided wherein the peptide comprises one or more polyethylene glycol chains, wherein the total molecular weight of the linked PEG is greater than 5,000 Daltons, and in one embodiment is greater than 10,000 Daltons, but less than 40,000 Daltons. Such modified glucagon peptides have a delayed or prolonged time of activity but without loss of the bioactivity. Accordingly, such compounds can be administered to extend the effect of the administered glucagon peptide.

Glucagon peptides that have been modified to be covalently bound to a PEG chain having a molecular weight of greater than 10,000 Daltons can be administered in conjunction with insulin to buffer the actions of insulin and help to maintain stable blood glucose levels in diabetics. The modified glucagon peptides of the present disclosure can be co-administered with insulin as a single composition, simultaneously administered as separate solutions, or alternatively, the insulin and the modified glucagon peptide can be administered at different time relative to one another. In one embodiment the composition comprising insulin and the composition comprising the modified glucagon peptide are administered within 12 hours of one another. The exact ratio of the modified glucagon peptide relative to the administered insulin will be dependent in part on determining the glucagon levels of the patient, and can be determined through routine experimentation.

In accordance with one embodiment a composition is provided comprising insulin and a modified glucagon peptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and glucagon agonist analogs thereof, wherein the modified glucagon peptide further comprises a polyethylene glycol chain covalently bound to an amino acid side chain at position 17, 21, 24 or 21 and 24. In one embodiment the composition is an aqueous solution comprising insulin and the glucagon analog. In embodiments where the glucagon peptide comprises the sequence of SEQ ID NO: 24 or SEQ ID NO: 25 the PEG chain is covalently bound at position 21 or 24 of the glucagon peptide. In one embodiment the polyethylene glycol chain has a molecular weight of about 10,000 to about 40,000.

In accordance with one embodiment the modified glucagon peptides disclosed herein are used to induce temporary paralysis of the intestinal tract. This method has utility for radiological purposes and comprises the step of administering an effective

amount of a pharmaceutical composition comprising a pegylated glucagon peptide, a glucagon peptide comprising a c-terminal extension or a dimer of such peptides. In one embodiment the glucagon peptide comprises a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 SEQ ID NO: 14 and SEQ ID NO: 15. In one embodiment the glucagon peptide further comprises a PEG chain, of about 1,000 to 40,000 Daltons is covalently bound to an amino acid residue at position 21 or 24. In one embodiment the glucagon peptide is selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15. In one embodiment the PEG chain has a molecular weight of about 500 to about 5,000 Daltons.

In a further embodiment the composition used to induce temporary paralysis of the intestinal tract comprises a first modified glucagon peptide and a second modified glucagon peptide. The first modified peptide comprises a sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25, optionally linked to a PEG chain of about 500 to about 5,000 Daltons, and the second peptide comprises a sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25, covalently linked to a PEG chain of about 10,000 to about 40,000 Daltons. In this embodiment the PEG chain of each peptide is covalently bound to an amino acid residue at either position 17, 21 or 24 of the respective peptide, and independent of one another.

Oxyntomodulin, a naturally occurring digestive hormone found in the small intestine, has been reported to cause weight loss when administered to rats or humans (see Diabetes 2005;54:2390-2395). Oxyntomodulin is a 37 amino acid peptide that contains the 29 amino acid sequence of glucagon (i.e. SEQ ID NO: 1) followed by an 8 amino acid carboxy terminal extension of SEQ ID NO: 27 (KRNRNNIA). Accordingly, applicants believe that the bioactivity of oxyntomodulin can be retained (i.e. appetite suppression and induced weight loss/weight maintenance), while improving the solubility and stability of the compound and improving the pharmacokinetics, by substituting the glucagon peptide portion of oxyntomodulin with the modified glucagon peptides disclosed herein. In addition applicants also believe that a truncated Oxyntomodulin molecule comprising a glucagon peptide of

the invention, having the terminal four amino acids of oxyntomodulin removed will also be effective in suppressing appetite and inducing weight loss/weight maintenance.

Accordingly, the present invention also encompasses the modified glucagon peptides of the present invention that have a carboxy terminal extension of SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28. These compounds can be administered to individuals to induce weight loss or prevent weight gain. In accordance with one embodiment a glucagon agonist analog of SEQ ID NO: 33 or SEQ ID NO: 20, further comprising the amino acid sequence of SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 linked to amino acid 29 of the glucagon peptide, is administered to individuals to induce weight loss or prevent weight gain. More particularly, the glucagon peptide comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13 SEQ ID NO: 14 and SEQ ID NO: 15, further comprising the amino acid sequence of SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 linked to amino acid 29 of the glucagon peptide.

Exendin-4, is a peptide made up of 39 amino acids. It is a powerful stimulator of a receptor known as GLP-1. This peptide has also been reported to suppress appetite and induce weight loss. Applicants have found that the terminal sequence of Exendin-4 when added at the carboxy terminus of glucagon improves the solubility and stability of glucagon without compromising the bioactivity of glucagon. In one embodiment the terminal ten amino acids of Exendin-4 (i.e. the sequence of SEQ ID NO: 26 (GPSSGAPPPS)) are linked to the carboxy terminus of a glucagon peptide of the present disclosure. These fusion proteins are anticipated to have pharmacological activity for suppressing appetite and inducing weight loss/weight maintenance. In accordance with one embodiment a glucagon agonist analog of SEQ ID NO: 33 or SEQ ID NO: 20, further comprising the amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS) or SEQ ID NO: 29 linked to amino acid 29 of the glucagon peptide, is administered to individuals to induce weight loss or prevent weight gain. More particularly, the glucagon peptide comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13 SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 55 and SEQ ID NO: 56 further comprising the amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS) or

SEQ ID NO: 29 linked to amino acid 29 of the glucagon peptide. In one embodiment the administered glucagon peptide analog comprises the sequence of SEQ ID NO: 64.

The present disclosure also encompasses multimers of the modified glucagon peptides disclosed herein. Two or more of the modified glucagon peptides can be linked together using standard linking agents and procedures known to those skilled in the art. For example, dimers can be formed between two modified glucagon peptides through the use of bifunctional thiol crosslinkers and bi-functional amine crosslinkers, particularly for the glucagon peptides that have been substituted with cysteine, lysine ornithine, homocysteine or acetyl phenylalanine residues (e.g. SEQ ID NO: 3 and SEQ ID NO: 4). The dimer can be a homodimer or alternatively can be a heterodimer. In one embodiment the dimer comprises a homodimer of a glucagon fusion peptide wherein the glucagon peptide portion comprises SEQ ID NO: 11 or SEQ ID NO: 20 and an amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS), SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 (KRNR) linked to amino acid 29 of the glucagon peptide. In another embodiment the dimer comprises a homodimer of a glucagon agonist analog of SEQ ID NO: 11, wherein the glucagon peptide further comprises a polyethylene glycol chain covalently bound to position 21 or 24 of the glucagon peptide.

In accordance with one embodiment a dimer is provided comprising a first glucagon peptide bound to a second glucagon peptide via a linker, wherein the first glucagon peptide comprises a peptide selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11 and the second glucagon peptide comprises SEQ ID NO: 20. In accordance with another embodiment a dimer is provided comprising a first glucagon peptide bound to a second glucagon peptide via a linker, wherein said first glucagon peptide comprises a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and the second glucagon peptide comprise SEQ ID NO: 11, and pharmaceutically acceptable salts of said glucagon polypeptides. In accordance with another embodiment a dimer is provided comprising a first glucagon peptide bound to a second glucagon peptide via a linker, wherein said first glucagon peptide is selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18 and the second glucagon

peptide is independently selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, and pharmaceutically acceptable salts of said glucagon polypeptides. In one embodiment the first glucagon peptide is selected from the group consisting of SEQ ID NO: 20 and the second glucagon peptide is independently selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 11. In one embodiment the dimer is formed between two peptides wherein each peptide comprises the amino acid sequence of SEQ ID NO: 11.

The modified glucagon peptides of the present invention can be provided in accordance with one embodiment as part of a kit. In one embodiment a kit for administering a glucagon agonist to a patient in need thereof is provided wherein the kit comprises a modified glucagon peptide selected from the group consisting of 1) a glucagon peptide comprising the sequence of SEQ ID NO: 20, SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO:11; 2) a glucagon fusion peptide comprising a glucagon agonist analog of SEQ ID NO: 11, SEQ ID NO: 20 or SEQ ID NO: 55, and an amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS), SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 (KRNR) linked to amino acid 29 of the glucagon peptide; and 3) a pegylated glucagon peptide of SEQ ID NO: 11 or SEQ ID NO: 51, further comprising an amino acid sequence of SEQ ID NO: 26 (GPSSGAPPPS), SEQ ID NO: 27 (KRNRNNIA) or SEQ ID NO: 28 (KRNR) linked to amino acid 29 of the glucagon peptide, wherein the PEG chain covalently bound to position 17, 21 or 24 has a molecular weight of about 500 to about 40,000 Daltons. In one embodiment the kit comprise a glucagon/GLP-1 co-agonist wherein the peptide comprises a sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.

In one embodiment the kit is provided with a device for administering the glucagon composition to a patient, e.g. syringe needle, pen device, jet injector or other needle-free injector. The kit may alternatively or in addition include one or more containers, *e.g.*, vials, tubes, bottles, single or multi-chambered pre-filled syringes, cartridges, infusion pumps (external or implantable), jet injectors, pre-filled pen devices and the like, optionally containing the glucagon peptide in a lyophilized form or in an aqueous solution. Preferably, the kits will also include instructions for use.

In accordance with one embodiment the device of the kit is an aerosol dispensing device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in one embodiment the sterile glucagon composition is prepackaged within the syringe.

The compounds of this invention may be prepared by standard synthetic methods, recombinant DNA techniques, or any other methods of preparing peptides and fusion proteins. Although certain non-natural amino acids cannot be expressed by standard recombinant DNA techniques, techniques for their preparation are known in the art. Compounds of this invention that encompass non-peptide portions may be synthesized by standard organic chemistry reactions, in addition to standard peptide chemistry reactions when applicable.

EXAMPLES

General Synthesis Protocol:

Glucagon analogs were synthesized using HBTU-activated "Fast Boc" single coupling starting from 0.2mmole of Boc Thr(OBzl)Pam resin on a modified Applied Biosystem 430 A peptide synthesizer. Boc amino acids and HBTU were obtained from Midwest Biotech (Fishers, IN). Side chain protecting groups used were: Arg(Tos), Asn(Xan), Asp(OcHex), Cys(pMeBzl), His(Bom), Lys(2Cl-Z), Ser(OBzl), Thr(OBzl), Tyr(2Br-Z), and Trp(CHO). The side-chain protecting group on the N-terminal His was Boc.

Each completed peptidyl resin was treated with a solution of 20% piperdine in dimethylformamide to remove the formyl group from the tryptophan. Liquid hydrogen fluoride cleavages were performed in the presence of p-cresol and dimethyl sulfide. The cleavage was run for 1 hour in an ice bath using an HF apparatus (Penninsula Labs). After evaporation of the HF, the residue was suspended in diethyl ether and the solid materials were filtered. Each peptide was extracted into 30-70ml aqueous acetic acid and a diluted aliquot was analyzed by HPLC [Beckman System Gold, 0.46x5cm Zorbax C8, 1ml/min, 45C, 214nm, A buffer =0.1%TFA, B=0.1%TFA/90%acetonitrile, gradient of 10% to 80%B over 10min].

Purification was done on a FPLC over a 2.2 x 25 cm Kromasil C18 column while monitoring the UV at 214nm and collecting 5 minute fractions. The homogeneous fractions were combined and lyophilized to give a product purity of

>95%. The correct molecular mass and purity were confirmed using MALDI-mass spectral analysis.

General Pegylation Protocol: (Cys-maleimido)

Typically, the glucagon Cys analog is dissolved in phosphate buffered saline (5-10mg/ml) and 0.01M ethylenediamine tetraacetic acid is added (10-15% of total volume). Excess (2-fold) maleimido methoxyPEG reagent (Nektar) is added and the reaction stirred at room temp while monitoring reaction progress by HPLC. After 8-24hrs, the reaction mixture, is acidified and loaded onto a preparative reverse phase column for purification using 0.1%TFA/acetonitrile gradient. The appropriate fractions were combined and lyophilized to give the desired pegylated analogs.

EXAMPLE 1

Synthesis of Glucagon Cys^{17}(1-29) and Similar MonoCys Analogs

0.2mmole Boc Thr(OBzl) Pam resin (SynChem Inc) in a 60ml reaction vessel and the following sequence was entered and run on a modified Applied Biosystems 430A Peptide Synthesizer using FastBoc HBTU-activated single couplings.

HSQGTFTSDYSKYLDSCRAQDFVQWLMNT (SEQ ID NO: 35)

The following side chain protecting groups were used: Arg(Tos), Asp(OcHex), Asn(Xan), Cys(pMeBzl), Glu(OcHex), His(Boc), Lys(2Cl-Z), Ser(Bzl), Thr(Bzl), Trp(CHO), and Tyr(Br-Z). The completed peptidyl resin was treated with 20% piperidine/dimethylformamide to remove the Trp formyl protection then transferred to an HF reaction vessel and dried in vacuo. 1.0ml p-cresol and 0.5 ml dimehyl sulfide were added along with a magnetic stir bar. The vessel was attached to the HF apparatus (Pennisula Labs), cooled in a dry ice/methanol bath, evacuated, and aprox. 10ml liquid hydrogen fluoride was condensed in. The reaction was stirred in an ice bath for 1hr then the HF was removed in vacuo. The residue was suspended in ethyl ether; the solids were filtered, washed with ether, and the peptide extracted into 50 ml aqueous acetic acid. An analytical HPLC was run [0.46 x 5 cm Zorbax C8, 1 ml/min, 45C, 214nm, A buffer of 0.1%TFA, B buffer of 0.1%TFA/90%ACN, gradient=10%B to 80%B over 10min.] with a small sample of the cleavage extract. The remaining extract was loaded onto a 2.2 x 25cm Kromasil C18 preparative reverse phase column and an acetonitrile gradient was run using a Pharmacia FPLC system. 5min fractions

were collected while monitoring the UV at 214nm (2.0A). A=0.1%TFA, B=0.1%TFA/50%acetonitrile. Gradient = 30%B to 100%B over 450min.

The fractions containing the purest product (48-52) were combined frozen, and lyophilized to give 30.1mg. An HPLC analysis of the product demonstrated a purity of >90% and MALDI mass spectral analysis demonstrated the desired mass of 3429.7. Glucagon Cys^{21}, Glucagon Cys^{24}, and Glucagon Cys^{29} were similarly prepared.

EXAMPLE 2

Synthesis of Glucagon-Cex and Other C-Terminal Extended Analogs.

285mg (0.2mmole) methoxybenzhydrylamine resin (Midwest Biotech) was placed in a 60ml reaction vessel and the following sequence was entered and run on a modified Applied Biosystems 430A peptide synthesizer using FastBoc HBTU-activated single couplings.

HSQGTFTSDYSKYLDSRRAQDFVQWLMNTGPSSGAPPPS (SEQ ID NO: 36)

The following side chain protecting groups were used: Arg(Tos), Asp(OcHex), Asn(Xan), Cys(pMeBzl), Glu(OcHex), His(Boc), Lys(2Cl-Z), Ser(Bzl), Thr(Bzl), Trp(CHO), and Tyr(Br-Z). The completed peptidyl resin was treated with 20% piperidine/dimethylformamide to remove the Trp formyl protection then transferred to HF reaction vessel and dried in vacuo. 1.0ml p-cresol and 0.5 ml dimehyl sulfide were added along with a magnetic stir bar. The vessel was attached to the HF apparatus (Pennisula Labs), cooled in a dry ice/methanol bath, evacuated, and aprox. 10ml liquid hydrogen fluoride was condensed in. The reaction was stirred in an ice bath for 1hr then the HF was removed in vacuo. The residue was suspended in ethyl ether; the solids were filtered, washed with ether, and the peptide extracted into 50 ml aqueous acetic acid. An analytical HPLC was run [0.46 x 5 cm Zorbax C8, 1 ml/min, 45C, 214nm, A buffer of 0.1%TFA, B buffer of 0.1%TFA/90%ACN, gradient=10%B to 80%B over 10min.] on an aliquot of the cleavage extract. The extract was loaded onto a 2.2 x 25cm Kromasil C18 preparative reverse phase column and an acetonitrile gradient was run for elution using a Pharmacia FPLC system. 5min fractions were collected while monitoring the UV at 214nm (2.0A). A=0.1%TFA,

B=0.1%TFA/50%acetonitrile. Gradient = 30%B to 100%B over 450min. Fractions 58-65 were combined, frozen and lyophilized to give 198.1mg.

HPLC analysis of the product showed a purity of greater than 95%. MALDI mass spectral analysis showed the presence of the desired theoretical mass of 4316.7 with the product as a C-terminal amide. Oxyntomodulin and oxyntomodulin-KRNR were similarly prepared as the C-terminal carboxylic acids starting with the appropriately loaded PAM-resin.

EXAMPLE 3

Glucagon Cys^{17} Mal-PEG-5K

15.1mg of Glucagon Cys^{17}(1-29) and 27.3mg methoxy poly(ethyleneglycol) maleimide avg. M.W.5000 (mPEG-Mal-5000,Nektar Therapeutics) were dissolved in 3.5ml phosphate buffered saline (PBS) and 0.5ml 0.01M ethylenediamine tetraacetic acid (EDTA) was added. The reaction was stirred at room temperature and the progress of the reaction was monitored by HPLC analysis [0.46 x 5 cm Zorbax C8, 1ml/min,45C, 214nm (0.5A), A=0.1%TFA, B=0.1%TFA/90%ACN, gradient=10%B to 80%B over 10min.].

After 5 hours, the reaction mixture was loaded onto 2.2 x 25 cm Kromasil C18 preparastive reverse phase column. An acetonitrile gradient was run on a Pharmacia FPLC while monitoring the UV wavelength at 214nm and collecting 5 min fractions. A=0.1%TFA, B=0.1%TFA/50% acetonitrile, gradient= 30%B to 100%B over 450 min. The fractions corresponding to the product were combined, frozen and lyophilized to give 25.9 mg.

This product was analyzed on HPLC [0.46 x 5 cm Zorbax C8, 1 ml/min, 45C, 214nm (0.5A), A=0.1%TFA, B=0.1%TFA/90%ACN, gradient=10%B to 80%B over 10min.] which showed a purity of aprox. 90%. MALDI (matrix assisted laser desorption ionization) mass spectral analysis showed a broad mass range (typical of PEG derivatives) of 8700 to 9500. This shows an addition to the mass of the starting glucagon peptide (3429) of approximately 5,000 a.m.u.

EXAMPLE 4

Glucagon Cys^{21} Mal-PEG-5K

21.6mg of Glucagon Cys^{21}(1-29) and 24mg mPEG-MAL-5000 (Nektar Therapeutics) were dissolved in 3.5ml phosphate buffered saline (PBS) and 0.5ml 0.01M ethylene diamine tetraacetic acid (EDTA) was added. The reaction was stirred at room temp. After 2hrs, another 12.7 mg of mPEG-MAL-5000 was added. After 8hrs, the reaction mixture was loaded onto a 2.2 x 25cm Vydac C18 preparative reverse phase column and an acetonitrile gradient was run on a Pharmacia FPLC at 4 ml/min while collecting 5min fractions. A=0.1%TFA, B=0.1%TFA/50%ACN. Gradient= 20% to 80%B over 450min.

The fractions corresponding to the appearance of product were combined frozen and lyophilized to give 34 mg. Analysis of the product by analytical HPLC [0.46 x 5 cm Zorbax C8, 1 ml/min, 45C, 214nm (0.5A), A=0.1%TFA, B=0.1%TFA/90%ACN, gradient=10%B to 80%B over 10min.] showed a homogeneous product that was different than starting glucagon peptide. MALDI (matrix assisted laser desorption ionization) mass spectral analysis showed a broad mass range (typical of PEG analogs) of 8700 to 9700. This shows an addition to the mass of the starting glucagon peptide (3470) of approximately 5,000 a.m.u.

EXAMPLE 5

Glucagon Cys^{24} Mal-PEG-5K

20.1mg Glucagon C^{24}(1-29) and 39.5mg mPEG-Mal-5000 (Nektar Therapeutics) were dissolved in 3.5ml PBS with stirring and 0.5 ml 0.01M EDTA was added. The reaction was stirred at room temp for 7 hrs, then another 40 mg of mPEG-Mal-5000 was added. After approximately 15 hr, the reaction mixture was loaded onto a 2.2 x 25 cm Vydac C18 preparative reverse phase column and an acetontrile gradient was run using a Pharmacia FPLC. 5 min. fractions were collected while monitoring the UV at 214nm (2.0A). A buffer = 0.1%TFA, B buffer = 0.1%TFA/50%ACN, gradient = 30%B to 100%B over 450min. The fractions corresponding to product were combined, frozen and lyophilized to give 45.8mg. MALDI mass spectral analysis showed a typical PEG broad signal with a maximum at 9175.2 which is approximately 5,000 a.m.u. more than Glucagon C^{24} (3457.8).

EXAMPLE 6

Glucagon Cys^{24} Mal-PEG-20K

25.7mg of Glucagon Cys^{24}(1-29) and 40.7mg mPEG-Mal-20K (Nektar Therapeutics) were dissolved in 3.5ml PBS with stirring at room temp. and 0.5 ml 0.01M EDTA was added. After 6hrs, the ratio of starting material to product was aprox. 60:40 as determined by HPLC. Another 25.1mg of mPEG-Mal-20K was added and the reaction allowed to stir another 16hrs. The product ratio had not significantly improved, so the reaction mixture was loaded onto a 2.2 x 25 cm Kromasil C18 preparative reverse phase column and purified on a Pharmacia FPLC using a gradient of 30%B to 100%B over 450min. A buffer =0.1%TFA, B buffer = 0.1%TFA/50%ACN, flow = 4ml/min, and 5 min fractions were collected while monitoring the UV at 214nm (2.0A). The fractions containing homogeneous product were combined, frozen and lyophilized to give 25.7 mg. Purity as determined by analytical HPLC was ~90%. A MALDI mass spectral analysis showed a broad peak from 23,000 to 27,000 which is approximately 20,000 a.m.u. more than starting Glucagon C^{24} (3457.8).

EXAMPLE 7

Glucagon Cys^{29} Mal-PEG-5K

20.0mg of Glucagon Cys^{29}(1-29) and 24.7 mg mPEG-Mal-5000 (Nektar Therapeutics) were dissolved in 3.5 ml PBS with stirring at room temperature and 0.5 ml 0.01M EDTA was added. After 4 hr, another 15.6 mg of mPEG-Mal-5000 was added to drive the reaction to completion. After 8 hrs, the reaction mixture was loaded onto a 2.2 x 25 cm Vydac C18 preparative reverse phase column and an acetonitrile gradient was run on a Pharmacia FPLC system. 5 min fractions were collected while monitoring the UV at 214nm (2.0A). A=0.1%TFA, B=0.1%TFA/50%ACN. Fractions 75-97 were combined frozen and lyophilized to give 40.0 mg of product that is different than recovered starting material on HPLC (fractions 58-63). Analysis of the product by analytical HPLC [0.46 x 5 cm Zorbax C8, 1 ml/min, 45C, 214nm (0.5A), A=0.1%TFA, B=0.1%TFA/90%ACN, gradient=10%B to 80%B over 10min.] showed a purity greater than 95%. MALDI mass spectral analysis showed the presence of a PEG component with a mass range of

8,000 to 10,000 (maximum at 9025.3) which is 5,540 a.m.u. greater than starting material (3484.8).

EXAMPLE 8

Glucagon Cys^{24} (2-butyrolactone)

To 24.7mg of Glucagon Cys^{24}(1-29) was added 4ml 0.05M ammonium bicarbonate/50%acetonitrile and 5.5 ul of a solution of 2-bromo-4-hydroxybutyric acid-γ-lactone (100ul in 900ul acetonitrile). After 3hrs of stirring at room temperature, another 105 ul of lactone solution was added to the reaction mixture which was stirred another 15hrs. The reaction mixture was diluted to 10ml with 10% aqueous acetic acid and was loaded onto a 2.2 x 25 cm Kromasil C18 preparative reverse phase column. An acetonitrile gradient (20%B to 80%B over 450min) was run on a Pharmacia FPLC while collecting 5min fractions and monitoring the UV at 214nm (2.0A). Flow =4ml/min, A=0.1%TFA, B=0.1%TFA/50%ACN. Fractions 74-77 were combined frozen and lyophilized to give 7.5mg. HPLC analysis showed a purity of 95% and MALDI mass spect analysis showed a mass of 3540.7 or 84 mass units more than starting material. This result consistent with the addition of a single butyrolactone moiety.



Molecular Weight =3541.91
Exact Mass =3538
Molecular Formula =C155H226N42O50S2

SEQ ID NO: 37

EXAMPLE 9

Glucagon Cys^{24}(S-carboxymethyl)

18.1mg of Glucagon Cys^{24}(1-29) was dissolved in 9.4ml 0.1M sodium phosphate buffer (pH=9.2) and 0.6ml bromoacetic acid solution (1.3mg/ml in acetonitrile) was added. The reaction was stirred at room temperature and the reaction progress was followed by analytical HPLC. After 1hr another 0.1ml bromoacetic acid solution was added. The reaction was stirred another 60min. then acidified with aqueous acetic acid and was loaded onto a 2.2 x 25cm Kromasil C18 preparative reverse phase column for purification. An acetonitrile gradient was run

on a Pharmacia FPLC (flow = 4ml/min) while collecting 5min fractions and monitoring the UV at 214nm (2.0A). A=0.1%TFA, B=0.1%TFA/50%ACN. Fractions 26-29 were combined frozen and lyophilized to give several mg of product. Analytical HPLC showed a purity of 90% and MALDI mass spectral analysis confirmed a mass of 3515 for the desired product.



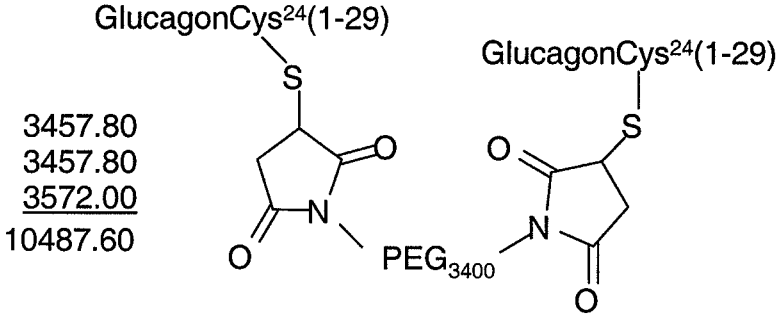
Molecular Weight =3515.87
Exact Mass =3512
Molecular Formula =C153H224N42O50S2

SEQ ID NO: 38

EXAMPLE 10

Glucagon Cys^{24} maleimido,PEG-3.4K-dimer

16mg Glucagon Cys^{24} and 1.02mg Mal-PEG-Mal-3400, poly(ethyleneglycol)-bis-maleimide avg. M.W. 3400, (Nektar Therpeutics) were dissolved in 3.5 phosphate buffered saline and 0.5ml 0.01M EDTA and the reaction was stirred at room temperature. After 16hrs, another 16mg of Glucagon Cys^{24} was added and the stirring continued. After approximately 40hrs, the reaction mixture was loaded onto a Pharmcia PepRPC 16/10 column and an acetonitrile gradient was run on a Pharmacia FPLC while collecting 2min fractions and monitoring the UV at 214nm (2.0A). Flow=2ml/min, A=0.1%TFA, B=0.1%TFA/50%ACN. Fractions 69-74 were combined frozen and lyophilized to give 10.4mg. Analytical HPLC showed a purity of 90% and MALDI mass spectral analysis shows a component in the 9500-11,000 range which is consistent with the desired dimer.



EXAMPLE 11

Synthesis of Glucagon Lactams

285 mg (0.2 mmole) methoxybenzhydrylamine resin (Midwest Biotech) was added to a 60 mL reaction vessels and the following sequence was assembled on a modified Applied Biosystems 430A peptide synthesizer using Boc DEPBT-activated single couplings.

HSQGTFTSDYSKYLDERRAQDFVQWLMNT-NH2 (12-16 Lactam; SEQ ID NO: 12)

The following side chain protecting groups were used: Arg(Tos), Asp(OcHx), Asn(Xan), Glu(OFm), His(BOM), Lys(Fmoc), Ser(Bzl), Thr(Bzl), Trp(CHO), Tyr(Br-Z). Lys(Cl-Z) was used at position 12 if lactams were constructed from 16-20, 20-24, or 24-28. The completed peptidyl resin was treated with 20% piperidine/dimethylformamide for one hour with rotation to remove the Trp formyl group as well as the Fmoc and OFm protection from Lys12 and Glu16. Upon confirmation of removal by a positive ninhydrin test, the resin was washed with dimethylformamide, followed by dichloromethane and than again with dimethylformamide. The resin was treated with 520 mg (1 mmole) Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) in dimethylformamide and diisopropylethylamine (DIEA). The reaction proceeded for 8-10 hours and the cyclization was confirmed by a negative ninhydrin reaction. The resin was washed with dimethylformamide, followed by dichloromethane and subsequently treated with trifluoroacetic acid for 10 minutes. The removal of the Boc group was confirmed by a positive ninhydrin reaction. The resin was washed with dimethylformamide and dichloromethane and dried before being transferred to a hydrofluoric acid (HF) reaction vessel. 500 μL p-cresol was added along with a magnetic stir bar. The vessel was attached to the HF apparatus (Peninsula Labs), cooled in a dry ice/methanol bath, evacuated, and approximately 10 mL of liquid hydrofluoric acid was condensed into the vessel. The reaction was stirred for 1 hour in an ice bath and the HF was subsequently removed in vacuo. The residue was suspended in ethyl ether; the solids were filtered, washed with ether, and the peptide was solubilized with 150 mL 20% acetonitrile/1% acetic acid.

An analytical HPLC analysis of the crude solubilized peptide was conducted under the following conditions [4.6 X 30 mm Xterra C8, 1.50 mL/min, 220 nm, A buffer 0.1% TFA/10% ACN, B buffer 0.1% TFA/100% ACN, gradient 5-95%B over 15 minutes]. The extract was diluted twofold with water and loaded onto a 2.2 X 25 cm Vydac C4 preparative reverse phase column and eluted using an acetonitrile gradient on a Waters HPLC system (A buffer of 0.1% TFA/10% ACN, B buffer of 0.1% TFA/10% CAN and a gradient of 0-100% B over 120 minutes at a flow of 15.00 ml/min. HPLC analysis of the purified peptide demonstrated greater than 95% purity and electrospray ionization mass spectral analysis confirmed a mass of 3506 Da for the 12-16 lactam. Lactams from 16-20, 20-24, and 24-28 were prepared similarly.

EXAMPLE 12

Glucagon Solubility Assays:

A solution (1mg/ml or 3mg/ml) of glucagon (or an analog) is prepared in 0.01N HCl. 100ul of stock solution is diluted to 1ml with 0.01N HCl and the UV absorbance (276nm) is determined. The pH of the remaining stock solution is adjusted to pH7 using
200-250ul 0.1M Na_2HPO_4 (pH9.2). The solution is allowed to stand overnight at 4°C then centrifuged. 100ul of supernatant is then diluted to 1ml with 0.01N HCl, and the UV absorbance is determined (in duplicate).

The initial absorbance reading is compensated for the increase in volume and the following calculation is used to establish percent solubility:

$$\frac{\text{Final Absorbance}}{\text{Initial Absorbance}} \times 100 = \text{percent soluble}$$

Results are shown in Table 1 wherein Glucagon-Cex represents wild type glucagon (SEQ ID NO: 1) plus a carboxy terminal addition of SEQ ID NO: 26 and Glucagon-Cex R^{12} represents SEQ ID NO: 39.

Table 1 Solubility date for glucagon analogs

Analog	Percent Soluble
Glucagon	16
Glucagon-Cex, R12	104
Glucagon-Cex	87
Oxyntomodulin	104
Glucagon, Cys17PEG5K	94
Glucagon, Cys21PEG5K	105
Glucagon, Cys24PEG5K	133

EXAMPLE 13

Glucagon Receptor Binding Assay

The affinity of peptides to the glucagon receptor was measured in a competition binding assay utilizing scintillation proximity assay technology. Serial 3-fold dilutions of the peptides made in scintillation proximity assay buffer (0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.1% w/v bovine serum albumin) were mixed in 96 well white/clear bottom plate (Corning Inc., Acton, MA) with 0.05 nM (3-[^{125}I]-iodotyrosyl) Tyr10 glucagon (Amersham Biosciences, Piscataway, NJ), 1-6 micrograms per well, plasma membrane fragments prepared from cells over-expressing human glucagon receptor, and 1 mg/well polyethyleneimine-treated wheat germ agglutinin type A scintillation proximity assay beads (Amersham Biosciences, Piscataway, NJ). Upon 5 min shaking at 800 rpm on a rotary shaker, the plate was incubated 12h at room temperature and then read on MicroBeta1450 liquid scintillation counter (Perkin-Elmer, Wellesley, MA). Non-specifically bound (NSB) radioactivity was measured in the wells with 4 times greater concentration of "cold" native ligand than the highest concentration in test samples and total bound radioactivity was detected in the wells with no competitor. Percent specific binding was calculated as following: % Specific Binding = ((Bound-NSB)/(Total bound-NSB)) X 100. IC_{50} values were determined by using Origin software (OriginLab, Northampton, MA).

EXAMPLE 14

Functional Assay- cAMP Synthesis

The ability of glucagon analogs to induce cAMP was measured in a firefly luciferase-based reporter assay. HEK293 cells co-transfected with either glucagon- or GLP-1 receptor and luciferase gene linked to cAMP responsive element were serum deprived by culturing 16h in DMEM (Invitrogen, Carlsbad, CA) supplemented with 0.25% Bovine Growth Serum (HyClone, Logan, UT) and then incubated with serial dilutions of either glucagon, GLP-1 or novel glucagon analogs for 5 h at 37°C, 5% CO_2 in 96 well poly-D-Lysine-coated "Biocoat" plates (BD Biosciences, San Jose, CA). At the end of the incubation 100 microliters of LucLite luminescence substrate reagent (Perkin-Elmer, Wellesley, MA) were added to each well. The plate was shaken briefly, incubated 10 min in the dark and light output was measured on MicroBeta-1450 liquid scintillation counter (Perkin-Elmer, Wellesley, MA). Effective 50% concentrations were calculated by using Origin software (OriginLab, Northampton, MA. Results are shown in Figs. 3-9 and in Tables 2 through 10.

Table 2

cAMP Induction by Glucagon Analogs with C-Terminus Extension

Peptide	cAMP Induction			
	Glucagon Receptor		GLP-1 Receptor	
	EC_{50}, nM	N*	EC_{50}, nM	N
Glucagon	0.22 ± 0.09	14	3.85 ±1.64	10
GLP-1	2214.00 ± 182.43	2	0.04 ± 0.01	14
Glucagon Cex	0.25 ± 0.15	6	2.75 ± 2.03	7
Oxyntomodulin	3.25 ± 1.65	5	2.53 ± 1.74	5
Oxyntomodulin KRNR	2.77 ± 1.74	4	3.21 ± 0.49	2
Glucagon R12	0.41 ± 0.17	6	0.48 ± 0.11	5
Glucagon R12 Cex	0.35 ± 0.23	10	1.25 ± 0.63	10
Glucagon R12 K20	0.84 ± 0.40	5	0.82 ± 0.49	5
Glucagon R12 K24	1.00 ± 0.39	4	1.25 ± 0.97	5
Glucagon R12 K29	0.81 ± 0.49	5	0.41 ± 0.24	6
Glucagon Amide	0.26 ± 0.15	3	1.90 ± 0.35	2
Oxyntomodulin C24	2.54 ± 0.63	2	5.27 ± 0.26	2
Oxyntomodulin C24 PEG 20K	0.97 ± 0.04	1	1.29 ± 0.11	1

* - number of experiments

Table 3

cAMP Induction by Pegylated Glucagon Analogs

Peptide	cAMP Induction			
	Glucagon Receptor		GLP-1 Receptor	
	EC_{50}, nM	N*	EC_{50}, nM	N
Glucagon	0.33 ± 0.23	18	12.71 ±3.74	2
Glucagon C17 PEG 5K	0.82 ± 0.15	4	55.86 ± 1.13	2
Glucagon C21 PEG 5K	0.37 ± 0.16	6	11.52 ± 3.68	2
Glucagon C24 PEG 5K	0.22 ± 0.10	12	13.65 ± 2.95	4
Glucagon C29 PEG 5K	0.96 ± 0.07	2	12.71 ± 3.74	2
Glucagon C24 PEG 20K	0.08 ± 0.05	3	Not determined	
Glucagon C24 Dimer	0.10 ± 0.05	3	Not determined	
GLP-1	> 1000		0.05 ± 0.02	4

* - number of experiments

Table 4
cAMP Induction by E16 Glucagon Analogs

Percent Potency Relative to Native Ligand		
Peptide	**GRec**	**GLP-1Rec**
E16 Gluc-NH2	187.2	17.8
Glucagon	100.0	0.8
Gluc-NH2	43.2	4.0
NLeu3, E16 Gluc-NH2	7.6	20.6
E3, E16 Gluc-NH2	1.6	28.8
Orn3, E16 Gluc-NH2	0.5	0.1
GLP-1	<0.1	100

Table 5
cAMP Induction by E16 Glucagon Analogs

Percent Potency Relative to Native Ligand		
Peptide	GRec	GLP-1Rec
E16 Gluc-NH2	187.2	17.8
E15, E16 Gluc-NH2	147.0	9.2
E16, K20 Gluc-NH2	130.1	41.5
Gluc-NH2	43.2	4.0

Table 6
EC50 values for cAMP Induction by E16 Glucagon Analogs

	Glucagon Receptor			**GLP-1 Receptor**		
Peptide	**EC50 (nM)**	StDev	n	**EC50 (nM)**	StDev	n
Glucagon	**0.28**	0.14	10	**4.51**	N/A	1
Glucagon-NH2	**0.53**	0.33	8	**1.82**	0.96	5
E16 Gluc-NH2	**0.07**	0.07	10	**0.16**	0.14	10
E16, G30 Gluc-NH2	**0.41**	0.36	5	**0.24**	0.10	5
E16, G30 Gluc-Cex	**0.51**	0.46	5	**1.19**	0.86	5
GLP-1	**2214**	N/A	1	**0.03**	0.02	9

Table 7
EC50 values for cAMP Induction by E16 Glucagon Analogs

	Glucagon Receptor			GLP-1 Receptor		
Peptide	EC50 (nM)	StDev	n	EC50 (nM)	StDev	n
E16 Glucagon NH2	**0.07**	0.07	10	**0.16**	0.14	10
$hCSO_3$16 Glucagon-NH2	**0.25**	0.12	2	**0.19**	0.02	2
hE16 Glucagon-NH2	**0.17**	0.08	2	**0.25**	0.03	2
H16 Glucagon-NH2	**0.45**	0.3	2	**0.38**	0.11	2
Q16 Glucagon-NH2	**0.22**	0.1	2	**0.39**	0.08	2
D16 Glucagon-NH2	**0.56**	0.15	2	**0.93**	0.28	2
(S16) Glucagon-NH2	**0.53**	0.33	8	**1.82**	0.96	5

Table 8
EC50 values for cAMP Induction by E16 Glucagon Analogs

	Glucagon Receptor			GLP-1 Receptor		
Peptide	EC50 (nM)	StD	n	EC50 (nM)	StDev	n
E16 Glucagon NH2	**0.07**	0.07	10	**0.16**	0.14	10
T16 Glucagon NH2	**0.10**	0.02	3	**1.99**	0.48	3
G16 Glucagon NH2	**0.10**	0.01	3	**2.46**	0.60	3
Glucagon NH2	**0.53**	0.33	4	**1.82**	0.96	5
GLP-1	2214	N/A	1	**0.03**	0.02	9

E16 Gluc NH_2 was 4-fold more potent at the glucagon receptor relative to G16-COOH and T16 Gluc NH_2,when the compounds were tested side by side.

Table 9
cAMP Induction by E16/Lactam Glucagon Analogs

Percent Potency Relative to Native Ligand		
Peptide	GRec	GLP-1Rec
E24K28 Gluc-NH2 Lac	196.4	12.5
E16K20 Gluc-NH2 Lac	180.8	63.0
K12E16 Gluc-NH2 Lac	154.2	63.3
K20E24 Gluc-NH2 Lac	120.2	8.1
E16 Gluc-NH2	187.2	17.8
E16, K20 Gluc-NH2	130.1	41.5
Glucagon	100.0	0.8
Gluc-NH2	43.2	4.0

Table 10
cAMP Induction by GLP-1 17-26 Glucagon Analogs

	Glucagon Receptor		GLP-1 Receptor	
Peptide	EC50(nM)	StD	EC50(nM)	StD
GLP-1			0.023	0.002
Gluc-NH2	0.159	0.023		
E16 GLP-1			0.009	0.000
E16 Glucagon-NH2	0.072	0.007		
E16 GLP(17-26)Glu(27-29)-NH2	0.076	0.004	0.014	0.001
E16 GLP(17-29)-NH2	0.46	0.023	0.010	0.000
E16 GLP(17-29)-NH2 E24, K28	0.23	0.020	0.007	
E16 GLP(17-29)-NH2 E24, K28 Lactam	0.16	0.017	0.007	0.000

EXAMPLE 15

Stability Assay for glucagon Cys-maleimido PEG analogs

Each glucagon analog was dissolved in water or PBS and an initial HPLC analysis was conducted. After adjusting the pH (4, 5, 6, 7), the samples were incubated over a specified time period at 37°C and re-analyzed by HPLC to determine the integrity of the peptide. The concentration of the specific peptide of interest was determined and the percent remaining intact was calculated relative to the initial analysis. Results for Glucagon Cys^{21}-maleimidoPEG$_{5K}$ are shown in Figs. 1 and 2.

EXAMPLE 16

The following glucagon peptides are constructed generally as described above in Examples 1-11:

In all of the following sequences, "a" means a C-terminal amide.

HSQGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 70)
HSQGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 71)
HSQGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 72)
HSQGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 73)
HSQGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 74)
HSQGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 75)
HSQGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 76)
HSQGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 77)
HSQGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 78)
HSQGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 79)
HSQGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 80)
HSQGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 81)
HSQGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 82)
HSQGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 83)
HSQGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 84)
HSQGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 85)

HSQGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 86)

X1SQGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 87)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 88)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 89)
X1SQGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 90)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 91)
X1SQGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 92)
X1SQGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 93)
X1SQGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 94)
X1SQGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 95)
X1SQGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 96)
X1SQGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 97)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 98)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 99)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 100)
X1SQGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 101)
X1SQGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 102)
X1SQGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 103)
Wherein in the preceding sequences, X1 = (Des-amino)His

HX2QGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 104)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 105)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 106)
HX2QGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 107)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 108)
HX2QGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 109)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 110)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 111)
HX2QGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 112)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 113)
HX2QGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 114)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 115)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 116)

HX2QGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 117)
HX2QGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 118)
HX2QGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 119)
HX2QGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 120)
Wherein in the preceding sequences X2 = Aminoisobutyric acid

HX2QGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 121)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 122)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 123)
HX2QGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 124)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 125)
HX2QGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 126)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 127)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 128)
HX2QGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 129)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 130)
HX2QGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 131)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 132)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 133)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 134)
HX2QGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 135)
HX2QGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 136)
HX2QGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 137)
Wherein in the preceding sequences X2 = (D-Ala)

HSEGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 138)
HSEGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 139)
HSEGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 140)
HSEGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 141)
HSEGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 142)
HSEGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 143)

HSEGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 144)

HSEGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 145)

HSEGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 146)

HSEGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 147)

HSEGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 148)

HSEGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 149)

HSEGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 150)

HSEGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 151)

HSEGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 152)

HSEGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 153)

HSEGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 154)

X1SEGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 155)

X1SEGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 156)

X1SEGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 157)

X1SEGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 158)

X1SEGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 159)

X1SEGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 160)

X1SEGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 161)

X1SEGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 162)

X1SEGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 163)

X1SEGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 164)

X1SEGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 165)

X1SEGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 166)

X1SEGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 167)

X1SEGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 168)

X1SEGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 169)

X1SEGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 170)
X1SEGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 171)
Wherein in the preceding sequences X1 = (Des-amino)His

HX2EGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 172)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 173)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 174)
HX2EGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 175)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 176)
HX2EGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 177)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 178)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 179)
HX2EGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 180)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 181)
HX2EGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 182)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 183)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 184)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 185)
HX2EGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 186)
HX2EGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 187)
HX2EGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 188)
Wherein in the preceding sequences X2 = Aminoisobutyric acid

HX2EGT FTSDY SKYLD ERRAQ DFVQW LMNTa (SEQ ID NO: 189)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMNTa (SEQ ID NO: 190)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 191)
HX2EGT FTSDY SKYLD ERRAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 192)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 193)
HX2EGT FTSDY SKYLD KRRAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 194)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMNTa (SEQ ID NO: 195)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 196)
HX2EGT FTSDY SKYLD ERAAQ DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 197)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMNTa (lactam @ 12-16; SEQ ID NO: 198)
HX2EGT FTSDY SKYLD KRAAE DFVQW LMNTa (lactam @ 16-20; SEQ ID NO: 199)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMNTa (SEQ ID NO: 200)

HX2EGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 12-16; SEQ ID NO: 201)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMNTa (lactam @ 16-20; SEQ ID NO: 202)
HX2EGT FTSDY SKYLD EQAAK EFIAW LVKGa (SEQ ID NO: 203)
HX2EGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 12-16; SEQ ID NO: 204)
HX2EGT FTSDY SKYLD EQAAK EFIAW LVKGa (lactam @ 16-20; SEQ ID NO: 205)
Wherein in the preceding sequences X2 = (D-Ala)

HSQGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 206)
HSQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 207)
HSQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 208)
HSQGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 209)
HSQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 210)
HSQGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 211)
HSQGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 212)
HSQGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 213)
HSQGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 214)
HSQGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 215)
HSQGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 216)
HSQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 217)
HSQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 218)
HSQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 219)
HSQGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 220)
HSQGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 221)
HSQGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 222)

X1SQGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 223)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 224)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 225)
X1SQGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 226)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 227)
X1SQGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 228)
X1SQGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 229)
X1SQGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 230)
X1SQGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 231)
X1SQGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 232)
X1SQGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 233)

X1SQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 234)
X1SQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 235)
X1SQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 236)
X1SQGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 237)
X1SQGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 238)
X1SQGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 239)
Wherein in the preceding sequences X1 = (Des-amino)His; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2QGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 240)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 241)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 242)
HX2QGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 243)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 244)
HX2QGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 245)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 246)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 247)
HX2QGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 248)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 249)
HX2QGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 250)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 251)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 252)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 253)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 254)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 255)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 256)
Wherein in the preceding sequences X2 = Aminoisobutyric acid; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2QGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 257)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 258)

HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 259)
HX2QGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 260)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 261)
HX2QGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 262)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 263)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 264)
HX2QGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 265)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 266)
HX2QGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 267)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 268)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 269)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 270)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 271)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 272)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 273)

Wherein in the preceding sequences X2 = (D-Ala); and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSEGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 274)
HSEGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 275)
HSEGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 276)
HSEGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 277)
HSEGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 278)
HSEGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 279)
HSEGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 280)
HSEGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 281)
HSEGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 282)
HSEGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 283)

HSEGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 284)
HSEGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 285)
HSEGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 286)
HSEGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 287)
HSEGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 288)
HSEGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 289)
HSEGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 290)

X1SEGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 291)
X1SEGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 292)
X1SEGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 293)
X1SEGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 294)
X1SEGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 295)
X1SEGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 296)
X1SEGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 297)
X1SEGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 298)
X1SEGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 299)
X1SEGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 300)
X1SEGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 301)
X1SEGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 302)
X1SEGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 303)
X1SEGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 304)
X1SEGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 305)
X1SEGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 306)
X1SEGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 307)

Wherein in the preceding sequences X1 = (Des-amino)His; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2EGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 308)
HX2EGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 309)
HX2EGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 310)
HX2EGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 311)
HX2EGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 312)

HX2EGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 313)
HX2EGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 314)
HX2EGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 315)
HX2EGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 316)
HX2EGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 317)
HX2EGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 318)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 319)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 320)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 321)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 322)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 323)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 324)
Wherein in the preceding sequences X2 = Aminoisobutyric acid; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2EGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (SEQ ID NO: 325)
HX2EGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 326)
HX2EGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 327)
HX2EGT FTSDY SKYLD ERRAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 328)
HX2EGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 329)
HX2EGT FTSDY SKYLD KRRAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 330)
HX2EGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 331)
HX2EGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 332)
HX2EGT FTSDY SKYLD ERAAQ DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 333)
HX2EGT FTSDY SKYLD ERAAK DFVC*W LMNTa (lactam @ 12-16; SEQ ID NO: 334)
HX2EGT FTSDY SKYLD KRAAE DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 335)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 336)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 12-16; SEQ ID NO: 337)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 338)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LVKGa (SEQ ID NO: 339)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 12-16; SEQ ID NO: 340)
HX2EGT FTSDY SKYLD EQAAK EFIC*W LVKGa (lactam @ 16-20; SEQ ID NO: 341)

Wherein in the preceding sequences X2 = (D-Ala); and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSQGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 342)
HSQGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 343)
HSQGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 344)
HSQGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 345)
X1SQGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 346)
X1SQGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 347)
X1SQGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 348)
X1SQGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 349)
Wherein X1 = (Des-amino)His; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2QGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 350)
HX2QGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 351)
HX2QGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 352)
HX2QGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 353)
Wherein X2 = Aminoisobutyric acid; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2QGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 354)
HX2QGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 355)
HX2QGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 356)
HX2QGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 357)
Wherein X2 = (D-Ala); and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol

of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSEGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 358)
HSEGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 359)
HSEGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 360)
HSEGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 361)
X1SEGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 362)
X1SEGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 363)
X1SEGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 364)
X1SEGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 365)
Wherein X1 = (Des-amino)His; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2EGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 366)
HX2EGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 367)
HX2EGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 368)
HX2EGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 369)
Wherein X2 = (D-Ala) ; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2EGT FTSDY SKYLD C*RRAK DFVQW LMNTa (SEQ ID NO: 370)
HX2EGT FTSDY SKYLD C*RAAK DFVQW LMNTa (SEQ ID NO: 371)
HX2EGT FTSDY SKYLD C*QAAK EFIAW LMNTa (SEQ ID NO: 372)
HX2EGT FTSDY SKYLD C*QAAK EFIAW LVKGa (SEQ ID NO: 373)
Wherein X2 = (D-Ala) ; and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSQGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 374)
HSQGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 375)
HSQGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 376)
HSQGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 377)
HSQGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 378)
HSQGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 379)
HSQGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 380)
HSQGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 381)
HSQGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 382)
HSQGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 383)
HSQGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 384)
HSQGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 385)
HSQGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 386)
HSQGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 387)

X1SQGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 388)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 389)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 390)
X1SQGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 391)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 392)
X1SQGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 393)
X1SQGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 394)
X1SQGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 395)
X1SQGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 396)
X1SQGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 397)
X1SQGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 398)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 399)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 400)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 401)
Wherein in the preceding sequences X1 = (Des-amino)His

HX2QGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 402)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 403)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 404)
HX2QGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 405)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 406)

HX2QGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 407)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 408)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 409)
HX2QGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 410)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 411)
HX2QGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 412)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 413)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 414)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 415)
Wherein in the preceding sequences X2 = Aminoisobutyric acid

HX2QGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 416)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 417)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 418)
HX2QGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 419)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 420)
HX2QGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 421)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 422)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 423)
HX2QGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 424)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 425)
HX2QGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 426)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 427)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 428)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 429)
Wherein in the preceding sequences X2 = (D-Ala)

HSEGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 430)
HSEGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 431)
HSEGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 432)
HSEGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 433)
HSEGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 434)
HSEGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 435)
HSEGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 436)
HSEGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 437)
HSEGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 438)

HSEGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 439)
HSEGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 440)
HSEGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 441)
HSEGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 442)
HSEGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 443)

X1SEGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 444)
X1SEGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 445)
X1SEGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 446)
X1SEGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 447)
X1SEGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 448)
X1SEGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 449)
X1SEGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 450)
X1SEGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 451)
X1SEGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 452)
X1SEGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 453)
X1SEGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 454)
X1SEGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 455)
X1SEGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 456)
X1SEGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 457)
Wherein in the preceding sequences X1 = (Des-amino)His

HX2EGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 458)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 459)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 460)
HX2EGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 461)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 462)
HX2EGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 463)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 464)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 465)
HX2EGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 466)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 467)
HX2EGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 468)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 469)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 470)

HX2EGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 471)
Wherein in the preceding sequences X2 = Aminoisobutyric acid

HX2EGT FTSDY SKYLD ERRAQ DFVQW LMDTa (SEQ ID NO: 472)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMDTa (SEQ ID NO: 473)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 474)
HX2EGT FTSDY SKYLD ERRAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 475)
HX2EGT FTSDY SKYLD ERRAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 476)
HX2EGT FTSDY SKYLD KRRAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 477)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMDTa (SEQ ID NO: 478)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 479)
HX2EGT FTSDY SKYLD ERAAQ DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 480)
HX2EGT FTSDY SKYLD ERAAK DFVQW LMDTa (lactam @ 12-16; SEQ ID NO: 481)
HX2EGT FTSDY SKYLD KRAAE DFVQW LMDTa (lactam @ 16-20; SEQ ID NO: 482)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMDTa (SEQ ID NO: 483)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 12-16; SEQ ID NO: 484)
HX2EGT FTSDY SKYLD EQAAK EFIAW LMDTa (lactam @ 16-20; SEQ ID NO: 485)
Wherein in the preceding sequences X2 = (D-Ala)

The following glucagon peptides with a GLP-1/glucagon activity ratio of about 5 or more are also constructed generally as described above in Examples 1-11. Generally, in these peptides, AIB at position 2 provides DPP IV resistance but also significantly reduces glucagon activity.

HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 486)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNC*a (SEQ ID NO: 487)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNGG PSSGA PPPSC*a (SEQ ID NO: 488)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNGG PSSGA PPPSC*a (lactam @ 16-20; SEQ ID NO: 489)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNGG PSSGA PPPSa (SEQ ID NO: 490)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNGG PSSGA PPPSa (lactam @ 16-20; SEQ ID NO: 491)
Wherein in the preceding sequences X2=AIB, and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a

polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNTa (SEQ ID NO: 492)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNC*a (SEQ ID NO: 493)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNGG PSSGA PPPSC*a (SEQ ID NO: 494)
HX2QGT FTSDY SKYLD ERAAK DFVQW LMNGG PSSGA PPPSC*a (lactam @ 16-20; SEQ ID NO: 495)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNGG PSSGA PPPSa (SEQ ID NO: 496)
HX2QGT FTSDY SKYLD ERAAK DFVC*W LMNGG PSSGA PPPSa (lactam @ 16-20; SEQ ID NO: 497)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 498)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNC*a (SEQ ID NO: 499)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNGG PSSGA PPPSC*a (SEQ ID NO: 500)
HX2QGT FTSDY SKYLD ERRAK DFVQW LMNGG PSSGA PPPSC*a (lactam @ 16-20; SEQ ID NO: 501)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNGG PSSGA PPPSa (SEQ ID NO: 502)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNGG PSSGA PPPSa (lactam @ 16-20; SEQ ID NO: 503)
Wherein in the preceding sequences X2=AIB, and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

The following glucagon peptides which are GLP-1/glucagon co-agonists are also constructed generally as described above in Examples 1-11. Formation of a lactam bridge between amino acids 16 and 20 restores the reduction in glucagon activity caused by the substitution at position 2.

HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 504)
Wherein in the preceding sequence X2=AIB, and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

X1SQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (lactam @ 16-20; SEQ ID NO: 505)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMNC*a (lactam @ 16-20; SEQ ID NO: 506)
X1SQGT FTSDY SKYLD EQAAK EFIAW LMNGG PSSGA PPPSC*a (lactam @ 16-20; SEQ ID NO: 507)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMNGG PSSGA PPPSC*a (lactam @ 16-20; SEQ ID NO: 508)
X1SQGT FTSDY SKYLD EQAAK EFIC*W LMNGG PSSGA PPPSa (lactam @ 16-20; SEQ ID NO: 509)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 510)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 511)
X1SQGT FTSDY SKYLD ERRAK DFVQW LMNC*a (lactam @ 16-20; SEQ ID NO: 512)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNGG PSSGA PPPSa (lactam @ 16-20; SEQ ID NO: 513)
Wherein in the preceding sequences X1=DMIA (alpha, alpha-dimethyl imidiazole acetic acid), and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSQGT FTSDY SKYLD EQAAK EFIC*W LMNTa (optionally with lactam @ 16-20; SEQ ID NO: 514)
Wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 517)
HX2QGT FTSDY SKYLD ERRAK DFVC*W LMNTa (lactam @ 16-20; SEQ ID NO: 528)
HX2QGT FTSDY SKYLD ERRAK EFIC*W LMNGG PSSGA PPPSC*a (SEQ ID NO: 531)
HX2QGT FTSDY SKYLD EQAAK EFIAW LMNGG PSSGA PPPSC*C*a (SEQ ID NO: 532)
HX2QGT FTSDY SKYLD EQAAK EFIC*W LMNGG PSSGA PPPSa (SEQ ID NO: 533)
Wherein in the preceding sequence X2=AIB, and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a

polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSQGT FTSDYSKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 518)
X1SQGT FTSDYSKYLD EQAAK EFIC*W LMNTa (SEQ ID NO: 519)
X1SQGT FTSDYSKYLD EQAAK EFIAW LMNC*a (SEQ ID NO: 520)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNGG PSSGA PPPSa (SEQ ID NO: 529)
X1SQGT FTSDY SKYLD ERRAK DFVC*W LMNTa (SEQ ID NO: 530)

Wherein in the preceding sequences X1=DMIA (alpha, alpha-dimethyl imidiazole acetic acid), and wherein the C* is a Cys, or a Cys attached to a hydrophilic polymer, or alternatively the C* is a Cys attached to a polyethylene glycol of about 20 kD average weight, or alternatively the C* is a Cys attached to a polyethylene glycol of about 40 kD average weight.

HSQGT FTSDYSKYLD SRRAQ DFVQW LMNTGPSSGAPPPSa (SEQ ID NO: 521)
HSQGT FTSDYSKYLD SRRAQ DFVQW LMNGGPSSGAPPPSa (SEQ ID NO: 522)
HSQGT FTSDYSKYLD SRRAQ DFVQW LMKGGPSSGAPPPSa (SEQ ID NO: 523)
HSQGT FTSDYSKYLD SRRAQ DFVQW LVKGGPSSGAPPPSa (SEQ ID NO: 524)
HSQGT FTSDYSKYLD SRRAQ DFVQW LMDGGPSSGAPPPSa (SEQ ID NO: 525)
HSQGT FTSDYSKYLD ERRAK DFVQW LMDGGPSSGAPPPSa (SEQ ID NO: 526)
HAEGT FTSDV SSYLE GQAAK EFIAW LVKGGa (SEQ ID NO: 527)

X1X2QGT FTSDY SKYLD ERX5AK DFVX3W LMNX4 (SEQ ID NO: 61)
wherein
X1=His, D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-histidine or alpha, alpha-dimethyl imidiazole acetic acid (DMIA) N-methyl histidine, alpha-methyl histidine, or imidazole acetic acid,
X2=Ser, D-serine, Ala, Val, glycine, N-methyl serine or aminoisobutyric acid (AIB), N-methyl alanine and D-alanine.
X3=Ala, Gln or Cys-PEG
X4=Thr-CONH2 or Cys-PEG or GGPSSGAPPPS (SEQ ID NO: 515) or GGPSSGAPPPSC-PEG (SEQ ID NO: 516)
Provided that when X3 is Cys-PEG, X4 is not Cys-PEG or GGPSSGAPPPSC-PEG (SEQ ID NO: 516), and when X2=Ser, X1 is not His.

X5=Ala or Arg

X1X2QGT FTSDY SKYLD EQ X5AK EFI X3W LMNX4 (SEQ ID NO: 62)
wherein
X1=His, D-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine, homo-histidine or alpha, alpha-dimethyl imidiazole acetic acid (DMIA), N-methyl histidine, alpha-methyl histidine, or imidazole acetic acid
X2=Ser, D-serine, Ala, Val, glycine, N-methyl serine or aminoisobutyric acid (AIB), N-methyl alanine and D-alanine.
X3=Ala, Gln or Cys-PEG
X4=Thr-CONH2 or Cys-PEG or GGPSSGAPPPS (SEQ ID NO: 515) or GGPSSGAPPPSC-PEG (SEQ ID NO: 516)
Provided that when X3 is Cys-PEG, X4 is not Cys-PEG or GGPSSGAPPPSC-PEG (SEQ ID NO: 516), and when X2=Ser, X1 is not His.
X5=Ala or Arg

Any of the preceding sequences can include additional modifications, e.g., 1, 2, 3, 4 or 5 modifications that do not destroy activity, including but not limited to W10 or R20 substitutions that can be used to enhance potency. Any of the preceding sequences can also be produced without the modifications that confer DPP IV resistance, i.e. in which the native His is at position 1 and the native Ser is at position 2. In addition, any of the preceding compounds may optionally be linked to a conjugate, such as a heterologous polypeptide, an immunoglobulin or a portion thereof (e.g. Fc region), a targeting agent, a diagnostic label, or a diagnostic or therapeutic agent.

EXAMPLE 17

The following glucagon peptides modified to comprise the c-terminal extension of SEQ ID NO: 26 linked to the carboxy terminus of the glucagon peptide were constructed generally as described above in Examples 1-11 and assayed for activity at the GLP-1 and glucagon receptors using the *in vitro* assay described in Example 14.

Table 11 represents the activity of various glucagon analogs at the glucagon and GLP-1 receptors. The data shows that for glucagon analogs comprising the c-terminal extension of SEQ ID NO: 26, amino acid substitutions at positions 16, 20, 28 and 29 can impact the analogs activity at the GLP-1 receptor.

Table 11

Glucagon-Cex
Structure Activity Relationship

Glucagon Peptide	Glucagon Receptor		GLP-1 Receptor	
	EC50 (nM)	Relative Potency (%)	EC50 (nM)	Relative Potency (%)
-MNT^{29} (SEQ ID NO: 1)	0.086	100		
-MNTG^{30} PSSGAPPPS (SEQ ID NO: 521)	0.14	61	1.19	2
-MNGG^{30} PSSGAPPPS (SEQ ID NO: 522)	0.28	30	0.31	8
-MKGG^{30} PSSGAPPPS (SEQ ID NO: 523)	0.61	14	0.80	3
-VKGG^{30} PSSGAPPPS (SEQ ID NO: 524)	1.16	7	0.21	12
-MDGG^{30} PSSGAPPPS (SEQ ID NO: 525)	0.12	72	0.13	19
E^{16}K^{20}-MDGG^{30} PSSGAPPPS (SEQ ID NO: 526)	0.22	39	0.020	125
GLP-1-VKGG^{30} (SEQ ID NO: 527)			0.025	100

EXAMPLE 18

Table 12 represents *in vitro* data accumulated for various glucagon peptides comparing their relative activities at the glucagon and GLP-1 receptors.

Table 12: COMPARISON OF AGONISTS AND CO-AGONISTS w/ and w/o PEG

	% Potency Relative to Native	
CONTROLS	**GR**	**GL-1R**
Glucagon	100	0.78
GLP-1	<0.01	100

	Parent w/o PEG		Parent w/PEG	
	% Potency Relative to Native		% Potency Relative to Native	
AGONISTS	GR	GLP-1R	GR	GLP-1R
Chimera AIB2, Cys24 (SEQ ID NO: 486)	15.4	160.6	2.6	82.5
Chimera AIB2, Cys29 (SEQ ID NO: 487)	20.1	124.6	5.6	54.3
Chimera AIB2, Gly29,30 Cys40 Cex (SEQ ID NO: 488)	2.2	359.1	0.3	68.8
Chimera AIB2, Gly29,30 Cys40 Cex Lactam (SEQ ID NO: 489)	14.2	169.6	3.2	63.6
Chimera AIB2, Gly29,30 Cys24 Cex (SEQ ID NO: 490)	2.5	457.8	0.2	95.4
Chimera AIB2, Gly29,30 Cys24 Cex Lactam (SEQ ID NO: 491)	25.2	381.5	1.4	96.4
E16, K20AIB2, A18 Cys24 (SEQ ID NO: 492)	--	--	1.1	73.5
E16, K20AIB2, A18 Gly29,30 Cys24 Cex (SEQ ID NO: 496)	--	--	0.1	88.5
CO-AGONISTS	GR	GLP-1R	GR	GLP-1R
Chimera DMIA1, Cys24 Lactam (SEQ ID NO: 505)	160.7	82.5	19.1	12.5
Chimera AIB2, Cys24 Lactam (SEQ ID NO: 504)	114.2	230.4	9.2	38.0
Chimera DMIA1, Cys29 Lactam (SEQ ID NO: 506)	--	--	--	--
Chimera DMIA1, Gly29,30 Cys40 Cex Lactam (SEQ ID NO: 507)	--	--	--	--
E16, K20 DMIA1, Gly29,30 Cys40 Cex Lactam (SEQ ID NO: 508)	--	--	--	--
Chimera DMIA1, Gly29,30 Cys24 Cex Lactam (SEQ ID NO: 509)	--	--	--	--
E16, K20 DMIA1, Cys24 Lactam (SEQ ID NO: 510)	--	--	64.1	9.3
E16, K20 AIB2, Cys24 Lactam (SEQ ID NO: 517)	108.3	96.9	15.8	31.0
Chimera Cys24 (SEQ ID NO: 518)	--	--	19.8	29.3
E16, K20 DMIA1, Gly29,30 Cys24 Cex Lactam (SEQ ID NO: 513)	116.0	78.3	12.6	11.3
Chimera DMIA1, Cys29 (SEQ ID NO: 520)	--	--	5.3	27.3
Chimera DMIA1, Cys24 (SEQ ID NO: 519)	28.9	64.5	6.9	19.3

Claims:

1. A non-native glucagon peptide comprising the sequence of SEQ ID NO: 55 or an analog of SEQ ID NO: 55, wherein said analog is not a naturally occurring peptide and said analog differs from SEQ ID NO: 55 by 1 to 3 amino acid modifications, selected from positions 1, 2, 3, 5, 7, 10, 11, 13, 14, 17, 18, 19, 21, 24, 27, 28, and 29, wherein said glucagon peptide exhibits at least 20% of the activity of native GLP-1 at the GLP-1 receptor.

2. The glucagon peptide of claim 1 wherein said glucagon peptide comprises SEQ ID No: 55.

3. The glucagon peptide of claim 1 wherein said glucagon peptide comprises SEQ ID NO: 56.

4. The glucagon peptide of any of claims 1, 2 or 3 wherein said glucagon peptide further comprises a peptide selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 29 and SEQ ID NO: 65 linked to the carboxy terminus of said glucagon peptide.

5. The glucagon peptide of claim 2 or 3 wherein said glucagon peptide further comprises SEQ ID NO: 26 linked to the carboxy terminus of said glucagon peptide.

6. The glucagon peptide of any of claims 1, 2 or 3 which comprises a lactam bridge between two amino acids selected from the group consisting of amino acids at positions 16 and 20, amino acids at positions 12 and 16, amino acids at positions 20 and 24, and amino acids at positions 24 and 28.

7. The glucagon peptide of claim 6 wherein the lactam bridge is between amino acids at positions 16 and 20.

8. The glucagon peptide of claim 1 wherein said glucagon peptide comprises SEQ ID No: 33.

9. The glucagon peptide of claim 1 wherein wherein the amino acid at position 28 is Asp, Asn or Lys and the amino acid at position 29 is Gly or Thr.

10. The glucagon peptide of claim 1 wherein said glucagon peptide comprises an analogof SEQ ID No: 55 that differs from SEQ ID NO: 55 by 1 to 2 amino acids.

11. The glucagon peptide of claim 1 wherein said glucagon peptide comprises an analog of SEQ ID No: 55 that differs from SEQ ID NO: 55 by 1 amino acid.

12. The glucagon peptide of claim 11 wherein said glucagon peptide comprises the sequence of SEQ ID NO: 53.

13. The glucagon peptide of claim 12 wherein the amino acid at position 3 is glutamine.

14. The glucagon peptide of claim 1 wherein the amino acid at position 3 is glutamic acid.

15. The glucagon peptide of any of claims 1-13 that exhibits at least 10% of the activity of native glucagon at the glucagon receptor and at least 50% of the activity of native GLP-1 at the GLP-1 receptor.

16. The glucagon peptide of any of claims 1-13 that exhibits at least 40% of the activity of native glucagon at the glucagon receptor and at least 40% of the activity of native GLP-1 at the GLP-1 receptor.

17. The glucagon peptide of any of claims 1-13 that exhibits at least 60% of the activity of native glucagon at the glucagon receptor and at least 60% of the activity of native GLP-1 at the GLP-1 receptor.

18. The glucagon peptide of any of claims 1-12 and 14 that exhibits 0.1-10% of the activity of native glucagon at the glucagon receptor and at least 20% of the activity of native GLP-1 at the GLP-1 receptor.

19. The glucagon peptide of any of claims 1-12 and 14 that exhibits 1-10% of the activity of native glucagon at the glucagon receptor and at least 20% of the activity of native GLP-1 at the GLP-1 receptor.

20. The glucagon peptide of any of claims 1-19 wherein the amino acid at position 16 is glutamic acid, the amino acid at position 20 is lysine, and the C-terminal carboxylic acid group is replaced with an amide, optionally with a lactam bridge between the glutamic acid at position 16 and the lysine at position 20.

21. The glucagon peptide of claim 20 wherein a polyethylene glycol chain is covalently linked to the side chain of an amino acid at position 17, 21 or 24 or at the C-terminal amino acid.

22. The glucagon peptide of claim 20 wherein the amino acid at position 28 or 29 comprises an acidic amino acid.

23. The glucagon peptide of any of claims 1, 5, 8, 20, 21, or 22 wherein the amino acid at position 1 or 2 is modified to exhibit reduced susceptibility to cleavage by dipeptidyl peptidase IV.

24. The glucagon peptide of claim 1 wherein the peptide comprises the sequence of X_1X_2QGT FTSDY SKYLD ERX_5AK DFVX_3W LMNX_4 (SEQ ID NO: 61) or X_1X_2QGT FTSDY SKYLD EQ X_5AK EFI X_3W LMNX_4 (SEQ ID NO: 62), with the proviso that when X_3 is Cys-PEG, X_4 is not Cys-PEG or GGPSSGAPPPSC-PEG, and when X_2 is serine, X_1 is not histidine.

25. The glucagon peptide of claim 24 wherein X_3 is Cys-PEG.

26. The glucagon peptide of claim 25 wherein X_4 is GGPSSGAPPPS.

27. The glucagon peptide of claim 24 wherein X_4 is GGPSSGAPPPSC-PEG.

28. The glucagon peptide of any of claims 24-27 which further comprises a lactam bridge between amino acids at positions 16 and 20.

29. The glucagon peptide of claim 1 wherein the peptide comprises the sequence of NH_2-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu- Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu- Xaa-Asn-Thr-COOH (SEQ ID NO: 9) wherein Xaa is selected from the group consisting of Met, Leu and Nle.

30. The glucagon peptide of claim 1 wherein the peptide comprises a sequence selected from the group consisting of SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44.

31. The glucagon peptide of claim 1 wherein the peptide comprises the sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15.

32. The glucagon peptide of claim 23 wherein amino acid at position 2 is selected from the group consisting of d-serine, alanine, D-alanine, valine, glycine, N-methyl serine, N-methyl alanine, and amino isobutyric acid.

33. The glucagon peptide of claim 23 wherein amino acid at position 1 is selected from the group consisting of d-histidine, desaminohistidine, hydroxyl-histidine, acetyl-histidine homo-histidine, N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, and alpha, alpha-dimethyl imidiazole acetic acid (DMIA).

34. The glucagon peptide of any of claims 1-23 further comprising a hydrophilic moiety covalently bound at position 17, 21 or 24 or at the C-terminal amino acid of the glucagon peptide.

35. The glucagon peptide of claim 34 wherein said hydrophilic moiety is a polyethylene glycol chain.

36. The glucagon peptide of claim 1 or 31 wherein the glucagon peptide further comprises a sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 65.

37. The glucagon peptide of claim 36 wherein the glucagon peptide comprises the sequence of SEQ ID NO: 33 wherein the amino acid at position 28 is aspartic acid, the amino acid at position 29 is glycine and the amino acid sequence of SEQ ID NO: 29 is covalently linked to the carboxy terminus of SEQ ID NO: 33.

38. The glucagon peptide of claim 37 wherein the peptide comprises the sequence of SEQ ID NO: 64.

39. The glucagon peptide of claim 1 wherein the glucagon peptide comprises SEQ ID NO: 20 wherein the amino acid at position 20 is selected from the group consisting of arginine, ornithine and citrulline.

40. The glucagon peptide of claim 1 wherein the peptide comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11,

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu- Met-Xaa-Xaa-R (SEQ ID NO: 66),

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Lys-Asp-Phe-Val-Gln-Trp-Leu- Met-Xaa-Xaa-R (SEQ ID NO: 67),

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Lys-Asp-Phe-Val-Glu-Trp-Leu- Met-Xaa-Xaa-R (SEQ ID NO: 68),

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Glu-Trp-Leu- Met-Lys-Thr-R (SEQ ID NO: 69),

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Lys-Asp-Phe-Val-Glu-Trp-Leu- Met-Asn-Thr-R (SEQ ID NO: 16),

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Gln-Asp-Phe-Val-Glu-Trp-Leu- Met-Lys-Thr-R (SEQ ID NO: 17), and

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Glu-Arg-Arg-Ala-Lys-Asp-Phe-Val-Glu-Trp-Leu- Met-Lys-Thr-R (SEQ ID NO: 18),

and SEQ ID NO: 55,

wherein the Xaa at position 28 of the peptide is asparagine or aspartic acid;

the Xaa at position 29 of the peptide is threonine or glycine; and

R is SEQ ID NO: 26, SEQ ID NO: 29, COOH or $CONH_2$.

41. The glucagon peptide of claim 40 wherein R is $CONH_2$.

42. The glucagon peptide of claim 40 wherein the peptide comprises the sequence of SEQ ID NO: 10.

43. The glucagon peptide of claim 40 wherein the peptide comprises the sequence of SEQ ID NO: 11.

44. The glucagon peptide of claim 41 wherein the peptide further comprises a polyethylene glycol chain covalently bound to an amino acid residue at position 16, 17, 21 or 24 or at the C-terminal amino acid, and pharmaceutically acceptable salts of said glucagon peptide, with the proviso that when the peptide comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17, 21 or 24, when the peptide comprises SEQ ID NO: 14 or SEQ ID NO: 15 the polyethylene glycol chain is covalently bound to an amino acid residue at position 16, 17 or 21, when the peptide comprises SEQ ID NO: 55, the polyethylene glycol chain is covalently bound to an amino acid position at position 21 or 24 and when the peptide comprises SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17 or 21.

45. The glucagon peptide of claim 44 wherein the polyethylene glycol chain has a molecular weight selected from the range of about 1,000 to about 5,000 Daltons.

46. The glucagon peptide of claim 44 wherein the polyethylene glycol chain has a molecular weight of about 40,000 Daltons.

47. The glucagon peptide of claim 39 wherein the peptide further comprises an additional amino acid added to the carboxy terminus of the peptide, said additional amino acid comprising an amide group in place of the terminal carboxylic acid group of the native amino acid.

48. The glucagon peptide of claim 40 further comprising an extension peptide bound to amino acid 29 of said glucagon peptide, said amino acid sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28.

49. The glucagon peptide of claim 48 wherein the amino acid at position 28 is aspartic acid, the amino acid at position 29 is glycine and the amino acid sequence of SEQ ID NO: 29 is covalently linked to amino acid 29 of said glucagon peptide.

50. A glucagon peptide comprising the sequence of

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Xaa-Xaa-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu- Xaa-Asn-Thr-R (SEQ ID NO: 2);

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Xaa-Arg-Arg-Ala-Gln-Xaa-Phe-Val-Gln-Trp-Leu- Xaa-Asn-Thr-R (SEQ ID NO: 3);

NH_2-His-Ser-Gln-Gly-Thr-Phe- Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Xaa-Arg-Arg-Ala-Gln-Asp-Phe-Val-Xaa-Trp-Leu-Xaa-Asn-Thr-R (SEQ ID NO: 4); and glucagon agonist analogs of SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO. 4, wherein R is COOH or $CONH_2$, and the side chain of an amino acid residue at

position 17, 21 or 24 or at the C-terminal amino acid of said glucagon peptide further comprises a hydrophilic moiety covalently bound to the amino acid residue, and pharmaceutically acceptable salts of said glucagon peptide.

51. The glucagon peptide of claim 50, wherein R is $CONH_2$ and said hydrophilic moiety is a polyethylene glycol chain.

52. The glucagon peptide of claim 51 wherein the peptide comprises a sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25.

53. The glucagon peptide of claim 52 wherein the polyethylene glycol chain has a molecular weight selected from the range of about 1,000 to about 5,000 Daltons.

54. The glucagon peptide of claim 52 wherein the polyethylene glycol chain has a molecular weight of at least about 20,000 Daltons.

55. A homo dimer comprising two glucagon peptides of claim 37 bound to one another through a linker.

56. A pharmaceutical composition comprising the glucagon agonist of claim 1, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

57. A kit for administering a glucagon agonist to a patient in need thereof, said kit comprising

a pharmaceutical composition of claim 56; and

a device for administering said composition to a patient.

58. A method of treating hypoglycemia using a pre-formulated aqueous composition, said method comprising the steps of administering an effective amount of a pharmaceutical composition of claim 56.

59. A method of treating diabetes, said method comprising administering an effective amount of a pharmaceutical composition of claim 56.

60. A method of causing temporary paralysis of the intestinal tract, said method comprising administering an effective amount of a pharmaceutical composition of claim 56.

61. A method of reducing weight gain or inducing weight loss, said method comprising administering an effective amount of a composition comprising a glucagon agonist comprising a glucagon peptide selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 55, wherein amino acid 29 of the glucagon peptide is bound to a second peptide through a peptide bond, and said second peptide comprises sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 65.

62. A method of reducing weight gain or inducing weight loss, said method comprising administering an effective amount of a composition comprising a glucagon peptide of claim 36.

63. The method of claim 61 wherein the glucagon agonist further comprise a polyethylene glycol chain bound to the glucagon peptide at position 17, 21 or 24 and said polyethylene chain has a molecular weight selected from the range of about 5,000 to about 40,000 Daltons, with the proviso that when the peptide comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13 the polyethylene glycol chain is covalently bound to an amino acid residue at position 16, 17, 21 or 24, when the peptide comprises SEQ ID NO: 14 or SEQ ID NO: 15 the polyethylene glycol chain is covalently bound to an amino acid residue at position 16, 17 or 21, when the peptide comprises SEQ ID NO: 55, the polyethylene glycol chain is covalently bound to an amino acid position at position 21 or 24, and when the peptide comprises SEQ

ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17 or 21.

64. The method of claim 58 wherein the glucagon agonist further comprise a polyethylene glycol chain bound to the glucagon peptide at position 16, 17, 21 or 24 and said polyethylene chain has a molecular weight selected from the range of about 5,000 to about 40,000 Daltons, with the proviso that when the peptide comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17, 21 or 24, when the peptide comprises SEQ ID NO: 14 or SEQ ID NO: 15 the polyethylene glycol chain is covalently bound to an amino acid residue at position 16, 17 or 21, and when the peptide comprises SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18 the polyethylene glycol chain is covalently bound to an amino acid residue at position 17 or 21.

65. A glucagon peptide comprising a sequence selected from and of the sequences designated by SEQ ID NO: 70 through SEQ ID NO: 514.