

patients (less than 10%) receiving Roux-en-Y gastric bypass (RYGB) surgeries subsequently develop hyperinsulinemic hypoglycemia, wherein blood glucose concentrations can become low enough (20-40 mg/dL) to cause seizures, altered mental status, loss of consciousness, cognitive dysfunction, disability, and death.

5 One approach to treating hyperinsulinemic-induced hypoglycemia is to administer a GLP-1 receptor antagonist. Such peptide antagonists block the ability of inappropriately elevated concentrations of plasma GLP-1 to stimulate insulin secretion, and function to normalize plasma glucose and reduce risk of cognitive impairment, vascular disease and potentially sudden death in patients experiencing
10 atypical hypoglycemia.

Exendin-4 (SEQ ID NO: 1) is a 39 amino acid agonist of the glucagon-like peptide 1 (GLP-1) receptor. Exendin-4 is present in the saliva of the Gila monster, *Heloderma suspectum*. Ex-4 (9-39)a (SEQ ID NO: 2) is an N-terminal truncated derivative of Exendin-4 that is known to function as a GLP-1 receptor antagonist.

15 However, Ex-4 (9-39)a suffers from two notable limitations regarding its potential use to treat chronic atypical hypoglycemia: its nonhuman amino acid sequence, and its relatively short *in vivo* duration of action.

 In accordance with one embodiment of the present disclosure a set of novel optimized GLP-1 antagonists are provided for use as drug candidates in treatment of
20 atypical hypoglycemia, including hyperinsulinemic induced hypoglycemia resulting after post-bariatric surgical procedures or resulting from congenital mutations.

SUMMARY

 As disclosed herein compositions and methods are provided for treating patients
25 experiencing atypical hypoglycemia, and more particularly in one [embodimentexample](#), treating patients who experience hyperinsulinemia induced hypoglycemia. In accordance with one [embodimentexample](#) compositions and methods are provided for treating hyperinsulinemia induced hypoglycemia resulting after post-bariatric surgery. In one [embodimentexample](#) the method comprises the administration of a glucagon-like
30 peptide-1 receptor antagonist (GLP1RA) in an amount effective to elevate blood glucose levels and alleviate associated acute symptoms and chronic outcomes associated with hypoglycemia.

In one aspect, the present disclosure refers to a GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of R₁₀-
DVX₁₁X₁₂YLY₁₅X₁₆QAX₁₉X₂₀EFX₂₃X₂₄WLVRRGGPSSGAPPPSX₄₀-R₂₀ SEQ ID NO: 97),
wherein

R₁₀ is NH₂ or an N-terminal extension of 1 or 2 amino acids, wherein one of the amino acids of the N-terminal extension is acylated with a C14-C20 fatty acid or diacid, optionally via a spacer, optionally wherein R₁₀ is a dipeptide of the structure: X₇X₈, wherein X₇ is an acylated amino acid, optionally acylated Lys or acylated dLys, and X₈ is Gly or a C₁-C₄ N-alkylated Gly;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Ser or an acylated amino acid, optionally acylated Lys;

X₁₅ is Glu or dGlu;

X₁₆ is Glu, or dGlu;

X₁₉ is Val;

X₂₀ is Arg;

X₂₃ is Ile, or dIle;

X₂₄ is Glu, or Ala; and

X₄₀ is a bond or an acylated amino acid, optionally an acylated Lys; and

R₂₀ is COOH or CONH₂, optionally wherein 1, 2 or 3 amino acids selected from positions 7, 10, 13, or 16 are substituted with Trp or dTrp, with the proviso that when one of X₁₀ or X₁₁ is Trp or dTrp then the other is not Trp or dTrp and that R₁₀ and X₁₂ cannot both comprise an acylated amino acid; optionally wherein 1, 2 or 3 amino acids at any of positions 16, 18, 19, 24, 26, or 28 are substituted with Aib.

In another aspect, the present disclosure refers to a GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

DX₁₀X₁₁X₁₂YLY₁₅X₁₆QAVREFX₂₃X₂₄WLVRRGGPSSGAPPPS (SEQ ID NO: 98);
wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Lys or Ser;

X₁₅ is Glu or dGlu;

X₁₆ is dGlu or Glu;

X₂₃ is Ile or dIle;

X₂₄ is Ala or Glu;

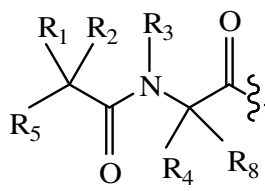
said GLP-1 receptor antagonist being acylated with a fatty acid or diacid group of sufficient size to bind serum albumin with high affinity, optionally wherein an amino acid of the GLP-1 receptor antagonist is acylated with a C16-C18 fatty acid or C16-C18 fatty diacid.

In another aspect, the present disclosure refers to a GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of
 $\text{DX}_{10}\text{X}_{11}\text{RYLX}_{15}\text{X}_{16}\text{QAVREFX}_{23}\text{EWLVRGGPSSGAPPSX}_{40}\text{R}_{20}$ (SEQ ID NO: 5),
wherein

- 5 X_{10} is Trp, dTrp or Val;
 X_{11} is Trp, dTrp or Ser;
 X_{15} is Glu or dGlu
 X_{16} is dGlu or Glu;
 X_{23} is Ile or dIle;
10 X_{40} is an acylated amino acid; and

R_{20} is COOH or CONH₂, optionally wherein the peptide comprises one or more substitutions of Aib at any of positions 16, 18, 19, 24, 26 or 28, or optionally a substitution of an acylated Lys at position 12, wherein said position number is relative to the native Exendin4 amino acid sequence.

- 15 In another aspect, the present disclosure refers to a derivative of the GLP-1 antagonist of any one of the claims 9-26 further comprising a dipeptide A-B:



- linked to said GLP-1 antagonist through an amide bond wherein
20 R_1 , R_2 , R_4 and R_8 are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O;
25 R_3 is selected from the group consisting of C₁-C₁₈ alkyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)NH₂, (C₁-C₁₈ alkyl)SH, (C₀-C₄ alkyl)(C₃-C₆)cycloalkyl, (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, and (C₁-C₄ alkyl)(C₃-C₉ heteroaryl) or R_4 and R_3 together with the atoms to which they are attached form a pyrrolidine ring;
30 R_5 is NHR₆ or OH;
 R_6 is H, C₁-C₈ alkyl; and
 R_7 is selected from the group consisting of H and OH
 wherein the chemical cleavage half-life ($t_{1/2}$) of A-B from said GLP-1 antagonist is at least about 1 hour to about 1 week in PBS under physiological conditions.

- 35 In another aspect, the present disclosure refers to a pharmaceutical composition comprising a GLP-1 antagonist as disclosed herein, or a derivative as disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient.

- In another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a patient suffering from atypical hypoglycemia.

- 40 In accordance with one embodiment example a GLP-1 receptor antagonist peptide is provided comprising the amino acid sequence

DX₁₀X₁₁RYLX₁₅X₁₆QAVREFX₂₃EWLVRGGPSSGAPPPSX₄₀R₂₀ (SEQ ID NO: 5), wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

5 X₁₅ is Glu or dGlu

X₁₆ is Trp, dTrp, dGlu or Glu;

X₂₃ is Ile or dIle;

X₄₀ is an acylated amino acid, and

10 R₂₀ is COOH or CONH₂, optionally wherein the peptide comprises one or more substitutions of Aib at any of positions 16, 18, 19, 24, 26 or 28, relative to the native Exendin4 sequence (SEQ ID NO: 1), or optionally a substitution of Lys at position 12, relative to the native Exendin4 sequence (SEQ ID NO: 1), optionally with the proviso that when one of X₁₀ or X₁₁ is Trp or dTrp, the other is not Trp or dTrp.

15 In accordance with one [embodimentexample](#) a GLP-1 receptor antagonist peptide is provided having the amino acid sequence of

DVWRYLX₁₅EQAVREFIEWLVRGGPSSGAPPPSX₄₀R₂₀ (SEQ ID NO: 96), wherein

X₁₅ is dGlu;

20 X₄₀ is an acylated Lys and R₂₀ is COOH or CONH₂, wherein the acyl group of the acylated Lys is a C16-C18 acid or diacid, optionally linked via a spacer to the Lys side chain. In one [embodimentexample](#) the spacer comprises a minipep or a gamma glutamic acid subunit or any multiple or combination of such minipep or gamma Glu molecules. In one [embodimentexample](#) the peptide of SEQ ID NO: 96 is modified with 1, 2 or 3 amino acid substitutions, including for example, substitution with amino
25 isobutyric acid (Aib) at one or more of positions 16, 18, 19, 24, 26 or 28, relative to the native Exendin4 sequence of SEQ ID NO: 1; or substitution of Lys at position 12.

30 In accordance with one [embodimentexample](#) a GLP-1 receptor antagonist peptide is provided comprising the amino acid sequence

DX₁₀X₁₁RYLX₁₅X₁₆QAVREFX₂₃EWLVRGGPSSGAPPPSX₄₀R₂₀ (SEQ ID NO: 5), wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₅ is Glu or dGlu

X₁₆ is Trp, dTrp, dGlu or Glu;

X₂₃ is Ile or dIle;;

X₄₀ is an acylated amino acid, and R₂₀ is COOH or CONH₂, optionally wherein the peptide comprises one or more substitutions of Aib at any of positions 16, 18, 19, 24, 26 or 28, relative to the native Exendin4 sequence (SEQ ID NO: 1), or optionally an N-terminal extension of the peptide of SEQ ID NO: 5 by X₇X₈, wherein X₇ is an acylated amino acid (e.g., Lys) and X₈ is Gly or C₁-C₃ N-alkyl Gly, with position numbering relative to the native Exendin4 sequence (SEQ ID NO: 1), optionally with the proviso that when one of X₁₀ or X₁₁ is Trp or dTrp, the other is not Trp or dTrp.

In accordance with one [embodimentexample](#) a GLP-1 receptor antagonist peptide is provided having the amino acid sequence of

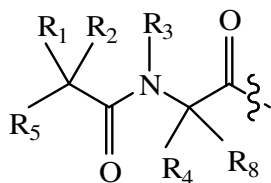
DVWRYLX₁₅EQAVREFIEWLVRGGPSSGAPPSX₄₀ R₂₀ (SEQ ID NO: 96),
wherein

X₁₅ is dGlu;

X₄₀ is an acylated Lys and R₂₀ is COOH or CONH₂, wherein the acyl group of the acylated Lys is a C16-C18 acid or diacid, optionally linked via a spacer to the Lys side chain. In one [embodimentexample](#) the spacer comprises a minipeg or a gamma glutamic acid subunit or any multiple or combination of such minipeg or gamma Glu molecules. In one [embodimentexample](#) the peptide of SEQ ID NO: 96 is modified with 1, 2 or 3 amino acid substitutions, including for example, substitution with amino isobutyric acid (Aib) at one or more of positions 16, 18, 19, 24, 26 or 28, relative to the native Exendin4 sequence of SEQ ID NO: 1; or by the addition of a dipeptide X₇X₈ to the N-terminus of SEQ ID NO: 96, wherein X₇ is an acylated amino acid (e.g., Lys) and X₈ is Gly or C₁-C₃ N-alkyl Gly, with position numbering relative to the native Exendin4 sequence (SEQ ID NO: 1).

In one [embodimentexample](#) 1 to 3 amino acids are added to the N-terminus of the peptide of SEQ ID NO: 5 or an analog thereof. In one [embodimentexample](#) one of the amino acids comprising the N-terminal extension is an acylated amino acid. In one [embodimentexample](#) the N-terminal extension is a dipeptide, X₇X₈, wherein X₇ is an acylated lysine, optionally wherein the lysine is in the D-conformation and X₈ is any amino acid. In one [embodimentexample](#) the N-terminal extension is a self-cleaving dipeptide linked to the N-terminal alpha amine of a 9-29 exendin4 analog (e.g., a peptide of SEQ ID

NO: 5) to form a prodrug of any of the GLP-1 antagonist of the present disclose. One advantage of using a prodrug derivative of a GLP-1 antagonist is that such an approach extends peptide's biological half-life based on a strategy of inhibiting recognition of the prodrug by the GLP-1 receptor. In one [embodimentexample](#) the prodrug derivative comprises a self-cleaving dipeptide (A-B) covalently linked to the GLP-1 antagonist wherein the dipeptide is cleaved under physiological conditions and in the absence of enzymatic activity to restore full activity to the GLP-1 antagonist. In one [embodimentexample](#) the GLP-1 antagonist is modified by the covalent linkage of one or more dipeptides (A-B) to an amine of GLP-1 antagonist, wherein A is an amino acid or a hydroxy acid and B is an N-alkylated amino acid linked to the GLP-1 antagonist through an amide bond between a carboxyl moiety of B and an amine of the GLP-1 antagonist. In one [embodimentexample](#), A-B comprises the structure:



linked to the GLP-1 antagonist through an amide bond between the carboxyl of A-B and an amine of the GLP-1 antagonist wherein

R_1 , R_2 , R_4 and R_8 are independently selected from the group consisting of H, C_1 - C_{18} alkyl, C_2 - C_{18} alkenyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)SH, $(C_2$ - C_3 alkyl)SCH₃, $(C_1$ - C_4 alkyl)CONH₂, $(C_1$ - C_4 alkyl)COOH, $(C_1$ - C_4 alkyl)NH₂, $(C_1$ - C_4 alkyl)NHC(NH₂⁺)NH₂, $(C_0$ - C_4 alkyl)(C_3 - C_6 cycloalkyl), $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl)R₇, $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl), and C_1 - C_{12} alkyl(W1) C_1 - C_{12} alkyl, wherein W1 is a heteroatom selected from the group consisting of N, S and O;

R_3 is selected from the group consisting of C_1 - C_{18} alkyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)NH₂, $(C_1$ - C_{18} alkyl)SH, $(C_0$ - C_4 alkyl)(C_3 - C_6)cycloalkyl, $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl)R₇, and $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl) or R_4 and R_3 together with the atoms to which they are attached form a pyrrolidine ring;

R_5 is NHR₆ or OH;

R_6 is H, C_1 - C_8 alkyl; and

R₇ is selected from the group consisting of H and OH, wherein the chemical cleavage half-life (t_{1/2}) of A-B from said GLP-1 antagonist is at least about 1 hour to about 1 week in PBS under physiological conditions. In one [embodimentexample](#) the dipeptide A-B is covalently linked to the N-terminal alpha amine of the GLP-1 antagonist amino acid sequence. In one [embodimentexample](#) wherein R₁ is H, C₁-C₄ alkyl, (C₁-C₄ alkyl)OH or (C₁-C₄ alkyl)NH₂; R₂ is H, R₃ is C₁-C₆ alkyl; R₄ is H, C₁-C₄ alkyl, or (CH₂)(C₆ aryl)R₇; R₅ is NH₂; R₇ is H or OH and R₈ is hydrogen.

The present disclosure moreover provides pharmaceutical compositions comprising any of the GLP-1 antagonist peptides and variant peptides described herein and a pharmaceutically acceptable carrier, diluent, or excipient. In one [embodimentexample](#) a method of treating a patient suffering from atypical hypoglycemia is provided wherein the method comprises the step of administering to a patient in need thereof a pharmaceutical composition comprising a GLP-1 antagonist peptide or variant peptides described herein in an amount effective to elevate blood glucose levels.

In accordance with one [embodimentexample](#) a method of treating atypical hypoglycemia is provided wherein the method comprises the steps of administering a GLP-1 receptor antagonist peptide having the amino acid sequence of

DVX₁₁RYLX₁₅X₁₆QAVREFX₂₃EVLVRGGPSSGAPPPSK (SEQ ID NO: 11), wherein X₁₁ is Trp, or dTrp; X₁₅ is Glu, or dGlu; X₁₆ is Glu, or dGlu; and X₂₃ is Ile, or dIle; in an amount therapeutically effective for increasing blood glucose levels. In one [embodimentexample](#) the GLP-1 receptor antagonist is acylated with a fatty acid or diacid group of sufficient size to bind serum albumin with high affinity.

In one [embodimentexample](#) a GLP-1 receptor antagonist is provided, wherein the antagonist comprises the amino acid sequence of

DX₁₀X₁₁X₁₂YLX₁₅X₁₆QAVREFX₂₃X₂₄WLVRRGGPSSGAPPPS (SEQ ID NO: 98);

wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Lys or Ser;

X₁₅ is Glu or dGlu;

X₁₆ is Trp, dTrp, dGlu or Glu;

X₂₃ is Ile or dIle;

X₂₄ is Ala or Glu; and

said GLP-1 receptor antagonist is acylated with a fatty acid or diacid group of sufficient size to bind serum albumin with high affinity, optionally wherein an amino acid of the GLP-1 receptor antagonist is acylated with a C16-C18 fatty acid or C16-C18 fatty diacid, optionally with the proviso that only one of X₁₀, X₁₁ or X₁₆ is Trp or dTrp.

In one [embodimentexample](#) a GLP-1 receptor antagonist is provided wherein the antagonist comprises the amino acid sequence of

DX₁₀X₁₁X₁₂YLX₁₅X₁₆QAVREFX₂₃X₂₄WLVRRGGPSSGAPPPS (SEQ ID NO:

98);

wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Lys or Ser;

X₁₅ is Glu or dGlu;

X₁₆ is Trp, dTrp, dGlu or Glu;

X₂₃ is Ile or dIle;

X₂₄ is Ala or Glu, further wherein the GLP-1 receptor antagonist is

acylated with a fatty acid or diacid group of sufficient size to bind serum albumin with high affinity, optionally wherein an amino acid of the GLP-1 receptor antagonist is acylated with a C16-C18 fatty acid or C16-C18 fatty diacid. In a further

[embodimentexample](#) a peptide of SEQ ID NO: 98 is provided wherein X₁₀ is Val, X₁₁ is Trp, dTrp or Ser, X₁₂ is Arg, X₁₅ is Glu or dGlu, X₁₆ is dGlu or Glu, X₂₃ is Ile or dIle, and X₂₄ is Ala or Glu, optionally wherein X₁₀ is Val, X₁₁ is Ser, X₁₂ is Arg, X₁₅ is Glu or dGlu, X₁₆ is dGlu or Glu, X₂₃ is Ile, and X₂₄ is Ala, optionally wherein X₁₀ is Val, X₁₁ is Ser, X₁₂ is Arg, X₁₅ is Glu, X₁₆ is Glu, X₂₃ is Ile, and X₂₄ is Ala.

In one [embodimentexample](#) the GLP-1 receptor antagonists of the present disclosure are acylated with a fatty acid or diacid group of sufficient size to bind serum albumin with high affinity, optionally wherein the acylated amino acid is the C-terminal amino acid, and optionally further modified by the linkage of a self-cleaving dipeptide via an amide bond, wherein an amino acid of the dipeptide is optionally acylated with a fatty-acyl group of sufficient size to bind serum albumin with high affinity. In one

embodimentexample an acylated amino acid is added to the C-terminus of SEQ ID NO: 11 at position 40 and optionally the added acylated amino acid is Lys acylated with a C14-C20 fatty acid or fatty diacid, optionally linked to the amino acid side chain via any of the spacers disclosed herein.

5 In one embodimentexample the pharmaceutical compositions for treating atypical hypoglycemia comprise any of the GLP-1 receptor antagonist disclosed herein in combination with any existing therapeutics useful for treating hypoglycemia. For example, the pharmaceutical composition may include a GLP-1 receptor antagonist of the present invention and one or more of the following: glucose
10 supplements (e.g., dextrose); glucose-elevating agents such as glucagon and glucagon analogs and inhibitors of insulin secretion (e.g., diazoxide, octreotide).

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph presenting the dose dependent change in blood glucose levels
15 over time after subcutaneous administration of a vehicle control, Ex-4 (a GLP-1 agonist having an amino acid sequence of SEQ ID NO: 1), or a GLP-1 antagonist peptide (DVSRYLEEQAVREFIEWLVRGGPSSGAPPPSK₄₀[mPEG-γE-C16]acid; SEQ ID NO: 16 to mice followed by intraperitoneal administration of glucose (1.5 g glucose per kg body weight), wherein the glucose is administered subcutaneously 4hr
20 after the GLP-1 antagonist.

Figs. 2A & 2B present data from a glucose tolerance test wherein mice are subcutaneously administered the GLP-1 antagonist followed four hours later by intraperitoneal administration of glucose (1.5 g glucose per kg body weight). The results demonstrate that the GLP-1 antagonist of SEQ ID NO: 16 (Fig. 2A) is a full
25 antagonist and has a much higher potency than the GLP-1 antagonist Ex-9-40 (DVSKQMEEEEAVRLFIEWLKNGGPSSGAPPPS; SEQ ID NO: 2); Fig. 2B).

Fig. 3 presents data from a glucose tolerance test investigating the effect of Aib substitutions on the efficacy of the GLP-1 antagonists. Mice are subcutaneously administered the GLP-1 antagonist followed four hours later by intraperitoneal
30 administration of glucose (1.5 g glucose per kg body weight). Fig. 3 demonstrates the activity of a GLP-1 antagonist peptide analog of 9-40Jant4-K₄₀ (DVSSYLEEQAVREFIAWLKGGPSSGAPPPSK; SEQ ID NO: 3) that comprises the sequence of (SEQ ID NO: 47), and derivatives thereof comprising a series of Aib

Claims:

1. A GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of R₁₀-DVX₁₁X₁₂YLX₁₅X₁₆QAX₁₉X₂₀EFX₂₃X₂₄WLVRRGGPSSGAPPPSX₄₀-R₂₀ SEQ ID NO: 97), wherein

R₁₀ is NH₂ or an N-terminal extension of 1 or 2 amino acids, wherein one of the amino acids of the N-terminal extension is acylated with a C₁₄-C₂₀ fatty acid or diacid, optionally via a spacer, optionally wherein R₁₀ is a dipeptide of the structure: X₇X₈, wherein X₇ is an acylated amino acid, optionally acylated Lys or acylated dLys, and X₈ is Gly or a C₁-C₄ N-alkylated Gly;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Ser or an acylated amino acid, optionally acylated Lys;

X₁₅ is Glu or dGlu;

X₁₆ is Glu, or dGlu, ~~Asp, homoglutamic acid or homocysteic acid;~~

X₁₉ is Val, ~~cyclopropane, cyclopentane, cyclohexane or phenyl glycine;~~

X₂₀ is Arg, ~~homolysine or citrulline;~~

X₂₃ is Ile, or dIle;

X₂₄ is Glu, or Ala; and

X₄₀ is a bond or an acylated amino acid, optionally an acylated Lys; and

R₂₀ is COOH or CONH₂, optionally wherein 1, 2 or 3 amino acids selected from positions 7, 10, 13, or 16 are substituted with Trp or dTrp, with the proviso that when one of X₁₀ or X₁₁ is Trp or dTrp then the other is not Trp or dTrp and that R₁₀ and X₁₂ cannot both comprise an acylated amino acid; optionally wherein 1, 2 or 3 amino acids at any of positions 16, 18, 19, 24, 26, or 28 are substituted with Aib.

2. The GLP-1 antagonist of claim 1 wherein said antagonist comprises the amino acid sequence of

R₁₀-DVX₁₁X₁₂YLX₁₅X₁₆QAX₁₉X₂₀EFX₂₃EWLVRRGGPSSGAPPPSX₄₀-R₂₀ SEQ ID NO: 23), wherein

R₁₀ is NH₂ or X₇X₈, wherein X₇ is an amino acid acylated with a C₁₄-C₂₀ fatty acid or diacid, optionally via a spacer and X₈ is Gly or a C₁-C₄ N-alkylated Gly;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Ser or an acylated amino acid, optionally acylated Lys;

X₁₅ is Glu or dGlu;

X₁₆ is Glu, ~~or dGlu, Asp, homoglutamic acid or homocysteic acid;~~

X₁₉ is Val, ~~cyclopropane, cyclopentane, cyclohexane or phenyl glycine;~~

X₂₀ is Arg, ~~homolysine or citrulline;~~

X₂₃ is Ile, or dIle; and

X₄₀ is an acylated amino acid, optionally an acylated Lys; and

R₂₀ is COOH or CONH₂, optionally wherein 1, 2 or 3 amino acids at any of positions 16, 18, 19, 24, 26, or 28 are substituted with Aib.

3. The GLP-1 antagonist of claim 1 wherein each acylated amino acid of the GLP-1 antagonist is an acylated Lys.

4. The GLP-1 antagonist of any one of claims 1-3 wherein X₇ is an acylated Lys and X₁₂ is Arg.

5. The GLP-1 antagonist of any one of claims 1-3 wherein R₁₀ is NH₂ and X₁₂ is an acylated Lys.

6. The GLP-1 antagonist of any one of claims 1-3 wherein X₇ is NH₂ and X₁₂ is Arg.

7. The GLP-1 antagonist of claim 1 wherein the antagonist comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, ~~SEQ ID NO: 35, SEQ ID NO: 36,~~ SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42.

8. The GLP-1 antagonist of any one of claims 1-7 wherein the acylated amino acids of said GLP-1 antagonist are independently a Lys residue acylated with a C16-C18 fatty acid or C16-C18 fatty diacid directly linked to the Lys side chain or optionally via a spacer comprising a

- i) gamma glutamic acid,
- ii) minipeptide polymer: -[COCH₂(OCH₂CH₂)_kNH]-, wherein k is 2, 4, 6 or 8,
- iii) or any multiplicity or combination of i) or ii).

9. A GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

DX₁₀X₁₁X₁₂YLX₁₅X₁₆QAVREFX₂₃X₂₄WLVRRGGPSSGAPPPS (SEQ ID NO: 98);

wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Lys or Ser;

X₁₅ is Glu or dGlu;

X₁₆ is ~~Trp, dTrp~~, dGlu or Glu;

X₂₃ is Ile or dIle;

X₂₄ is Ala or Glu;

said GLP-1 receptor antagonist being acylated with a fatty acid or diacid group of sufficient size to bind serum albumin with high affinity, optionally wherein an amino acid of the GLP-1 receptor antagonist is acylated with a C16-C18 fatty acid or C16-C18 fatty diacid.

10. A GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

DX₁₀X₁₁RYLX₁₅X₁₆QAVREFX₂₃EWLVRRGGPSSGAPPPSX₄₀R₂₀ (SEQ ID NO: 5), wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₅ is Glu or dGlu

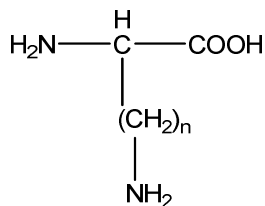
X₁₆ is ~~Trp, dTrp~~, dGlu or Glu;

X₂₃ is Ile or dIle;

X₄₀ is an acylated amino acid; and

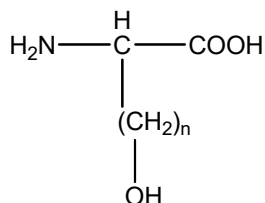
R₂₀ is COOH or CONH₂, optionally wherein the peptide comprises one or more substitutions of Aib at any of positions 16, 18, 19, 24, 26 or 28, or optionally a substitution of an acylated Lys at position 12, wherein said position number is relative to the native Exendin4 amino acid sequence.

11. The GLP-1 antagonist of claim 10 wherein X₁₁ is Trp or dTrp.
12. The GLP-1 antagonist of claim 9, 10 or 11 wherein an amino acid at position 16, 18, 19, 24, 26 or 28 is substituted with Aib.
13. The GLP-1 antagonist of claim 10 or 11 wherein the amino acid at position 12 is substituted with an acylated Lys.
14. The GLP-1 antagonist of any one of claims 9-13 wherein
X₁₅ is dGlu;
X₁₆ is Glu; and
X₂₃ is Ile.
15. The GLP-1 antagonist of any one of claims 9-13 wherein
X₁₅ is Glu;
X₁₆ is Glu; and
X₂₃ is Ile.
16. The GLP-1 antagonist of any one of claims 10-15 wherein X₄₀ is an amino acid having an acyl group linked to the side chain of the amino acid, optionally via a spacer.
17. The GLP-1 antagonist of claim 16 wherein X₄₀ is an amino acid comprising a structure of Formula I (optionally, Lys), Formula II (optionally, Cys), or Formula III (optionally, Ser), wherein each of Formulae I, II, and III, is:



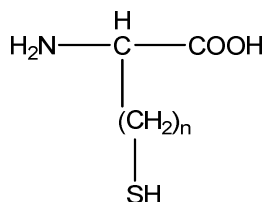
wherein n = 1 to 4

[Formula I];



wherein n = 1 to 4

[Formula II]; and



wherein n = 1 to 4

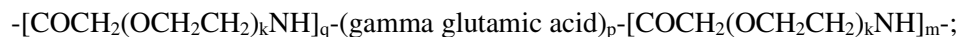
[Formula III].

18. The GLP-1 antagonist of claim 15 or 16 wherein X₄₀ is an acylated lysine.
19. The GLP-1 antagonist of any one of claims 10-18 wherein the acyl group of the acylated amino acid is selected from (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀CH₃, (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀CH₃, (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀COOH or (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀COOH.
20. The GLP-1 antagonist of any one of claims 10-19 wherein the acyl group of the acylated amino acid is covalently linked to the amino acid side chain of the acylated amino acid via a spacer.
21. The GLP-1 antagonist of any one of claims 16-20 wherein the spacer is an amino acid or dipeptide.
22. The GLP-1 antagonist of any one of claims 16-20, wherein the spacer comprises a
 - i) gamma glutamic acid,
 - ii) minipeeg polymer: -[COCH₂(OCH₂CH₂)_kNH]-, wherein k is 2, 4, 6 or 8,
 - iii) or any multiplicity or combination of i) and/or ii).

23. The GLP-1 antagonist of any one of claims 10-22, wherein the acylated amino acid is linked to a C16 to C18 fatty acid or C16 to C18 fatty diacid, optionally wherein the acid or diacid is linked via a spacer comprising the structure:



or



wherein k is an integer selected from 2, 4 or 8, m and n are independently an integer selected from 0, 1 or 2, and p and q are independently an integer selected from 1, 2, 4 or 8.

24. The GLP-1 antagonist of 23, wherein the acylated amino acid is Lys having a C16 to C18 fatty acid linked to the lysine side chain via a spacer comprising the structure:



wherein k is 2, and p and q are independently an integer selected from 1 or 2.

25. The GLP-1 antagonist of claim 10 wherein

X₁₁ is Trp or dTrp;

X₁₅ is Glu or dGlu;

X₁₆ is Glu or dGlu;

X₂₃ is Ile or dIle; and

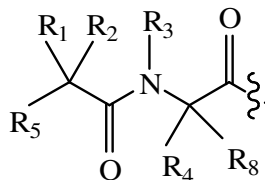
X₄₀ is Lys acylated with a C16 or C18 diacid, optionally wherein the diacid is linked via a spacer comprising the structure:



wherein k is 2, and p and q are independently an integer selected from 1 or 2.

26. The GLP-1 antagonist of any one of claims 10-20 wherein R₂₀ is CONH₂.

27. A derivative of the GLP-1 antagonist of any one of the claims 9-26 further comprising a dipeptide A-B:



linked to said GLP-1 antagonist through an amide bond wherein

R₁, R₂, R₄ and R₈ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O;

R₃ is selected from the group consisting of C₁-C₁₈ alkyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)NH₂, (C₁-C₁₈ alkyl)SH, (C₀-C₄ alkyl)(C₃-C₆)cycloalkyl, (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, and (C₁-C₄ alkyl)(C₃-C₉ heteroaryl) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine ring;

R₅ is NHR₆ or OH;

R₆ is H, C₁-C₈ alkyl; and

R₇ is selected from the group consisting of H and OH

wherein the chemical cleavage half-life (t_{1/2}) of A-B from said GLP-1 antagonist is at least about 1 hour to about 1 week in PBS under physiological conditions.

28. The derivative of claim 27 wherein the dipeptide A-B is covalently linked to the N-terminal alpha amine of the GLP-1 antagonist amino acid sequence.

29. The derivative of claim 27 or 28 wherein

R₁ and R₈ are independently H or C₁-C₈ alkyl;

R₂ and R₄ are independently selected from the group consisting of H, C₁-C₈ alkyl, (C₁-C₄ alkyl)OH, (C₁-C₄ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, and (C₁-C₄ alkyl)(C₆ aryl)R₇;

R₃ is C₁-C₆ alkyl;

R₅ is NH₂; and

R₇ is selected from the group consisting of hydrogen, and OH.

30. The derivative of claim 27 or 28, wherein

R₁ comprises a side chain selected from the group consisting of C₁-C₈ alkyl, (C₁-C₄ alkyl)OH, (C₁-C₄ alkyl)SH, (C₁-C₄ alkyl)COOH, and (C₁-C₄ alkyl)NH₂, optionally wherein a C₁₆-C₃₀ fatty acid or C₁₆-C₃₀ diacid is covalently linked to said side chain, optionally via a spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a gamma glutamic acid-[COCH₂(OCH₂CH₂)_kNH]_q-gamma glutamic acid, wherein

k is an integer selected from the range of 1-8; and

q is an integer selected from the range of 1-8, optionally wherein k is 2 and q is selected from the range of 1-8;

R₂, R₄ and R₈ are independently H, or C₁-C₄ alkyl;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂.

31. The derivative of claim 27 or 28, wherein

R₁ is H, C₁-C₄ alkyl, (C₁-C₄ alkyl)OH or (C₁-C₄ alkyl)NH₂;

R₂ is H,

R₃ is C₁-C₄ alkyl;

R₄ is H, or C₁-C₄ alkyl;

R₅ is NH₂; and

R₈ is hydrogen.

32. The derivative of claim 27 or 28, wherein said dipeptide A-B comprises an acylated Lys residue and an N-alkylated Gly residue, wherein said Lys and N-alkylated Gly residues are linked via a peptide bond, optionally wherein said Lys residue is in the D-conformation.

33. The derivative of claim 27 or 28, wherein

R₁ is (C₄ alkyl)NH₂ or (C₄ alkyl)NH(mPeg-γE-diacid)-C₁₈;

R₂, R₄ and R₈ are each H;

R₃ is C₁-C₄ alkyl; and

R₅ is NH₂;

R₅ is an amine.

34. A pharmaceutical composition comprising a GLP-1 antagonist of any one of claims 1-26, or a derivative of any one of claims 27-33 and a pharmaceutically acceptable carrier, diluent, or excipient.

35. Use of the pharmaceutical composition of claim 34 in the manufacture of a medicament for ~~A method of~~ treating a patient suffering from atypical hypoglycemia ~~said method comprising the step of administering to a patient in need thereof a pharmaceutical composition of claim 34 in an amount effective to elevate blood glucose levels.~~