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(54) GLUCAGON-LIKE PEPTIDE-2 ANALOGS

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(57) **ABSTRACT**

Analogs of glucagon-like peptide 2, a product of glucagon gene expression, have been identified as intestinal tissue growth factors. Their formulation as pharmaceutical, and therapeutic use in treating disorders of the small bowel, are described.

GLUCAGON-LIKE PEPTIDE-2 ANALOGS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of application Ser. No. 08/631,273, filed Apr. 12, 1996, and application Ser. No. 08/632,533, filed Apr. 12, 1996, and a continuation-in-part of application Ser. No. 08/422,540, filed Apr. 14, 1995, the disclosures of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] This invention relates to glucagon-related peptides having intestinal tissue growth promoting properties, and to their use therapeutically to treat various medical conditions resulting from the impaired growth or loss of such tissue.

BACKGROUND TO THE INVENTION

[0003] Expression of the glucagon gene yields a tissue-determined variety of peptide products that are processed from the 160 residue proglucagon product. The organization of these peptides within the proglucagon precursor was elucidated by the molecular cloning of preproglucagon cDNAs from the rat, hamster and bovine pancreas. These analyses revealed that preproglucagon contains not only the sequence of glucagon and glicentin, but also two additional glucagon-like peptides (GLP-1 and GLP-2) separated from glucagon and each other by two spacer or intervening peptides (IP-I and IP-II). These peptides are flanked by pairs of basic amino acids, characteristic of classic prohormone cleavage sites, suggesting they might be liberated after posttranslational processing of proglucagon (Drucker, Pancreas, V1990, 5(4):484).

[0004] Analysis of the peptides liberated from proglucagon in the pancreatic islets of Langerhans, for instance, suggests the primary pancreatic peptide liberated is the 29-mer glucagon, whereas glicentin, oxyntomodulin, IP-II and the glucagon-like peptides are more prevalent in the small and large intestines. This demonstration that the glucagon-like peptides are found in the bowel has prompted research into the precise structure and putative function(s) of these newly discovered gut peptides. Most studies have focussed on GLP-1, because several lines of evidence suggested that GLP-1 may be an important new regulatory peptide. Indeed, it has been determined that GLP-1 is one of the most potent known peptidergic stimulus for insulin release, an action mediated in a glucose-dependent manner through interaction with receptors on pancreatic B cells. GLP-1 and its derivatives are in development for use in the treatment of diabetics.

[0005] The physiological roles of glicentin and oxyntomodulin, the so-called "enteroglucagons", are also under investigation, particularly with respect to regulation of acid secretion and the growth of intestinal cells. Oxyntomodulin is capable of inhibiting pentagastrin-stimulated gastric acid secretion in a dose-dependent manner. The role of glicentin in mediating the changes of intestinal adaptation and growth of the intestinal mucosa has been investigated, and the intestinotrophic effect of glicentin and its therapeutic use have recently been reported by Matsuno et al in EP 612,531 published Aug. 31, 1994.

[0006] In contrast to GLP-1 and other glucagon-related peptides, the physiological role of glucagon-like peptide GLP-2 remains poorly understood despite the isolation and sequencing of various GLP-2 homologues including human, rat, bovine, porcine, guinea pig and hamster. Using GLP-2 antisera raised against synthetic versions of GLP-2, various groups have determined that GLP-2 is present primarily in intestinal rather than pancreatic extracts (see Mojsov et al, J. Biol. Chem., 1986, 261(25):11880; Orskov et al in Endocrinology, 1986, 119(4):1467 and in Diabetologia, 1987, 30:874 and in FEBS Letters, 1989, 247(2):193; George et al, FEBS Letters, 1985, 192(2):275). With respect to its biological role, Hoosein et al report (FEBS Letters, 1984, 178(1):83) that GLP-2 neither competes with glucagon for binding to rat liver and brain tissues, nor stimulates adenylate cyclase production in liver plasma membranes, but, enigmatically, can stimulate adenylate cyclase in both rat hyopthalamic and pituitary tissue, at 30-5 OpM concentrations. An elucidation of the physiological role of GLP-2 would clearly be desirable.

SUMMARY OF THE INVENTION

[0007] There have now been discovered analogs of GLP-2 which promote growth of small bowel tissue. It is accordingly a general object of the present invention to provide such GLP-2 analogs and to provide for their use therapeutically and for related purposes.

[0008] In one aspect of the invention, the GLP-2-analogs exhibit intestinotrophic activity and conform to the structural Formula 1:

R1-(Y1)m-X1-X2-X3-X4-Ser5-Phe6-Ser7-Asp8-(P1) -Leul4- Asp15-Asn16-Leu17-Ala18-X19-X20-Asp21-Phe22-(P2)-Trp25-Leu26-Ile27-Gln28-Thr29-Lys30-(P3)-(Y2)n-R2,

[0009] wherein

[0010] X1 is His or Tyr

[0011] X2 is Ala or an Ala-replacement amino acid conferring on said analog resistance to DPP-IV enzyme;

[0012] X3 is Pro, HPro, Asp or Glu;

[0013] X4 is Gly or Ala;

[0014] P1 is Glu-X10-Asn-Thr-Ile or Tyr-Ser-Lys-Tyr;

[0015] X10 is Met or an oxidatively stable Met-replacement amino acid;

[0016] X19 is Ala or Thr;

[0017] X20 is Arg, Lys, His or Ala;

[0018] P2 is Ile-Asn, Ile-Ala or Val-Gln;

[0019] P3 is a covalent bond, or is Ile, Ile-Thr or Ile-Thr-Asn;

[0020] R1 is H or an N-terminal blocking group;

[0021] R2 is OH or a C-terminal blocking group;

[0022] Y1 is one or two basic amino acids selected from the group Arg, Lys, and His;

[0023] Y2 is one or two basic amino acids selected from the group Arg, Lys, and His; and

[0024] m and n, independently, are 0 or 1; and

[0025] wherein at least one of X1, X2, X3, X4, P1, X10, X19, X20, P2 and P3 is other than a wild type, mammalian GLP-2 residue.

[0026] Particularly preferred analogs according to Formula 1 are those which are rendered resistant to cleavage by human DPP-IV enzyme by replacing the Ala at position X2 with an alternative amino acid. Other analogs of the invention are those which replace the oxidatively sensitive Met at position X10 with an amino acid residue which is oxidatively stable. In this manner, the analog peptides have increased stability compared to GLP-2 peptides with the wild-type Met residue at this position. Yet another preferred embodiment of the invention is the incorporation at position X20 of a basic amino acid selected from His or Lys. This substitution is advantageous when the GLP-2 analogs are chemically synthesized. The Arg residue which normally occurs at this position tends to strongly bind solvents used in peptide synthesis procedures. Substitution of the Arg allows easier formulation of the synthetically produced GLP-2 analogs into pharmaceutically acceptable compositions.

[0027] More particularly, and according to one aspect of the invention, there are provided analogs of a GLP-2 peptide selected from a mammalian GLP-2 species and N- and/or C-terminally modified forms thereof, the analogs having intestinotrophic activity and incorporating, relative to said mammalian GLP-2 peptide, at least one amino acid substitution at a position which is conserved in mammalian GLP-2's. In a preferred aspect, the GLP-2 analogs incorporate a substitution selected from:

[0028] 1) incorporation at position 2 or at position 3 a replacement amino acid conferring on said analog resistance to Dipeptidyl Peptidase-IV (hereinafter referred to as DPP-IV); and

[0029] 2) incorporation at position 10 of an oxidatively stable Met-replacement amino acid; and

[0030] 3) incorporation at X20 of a replacement amino acid other than Arg.

[0031] In still another of its aspects, the invention provides intestinotrophic analogs of a mammalian GLP-2, preferably human GLP-2, in which either the N- and/or C-terminus is modified by a blocking group, or additional amino acids, or the substitution of a modified or alternative amino acid. In yet another aspect, there are provided intestinotrophic analogs of mammalian GLP-2, preferably human GLP-2, in which positions X1, X5, X7, X16-X18, X25, X30, or X33 incorporate a replacement amino acid other than that found in naturally occurring mammalian GLP-2's. Optionally, in a further aspect, the invention provides intestinotrophic analogs in which from 4 to 8 residues are deleted from the C-terminus.

[0032] In another of its aspects, the invention provides a pharmaceutical composition comprising a GLP-2 analog of the present invention in a therapeutically effective amount, and preferably in an intestinotrophic amount, and a pharmaceutically acceptable carrier.

[0033] In a further aspect, the invention provides a method for promoting growth of small bowel tissue in a patient in need thereof, comprising the step of delivering to the patient an intestinotrophic amount of a GLP-2 analog of the present invention.

[0034] Besides promoting bowel growth, in another of its aspects the invention provides a method for treating a gastrointestinal disease by administering to a patient suffering from gastrointestinal disease a therapeutically effective amount of a GLP-2 analog of the invention, together with a pharmaceutically acceptable carrier, in order to reduce a pathological effect or symptom of the gastrointestinal disease.

[0035] In still another aspect of the invention, there is provided a method useful to identify intestinotrophic analogs of GLP-2, comprising the steps of:

[0036] 1) obtaining a GLP-2 analog conforming to Formula 1 represented above;

[0037] 2) treating a mammal with said analog using a regimen capable of eliciting an intestinotrophic effect when utilized for rat GLP-2; and

[0038] 3) determining the effect of said analog on small bowel weight relative to a mock treated control mammal, whereby said intestinotrophic analog of GLP-2 is identified as an analog which elicits an increase in said weight.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The invention relates to therapeutic and related uses of GLP-2 analogs, particularly for promoting growth of tissue of the small bowel. The effect on growth elicited by the present GLP-2 analogs manifests as an increase in small bowel weight, relative to a mock-treated control. In particular, GLP-2 analogs are considered to have "intestinotrophic" activity if, when assessed in the murine model exemplified herein, the analog mediates an increase in small bowel weight of at least 10% relative to a control animal receiving vehicle alone. Particularly suitable for therapeutic use are those analogs which mediate an increase of at least 20% in small bowel weight; preferred for therapeutic use are those which mediate an increase in small bowel weight of 50% or more. Intestinotrophic activity is noted most significantly in relation to the jejunum, including the distal jejunum and particularly the proximal jejunum, and is also noted in the ileum.

[0040] In addition to exhibiting intestinotrophic activity as just defined, the GLP-2 analogs of the present invention incorporate an amino acid substitution at one or more sites within a GLP-2 peptide "background", which is either a mammalian GLP-2 species per se, or is a variant of a mammalian GLP-2 species in which the C-terminus and/or the N-terminus has been altered by addition of one or two basic residues, or has been modified to incorporate a blocking group of the type used conventionally in the art of peptide chemistry to protect peptide termini from undesired biochemical attack and degradation in vivo. Thus, the present peptides incorporate an amino acid substitution in the context of any mammalian GLP-2 species, including but not limited to human GLP-2, bovine GLP-2, rat GLP-2, degu GLP-2, ox GLP-2, porcine GLP-2, guinea pig GLP-2 and hamster GLP-2, the sequences of which have been reported by many authors, including Buhl et al, J. Biol. Chem., 1988, 263(18):8621.

[0041] In one aspect of the invention, the intestinotrophic analogs of GLP-2 conform to the sequence of Formula 1 as follows:

R1-(Y1)m-X1-X2-X3-X4-Ser5-Phe6-Ser7-Asp8-(P1)-Leu14-Asp15-Asn16-Leu17-Ala18-X19-X20-Asp21-Phe22-(P2)-Trp25-Leu26-Ile27-Gln-28-Thr29-Lys30-(P3)-(Y2)n-R2,

[0042] wherein

[0043] X1 is His or Tyr

[0044] X2 is Ala or an Ala-replacement amino acid conferring on said analog resistance to DPP-IV enzyme;

[0045] X3 is Pro, HPro, Asp or Glu;

[0046] X4 is Gly or Ala;

[0047] P1 is Glu-XlO-Asn-Thr-Ile or Tyr-Ser-Lys-Tyr;

[0048] X10 is Met or an oxidatively stable Met-replacement amino acid;

[0049] X19 is Ala or Thr;

[0050] X20 is Arg, Lys, His or Ala;

[0051] P2 is Ile-Asn, Ile-Ala or Val-Gln;

[0052] P3 is a covalent bond, or is Ile, Ile-Thr or Ile-Thr-Asn;

[0053] R1 is H or an N-terminal blocking group;

[0054] R2 is OH or a C-terminal blocking group;

[0055] Y1 is one or two basic amino acids selected from the group Arg, Lys, and His;

[0056] Y2 is one or two basic amino acids selected from the group Arg, Lys, and His; and

[0057] m and n, independently, are 0 or 1; and

[0058] wherein at least one of X1, X2, X3, X4, P1, X10, X19, X20, P2 and P3 is other than a wild type, mammalian GLP-2 residue.

[0059] Wild-type mammalian GLP-2 residues which occur at a specific position are determined by aligning the sequences of GLP-2's isolated from different mammalian species and comparing the sequence to the human sequence, reproduced below, for convenience:.

[0060] His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-1 5 10

[0061] Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-15 20

[0062] Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp 25 30

[0063] The amino acid residues which, for purposes of this application, are known to occur at specific positions in wild type mammalian GLP-2's are the following: position X13 may be Ile or Val; Position X16 may be Asn or Ser; position X19 may be Alanine or Threonine; position X20 may be Arg or Lys; position X27 may be Ile or Leu; and position X28 may be Gln or His.

[0064] The present GLP-2 analogs may incorporate desired amino acid substitutions into a "background" which is an N-terminally or C-terminally modified form of a mammalian GLP-2 peptide. Such analogs are represented in Formula 1 as those in which R1 constitutes an N-terminal blocking group, and/or when m is 1 then Y1 is one or two basic amino acids such as Arg or Lys; and/or R2 is a C-terminal blocking group; and/or when n is 1 then Y2 is independently, one or two basic amino acids such as Arg or Lys.

[0065] In preferred embodiments of the invention, the GLP-2 analog is an analog of full length GLP-2, i.e., GLP-2(1-33), and P3 is accordingly the sequence Ile-Thr-Asn. Alternatively, the GLP-2 analogs may be C-terminally truncated, to yield GLP-2(1-32) forms in which P3 is Ile-Thr, or GLP-2(1-31) forms in which P3 is Ile, or GLP-2(1-30) forms in which P3 is a covalent bond.

[0066] The "blocking groups" represented by R1 and R2 are chemical groups that are routinely used in the art of peptide chemistry to confer biochemical stability and resistance to digestion by exopeptidase. Suitable N-terminal protecting groups include, for example, C_{1-5}alkanoyl groups such as acetyl. Also suitable as N-terminal protecting groups are amino acid analogues lacking the amino function, for example, desamino-Tyrl. Suitable C-terminal protecting groups include groups which form ketones or amides at the carbon atom of the C-terminal carboxyl, or groups which form esters at the oxygen atom of the carboxyl. Ketone and ester-forming groups include alkyl groups, particularly branched or unbranched C_{1-5}alkyl groups, e.g., methyl, ethyl and propyl groups, while amide-forming groups include amino functions such as primary amine, or alkylamino functions, e.g., mono-C_{1-5}salkylamino and di-C_{1-5}alkylamino groups such as methylamino, ethylamino, dimethylamino, diethylamino, methylethylamino and the like. Amino acid analogues are also suitable for protecting the C-terminal end of the present compounds, for example, decarboxylated amino acid analogues such as agmatine.

[0067] Embodiments of the invention specifically include such analogs in which m is 0 and R1 is a blocking group such as acetyl; and analogs in which m is 0 and R2 is a C-terminal blocking group such as an amide or an amine, e.g., -NH2.

[0068] In a preferred aspect of the invention, the GLP-2 analogs are analogs of either human GLP-2 or of rat GLP-2. Human GLP-2 is herein referred to interchangably as hGLP-2(1-33). Rat GLP-2 has the amino acid sequence of human GLP-2, but incorporates at position 19 a Thr residue instead of an Ala residue. Rat GLP-2 is accordingly referenced herein either as rGLP-2(1-33) or as the Thr^{19} analog of human GLP-2, i.e., as [Thr^{19}]hGLP-2(1-33).

[0069] In particularly preferred embodiments of the invention, with respect to both the Formula 1 analogs and the more specific human or rat GLP-2 analogs, the GLP-2 analogs incorporate an amino acid substitution selected from:

[0070] 1) incorporation at X2 and/or at X3 of a replacement amino acid which renders said analog resistant to cleavage by DPP-IV enzyme;

[0071] 2) incorporation at X10 of an oxidatively stable Met-replacement amino acid; and

[0072] 3) incorporation at X20 of a replacement amino acid other than Arg.

[0073] In still another of its aspects, the invention provides intestinotrophic analogs of a mammalian GLP-2, preferably human GLP-2, in which either the N- and/or C-terminus is modified by a blocking group, or additional amino acids, or the substitution of a modified or alternative amino acid. In yet another aspect, there are provided intestinotrophic analogs of mammalian GLP-2, preferably human GLP-2, in which positions X1, X5, X7, X16-X18, X25, X30, or X33 incorporate a replacement amino acid other than that found in naturally occurring mammalian GLP-2's. Optionally, in a further aspect, the invention provides intestinotrophic analogs in which from 4 to 8 residues are deleted from the C-terminus.

[0074] The DPP-IV-resistant class of GLP-2 analogs possess particularly advantageous properties. As is demonstrated herein, mammalian GLP-2 species have been found to be sensitive to cleavage by DPP-IV enzyme. It has also been found that this sensitivity to DPP-IV is the result of the recognition sequence Ala^2 Asp^3 found in all mammalian forms of GLP-2. There are accordingly provided by the present invention a class of GLP-2 analogs which incorporate at X2 and/or X3 a replacement amino acid which confers on the GLP-2 analog relative resistance to DPP-IV mediated cleavage, as determined by any convenient in vitro or in vivo assessment technique that is able to detect the presence of GLP-2 digestion products. A DPP-IV resistant GLP-2 analog is revealed as that GLP-2 analog which is processed or degraded at a rate that is measurably slower than the rate at which human GLP-2 is processed or degraded, under the same conditions.

[0075] An assay suitable for assessing DPP-IV sensitivity and resistance is described below in Example 3, in the context of results actually obtained.

[0076] The X2 class of GLP-2 analogs is preferred herein. These Ala^2-substituted GLP-2 analogs can incorporate at X2 a structurally wide variety of Ala-replacement amino acids to achieve relative resistance to DPP-IV digestion. A similarly wide variety of Ala-replacement amino acids allow also for the retention by the analog of intestinotrophic activity. For purposes of identifying those DPP-IV-resistant X2 analogs that also retain intestinotrophic activity, the X2 analogs showing DPP-IV resistance are screened in the assay of intestinotrophic activity described below in Example 4.

[0077] In embodiments of the present invention, the Ala^2 replacements include stereoisomers of amino acids that would otherwise be substrates for DPP-IV, for example D-Ala, D-HPr, βAla, t-butyl-Gly, L-penicillamin, α-aminobutyric acid, aminoisobutyric acid, norvaline, L-phenyl-Gly, and D-Pro, and naturally occurring amino acids, for example, Glu, Lys, Ile, Ser, Asp, Phe, Lys, Met, Asn, Pro, Gln, Thr, Tyr, Gly and Val. In specific embodiments of the invention, there are provided the following Ala^2-substituted GLP-2 analogs: [D-Ala^2]rGLP-2(1-33), [Gly^2]rGLP-2(1-33), [Val^2]rGLP-2(1-33) [Gly^2]hGLP-2(1-33), [tBuGly^2]hGLP-2, [ASP^2]hGLP-2(1-33), [Glu^2]hGLP-2(1-33), [Phe^2]hGLP-2(1-33), [His^2]hGLP-2(1-33) , [Ile^2]hGLP-2(1-33), [Lys^2]hGLP-2(1-33), [Met^2]hGLP-2(1-33), [Asn^2]hGLP-2(1-33), [Pro^2]hGLP-2(1-33), [Gln^2]hGLP-2(1-33), [Ser^2]hGLP-2(1-33), [Thr^2]hGLP-2(1-33), [Val^2]hGLP-2(1-33), [Tyr^2]hGLP-2(1-33), [D-Ala^2]hGLP-2(1-33), [Pen^2]hGLP-2(1-33), [bAla^2]hGLP-2(1-33), [aAbu^2]hGLP-2(1-33), [$Nval^2$]hGLP-2(1-33), [PhGly^2]hGLP-2(1-33), and [Aib^2]hGLP-2(1-33).

[0078] The X2 GLP-2 analogs may incorporate amino acid replacements at other positions. In embodiments of the invention, such analogs include those carrying amino acid substitutions also at one or more of positions X1, X3, X4, X10, X19, X20 and X24, and therefore include those which, according to Formula 1, include at least one of the following substitutions: X1 is Tyr; X3 is Glu; X4 is Ala; P1 is Glu-X10-Asn-Thr-Ile where X10 is other than Met or P1 is Tyr-Ser-Lys-Tyr; X10 is an oxidatively stable Met-replacement amino acid; X19 is Thr; X20 is Lys or Ala; P2 is Val-Gln and P3 is a covalent bond, Ile, or Ile-Thr or Ile-Thr-Asn.

[0079] In embodiments of the present invention, the X2 analogs of GLP-2 include those which also incorporate one of the following substitutions: X1 is Ala; X3 is Ala; X4 is Ala; X10 is an oxidatively stable Met-replacement amino acid such as Val, Ile, Asn, Glx, Tyr, Phe, and preferably Leu, Nle, Ala, Ser, and Gly; and P2 is Ile-Ala. In specific embodiments of the invention, there are provided the following GLP-2 analogs: [D-Ala^2Thr^{19}]hGLP-2(1-33); [Gly^2Thr^{19}]hGLP-2 (1-33); [Val^2Thr^{19}]hGLP-2 (1-33); (Gly^2Ala^{24}]hGLP-2(1-33); [Gly^2Ala^{10}]hGLP-2 (1-33); [Ala^1Gly^2]hGLP-2(1-33); (Gly^2Ala^3]hGLP-2(1-33) and [Gly^2Ala^4]hGLP-2(1-33).

[0080] In a related embodiment, the X2 analogs include those which include the following substitutions: GLP-2 [Gly^2Ala^8]hGLP-2; [Gly^2Ala^{11}]hGLP-2; [Gly^2Ala^{21}]hGLP-2; [Gly^2Ala^9]hGLP-2; [Gly^2Ala^{16}]hGLP-2; [Gly^2Ala^{17}]hGLP-2; [Gly^2Ala^{28}]hGLP-2; [Gly^2Ala^5]hGLP-2; [Gly^2Ala^{31}]hGLP-2; [Gly^2Ala^{27}]hGLP-2; [Gly^2Ala^{12}]hGLP-2; [Gly^2Ala^{13}]hGLP-2; [Gly^2Ala^7]hGLP-2; [Gly^2Ala^6]hGLP-2; [Ser^2,Gln^3]hGLP-2; (1-33); [Gly^2, Ala^{25}]hGLP-2; (1-33); [Gly^2, Ala^{26}]hGLP-2; (1-33); [Gly^2, Ala^{14}]hGLP-2 (1-33); [Gly^2, Ala^{23}]hGLP-2 (1-33); [Gly^2, Ala^{30}]hGLP-2(1-33); [Tyr^1, Gly^{26}]hGLP-2(1-33); [Gly^2, Ala^{34}]hGLP-2(1-33); and [Gly^2, Tyr^{34}]hGLP-2(1-34);

[0081] Alternatively, in the case where X2 is Ala, hPr or Pro, DPP-IV resistance can be conferred by replacing Asp^3 with an Asp-replacement amino acid, X3 which is Pro or hPr.

[0082] The present invention also provides, as another class of GLP-2 analogs, those analogs in which X10 represents an oxidatively stable, Met-replacement amino acid. It has been found that the intestinotrophic activity of mammalian GLP-2 is reduced when the Met in position 10 is oxidized, and also that intestinotrophic activity is retained when replacement amino acids insensitive to oxidation are substituted for Met^{10} replacement. Such Met^{10}-substituted GLP-2 analogs are accordingly more stable during synthesis, work-up and storage. In embodiments of the present invention, such X10 analogs of GLP-2 are analogs of human GLP-2. In a specific embodiments of the invention, such analogs include: [Ser^{10}]hGLP-2(1-33); [Nle^{10}]hGLP-2(1-33); [Ala^{10}]hGLP-2(1-33); [Leu^{10}]rGLP-2(1-33); [$Met(O)^{10}$]ratGLP-2(1-33); and [Nle^{10}]ratGLP-2 (1-33).

[0083] The X10 analogs may also incorporate amino acid substitutions at one or more positions other than X10. As noted above, such analogs include, for instance, those incorporating substitutions at X2, and those in which P1 is is Tyr-Ser-Lys-Tyr, as well as those in which any one of the X substituents or the P substituents represented in Formula 1

are other than wild type residues or sequences. In specific embodiments, the GLP-2 analogs include [Gly^2Ala^{10}]hGLP-2(1-33) and [$Tyr^9Ser^{10}Lys^{11}Tyr^{12}desIle^{13}$]hGLP-2(1-33).

[0084] In other embodiments of the invention, the GLP-2 analogs incorporate single amino acid substitutions in the context of the mammalian GLP-2 peptide background in which they are introduced. Such analogs include, for example, those mammalian GLP-2 analogs and particularly the human GLP-2 analogs in which X1 is Tyr; X3 is Glu; X4 is Ala; P1 is Tyr-Ser-Lys-Tyr-(desIle); P2 is Ile-Ala or Val-Gln; and P3 is a covalent bond, or is Ile or Ile-Thr.

[0085] Still other GLP-2 analogs of the invention incorporate alterations of the N- or C-terminus by the addition of amino acids, by blocking groups, or by substituted amino acids. There are also provided intestinotrophic analogs of mammalian GLP-2, preferably human GLP-2, in which positions X1, X5, X7, X16-X18, X25, X30, or X33 incorporate a replacement amino acid other than that found in naturally occurring mammalian GLP-2's. optionally, the invention provides intestinotrophic analogs in which from 4 to 8 residues are deleted from the C-terminus. Specific embodiments of such intestinotrophic analogs include: Ac-ratGLP-2(1-33); ratGLP-2(1-30); ratGLP-2(1-25); ratGLP-2(1-33)amide; [Arg^{-2}, Arg^{-1}]ratGLP-2(2-33) [Pro^1]hGLP-2(1-33); [Gln^{20}]hGLP-2(1-33); [Asp^1]hGLP-2(1-33); [Tyr^{34}]hGLP-2(1-34); [desNH2Tyr^1]hGLP-2(1-33); [Thr^5]hGLP-2(1-33); [Ser^{16},Arg^{17},Arg^{18}]hGLp-2(1-33); [Agm^{34}]hGLP-2(1-34); [Arg^{30}]hGLP-2(1-33); [Ala^5, Ala^7]hGLP-2(1-33); [Glu^{33}]hGLP-2(1-33); (Phe^{25}]hGLP-2(1-33); and [Tyr^{25}]hGLP-2(1-33).

[0086] The present GLP-2 analogs can be synthesized using standard techniques of peptide chemistry and can be assessed for intestinotrophic activity, all according to the guidance provided herein. With respect to synthesis, the selected GLP-2 analog can be prepared by a variety of techniques well known for generating peptide products. Those GLP-2 analogs that incorporate only L-amino acids can be produced in commercial quantities by application of recombinant DNA technology. For this purpose, DNA coding for the desired GLP-2 analog is incorporated into an expression vector and transformed into a microbial, e.g., yeast, or other cellular host, which is then cultured under conditions appropriate for GLP-2 expression. A variety of gene expression systems have been adapted for this purpose, and typically drive expression of the desired gene from expression regulatory elements used naturally by the chosen host. Because GLP-2 does not require post translational glycosylation for its activity, its production may most conveniently be achieved in bacterial hosts such as E. coli. For such production, DNA coding for the selected GLP-2 peptide may usefully be placed under expression controls of the lac, trp or PL genes of E. coli. As an alternative to expression of DNA coding for the GLP-2 per se, the host can be adapted to express GLP-2 peptide as a fusion protein in which the GLP-2 is linked releasably to a carrier protein that facilitates isolation and stability of the expression product.

[0087] In an approach universally applicable to the production of a selected GLP-2 analog, and one used necessarily to produce GLP-2 analogs that incorporate non-genetically encoded amino acids and N- and C-terminally derivatized forms, the well established techniques of automated peptide synthesis are employed, general descriptions of which appear, for example, in J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd Edition, 1984, Pierce Chemical Company, Rockford, Ill.; and in M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis, 1984, Springer-Verlag, N.Y.; Applied Biosystems 430A Users Manual, 1987, ABI Inc., Foster City, Calif. In these techniques, GLP-2 analog is grown from its C-terminal, resin-conjugated residue by the sequential addition of appropriately protected amino acids, using either the Fmoc or tBoc protocols, as described for instance by Orskov et al, 1989, supra.

[0088] For the incorporation of N- and/or C- blocking groups, protocols conventional to solid phase peptide synthesis methods can also be applied. For incorporation of C-terminal blocking groups, for example, synthesis of the desired peptide is typically performed using, as solid phase, a supporting resin that has been chemically modified so that cleavage from the resin results in a GLP-2 peptide having the desired C-terminal blocking group. To provide peptides in which the C-terminus bears a primary amino blocking group, for instance, synthesis is performed using a p-methylbenzhydrylamine (MBHA) resin so that, when peptide synthesis is completed, treatment with hydrofluoric acid releases the desired C-terminally amidated peptide. Similarly, incorporation of an N-methylamine blocking group at the C-terminus is achieved using N-methylaminoethyl-derivatized DVB resin, which upon HF treatment releases peptide bearing an N-methylamidated C-terminus. Protection of the C-terminus by esterification can also be achieved using conventional procedures. This entails use of resin/blocking group combination that permits release of side-chain protected peptide from the resin, to allow for subsequent reaction with the desired alcohol, to form the ester function. FMOC protecting groups, in combination with DVB resin derivatized with methoxyalkoxybenzyl alcohol or equivalent linker, can be used for this purpose, with cleavage from the support being effected by TFA in dichloromethane. Esterification of the suitably activated carboxyl function, e.g., with DCC, can then proceed by addition of the desired alcohol, followed by deprotection and isolation of the esterified GLP-2 peptide.

[0089] Incorporation of N-terminal blocking groups can be achieved while the synthesized GLP-2 peptide is still attached to the resin, for instance by treatment with suitable anhydride and nitrile. To incorporate an acetyl blocking group at the N-terminus, for instance, the resin-coupled peptide can be treated with 20% acetic anhydride in acetonitrile. The N-blocked GLP-2 analog can then be cleaved from the resin, deprotected and subsequently isolated.

[0090] Once the desired GLP-2 analog has been synthesized, cleaved from the resin and fully deprotected, the peptide is then purified to ensure the recovery of a single oligopeptide having the selected amino acid sequence. Purification can be achieved using any of the standard approaches, which include reversed-phase high-pressure liquid chromatography (RP-HPLC) on alkylated silica columns, e.g., C_4, C_8-, or C_{18}- silica. Such column fractionation is generally accomplished by running linear gradients, e.g., 10-90%, of increasing % organic solvent, e.g., acetonitrile, in aqueous buffer, usually containing a small amount (e.g., 0.1%) of pairing agent such as TFA or TEA. Alternatively, ion-exchange HPLC can be employed to separate peptide

species on the basis of their charge characteristics. Column fractions are collected, and those containing peptide of the desired/required purity are optionally pooled. In one embodiment of the invention, the GLP-2 analog is then treated in the established manner to exchange the cleavage acid (e.g., TFA) with a pharmaceutically acceptable acid, such as acetic, hydrochloric, phosphoric, maleic, tartaric, succinic and the like, to generate a pharmaceutically acceptable acid addition salt of the peptide.

[0091] For administration to patients, the GLP-2 analog or its salt is desirably provided in pharmaceutically acceptable form, e.g., as a preparation that is sterile-filtered, e.g., through a 0.22 μ filter, and substantially pyrogen-free. Desirably, the GLP-2 analog to be formulated migrates as a single or individualized peak on HPLC, exhibits uniform and authentic amino acid composition and sequence upon analysis thereof, and otherwise meets standards set by the various national authorities which regulate quality of pharmaceutical products.

[0092] For therapeutic use, the chosen GLP-2 analog is formulated with a carrier that is pharmaceutically acceptable and is appropriate for delivering the peptide by the chosen route of administration. Suitable pharmaceutically acceptable carriers are those used conventionally with peptide-based drugs, such as diluents, excipients and the like. Reference may be made to "Remington's Pharmaceutical Sciences", 17th Ed., Mack Publishing Company, Easton, Pa., 1985, for guidance on drug formulations generally. In one embodiment of the invention, the compounds are formulated for administration by infusion, e.g., when used as liquid nutritional supplements for patients on total parenteral nutrition therapy, or by injection, e.g., sub-cutaneously, intramuscularly or intravenously, and are accordingly utilized as aqueous solutions in sterile and pyrogen-free form and optionally buffered to physiologically tolerable pH, e.g., a slightly acidic or physiological pH. Thus, the compounds may be administered in a vehicle such as distilled water or, more desirably, in saline, phosphate buffered saline or 5% dextrose solution. Water solubility of the GLP-2 analog may be enhanced, if desired, by incorporating a solubility enhancer, such as acetic acid.

[0093] The aqueous carrier or vehicle can be supplemented for use as injectables with an amount of gelatin that serves to depot the GLP-2 analog at or near the site of injection, for its slow release to the desired site of action. Concentrations of gelatin effective to achieve the depot effect are expected to lie in the range from 10-20%. Alternative gelling agents, such as hyaluronic acid, may also be useful as depoting agents.

[0094] The GLP-2 analogs of the invention may also be formulated as a slow release implantation device for extended and sustained administration of GLP-2 analog. Examples of such sustained release formulations include composites of biocompatible polymers, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including, A. Domb et al., *Polymers for Advanced Technologies* 3:279-292 (1992). Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in the text by M. Chasin and R. Langer (eds.), "Biodegradable Polymers as Drug Delivery Systems," Vol. 45 of "Drugs and the Pharmaceutical Sciences," M. Dekker, N.Y., 1990. Liposomes may also be used to provide for the sustained release of a GLP-2 analog. Details concerning how to use and make liposomal formulations of drugs of interest can be found in, among other places, U.S. Pat. No 4,944,948; U.S. Pat. No. 5,008,050; U.S. Pat. No. 4,921,706; U.S. Pat. No. 4,927,637; U.S. Pat. No. 4,452,747; U.S. Pat. No. 4,016,100; U.S. Pat. No. 4,311,712; U.S. Pat. No. 4,370,349; U.S. Pat. No. 4,372,949; U.S. Pat. No. 4,529,561; U.S. Pat. No. 5,009,956; U.S. Pat. No. 4,725,442; U.S. Pat. No. 4,737,323; U.S. Pat. No. 4,920,016. Sustained release formulations are of particular interest when it is desirable to provide a high local concentration of a GLP-2 analog.

[0095] The GLP-2 analog can be utilized in the form of a sterile-filled vial or ampoule, that contains an intestinotrophic amount of the peptide, in either unit dose or multi-dose amounts. The vial or ampoule may contain the GLP-2 analog and the desired carrier, as an administration-ready formulation. Alternatively, the vial or ampoule may contain the GLP-2 peptide in a form, such as a lyophilized form, suitable for reconstitution in a suitable carrier, such as phosphate-buffered saline.

[0096] As an alternative to injectable formulations, the GLP-2 analog may be formulated for administration by other routes. Oral dosage forms, such as tablets, capsules and the like, can be formulated in accordance with standard pharmaceutical practice. According to the present invention, the GLP-2 analog is administered to treat patients that would benefit from growth of small bowel tissue. The effects of GLP-2 analog on this tissue, as evidenced by the results exemplified herein, is dramatic and would clearly benefit those patients suffering from diseases or conditions marked by abnormalities in the small intestinal tract mucosa, which include ulcers and inflammatory disorders; congenital or acquired digestion and absorption disorders including malabsorption syndromes; and diseases and conditions caused by loss of small bowel mucosal function particularly in patients undergoing extended parenteral feeding or who, as a result of surgery, have undergone resection of the small bowel and suffer from short-gut syndrome and cul-de-sac syndrome. Therapeutic treatment with GLP-2 analog is administered so as to reduce or eliminate the disease symptoms and/or improve the nutritional status in these patients associated with their reduced intestinal tract mucosal function. For example, GLP-2 analog is administrated to a patient with an inflammatory bowel condition in an amount sufficient to ameliorate the intestinal discomfort and diarrhea caused by the condition. Additionally, GLP-2 analog may be administered to patients with malabsorption disorders so as to enhance the nutritional absorption and thereby improve the nutritional status of such patients.

[0097] In general, patients who would benefit from increased small intestinal mass and consequent increased small bowel mucosal function are candidates for treatment with GLP-2 analog. Particular conditions that may be treated with GLP-2 analog include the various forms of sprue including celiac sprue which results from a toxic reaction to α-gliadin from wheat, and is marked by a tremendous loss of villae of the small bowel; tropical sprue which results from infection and is marked by partial flattening of the villae; hypogammaglobulinemic sprue which is observed commonly in patients with common variable immunodefi-

ciency or hypogammaglobulinemia and is marked by significant decrease in villus height. The therapeutic efficacy of the GLP-2 analog treatment may be monitored by enteric biopsy to examine the villus morphology, by biochemical assessment of nutrient absorption, by patient weight gain, or by amelioration of the symptoms associated with these conditions. Other conditions that may be treated with GLP-2 analog, or for which GLP-2 analog may be useful prophylactically, include radiation enteritis, infectious or post-infectious enteritis, regional enteritis (Crohn's disease), small intestinal damage due to toxic or other chemotherapeutic agents, and patients with short bowel syndrome.

[**0098**] The therapeutic dosing and regimen most appropriate for patient treatment will of course vary with the disease or condition to be treated, and according to the patient's weight and other parameters. The results presented hereinbelow demonstrate that a dose of GLP-2 peptide equivalent to about 100 μg/kg (or less) administered twice daily over 10 days can generate very significant increases in small bowel mass. It is expected that much smaller doses, e.g., in the μg/kg range, and shorter or longer duration or frequency of treatment, will also produce therapeutically useful results, i.e., a statistically significant increase particularly in small bowel mass. Also, it is anticipated that the therapeutic regimen will include the administration of maintenance doses appropriate for reversing tissue regression that occurs following cessation of initial treatment. The dosage sizes and dosing regimen most appropriate for human use are guided by the results herein presented, and can be confirmed in properly designed clinical trials.

[**0099**] An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Primary among these is the amount of GLP-2 normally circulating in the plasma, which is on the order of 151 pmol/ml in the resting state, rising to 225 pmol/ml after nutrient ingestion for healthy adult humans. Orskow, C. and Helst, J.J., 1987, Scand. J. Clin. Lav. Invest. 47:165. Additional factors include the size of the patient, the age of the patient, the general condition of the patient, the particular disease being treated, the severity of the disease, the presence of other drugs in the patient, the in vivo activity of the GLP-2 analog and the like. The trial dosages would be chosen after consideration of the results of animal studies and the clinical literature. It will be appreciated by the person of ordinary skill in the art that information such as binding constants and Ki derived from in vitro GLP-2 binding competition assays may also be used in calculating dosages, as well as the calculated half-life of the GLP-2 analog in vivo.

[**0100**] A typical human dose of a GLP-2 peptide would be from about 10 μg/kg body weight/day to about 10 mg/kg/day, preferably from about 50 μg/kg/day to about 5 mg/kg/day, and most preferably about 100 μg/kg/day to 1 mg/kg/day. As the GLP-2 analogs of the invention can be up to 10 to even 100 times more potent than GLP-2, a typical dose of such a GLP-2 analog may be lower, for example, from about 100 ng/kg body weight/day to 1 mg/kg/day, preferably 1 μg/kg/day to 500 μg/kg/day, and even more preferably 1 μg/kg/day to 100 μg/kg/day.

[**0101**] Similarly, the intestinotrophic GLP-2 analogs of the invention are also useful for both augmenting bowel growth and treating inflammatory disorders in livestock and pets.

EXAMPLE 1

GLP-2 analog synthesis

[**0102**] Solid phase peptide synthesis (SPPS) is carried out manually in a 300 milliliter (ml) vessel on a 3 millimole (mmole) scale using 6 grams (g) of chloromethyl (Merrifield) resin (for C-terminal free acid peptides) with a substitution of 0.5 milliequivalents (meq) per gram. Amino acids are protected at the amino-terminus with the t-butyloxycarbonyl (tBoc) group. The side-chains of trifunctional amino acids are protected with the benzyl (Bz, for serine and threonine), benzyloxymethyl (BOM, for histidine), 2-bromobenzyloxycarbonyl (2-BrZ, for tyrosine), 2-chlorobenzyloxycarbonyl (2-ClZ, for lysine), cyclohexyl (cHex, for aspartic and glutamic acids), and tosyl (Tos, for arginine) groups. The first amino acid is coupled to the chloromethyl resin through esterification of the protected amino acid in the presence of potassium fluoride (KF). C-terminal amide peptides are synthesized on a 4-methylbenzhydrylamine (MBHA) resin on a 3 mmol scale using 6 g of resin with a substitution of 0.5 meq/g. The first amino acid is coupled to the MBHA resin according to the procedure described for peptide elongation.

[**0103**] Amino-group deprotection is carried out using 50% trifluoroacetic acid (TFA) in dichloromethane (CH_2Cl_2), followed by neutralization using two washes of 10% triethylamine (Et_3N) in CH_2Cl_2. Peptide elongation is carried out using N,N-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt) activation in CH_2Cl_2/dimethylformamide (DMF). The growing peptide chain is capped after each elongation step with 20% Ac_2O in CH_2Cl_2. The peptide-resin is washed after each elongation, capping and deprotection step with isopropanol (iPrOH) and methanol (MeOH). The washes are repeated once. N-terminal acetyl peptides are prepared by acetylation of the terminal amino-group with 20% Ac_2O in CH_2Cl_2 after deprotection and neutralization as described. Resin-bound products are routinely cleaved by a low-high procedure using hydrogen fluoride (HF) containing dimethylsulfide (DMS) and p-cresol as scavengers.

[**0104**] Crude peptides are purified by preparative high pressure liquid chromatography (HPLC) using a Vydac C18, 15-20 μm wide-pore, 2 inch×12 inch, reverse-phase silica column using gradient elution with 0.1% TFA in water modified with acetonitrile. Elution is monitored at 220 nanometers (nm). Each fraction collected is analyzed for purity by analytical HPLC using a Vydac C18, 5 μm, 4.6×254 millimeter (mm), reverse-phase silica column by gradient elution using 0.1% TFA in water modified with acetonitrile, and monitored at 215 nm. Fractions demonstrating greater than 95% purity are combined and lyophilized. Acetate salts of the peptides are prepared from the TFA salts by dissolution of the lyophilized powder in water, with addition of acetonitrile to aid in dissolution where necessary. The solution is passed through a protonated Bio-Rex-70 cation exchange resin. The resin is washed with 5 bed-volumes of water, and the resin-bound peptide is eluted with 50% acetic acid in water. The eluent is diluted with water and lyophilized.

[0105] The final lyophilized powder is analyzed for purity by two analytical reverse-phase HPLC methods using a Vydac C18, 5 μm, 4.6×254 mm reverse-phase silica column. The two solvent systems used are a gradient of water adjusted to pH 2.25 with triethylamine phosphate, modified with acetonitrile, and a gradient of 0.1% TFA in water, modified with acetonitrile. The column eluent is monitored at 215 nm. The identity of each product is confirmed by amino acid analysis and by electrocopy-mass spectroscopy.

[0106] The GLP-2 analogs are next formulated as described below in Example 2. Each of the GLP-2 analogs is fully soluble in water at room temperature unless otherwise noted.

EXAMPLE 2

GLP-2 analog formulation

[0107] The GLP-2 analogs were formulated for injection either in phosphate buffered saline or as a gelatin-containing depot formulation. For the PBS-formulated GLP-2 analog preparations, a 10X stock PBS solution was first prepared, using 80 g NaCl (BDH ACS 783), 2 g KCl (BDH ACS 645), 11.5 g Na_2HPO_4 (Anachemia AC-8460), and 2 g KH_2PO_4 (Malinckrodt AR7100), which was brought to a total volume of one litre with sterile distilled water. The final working solution was obtained by 10:1 dilution of the stock solution with sterile distilled water and adjusted to pH 7.3-7.4 if necessary, using sufficient volumes of 10 N Na OH. The working solution was then autoclaved for 30 minutes. In the final working PBS solution, concentrations were 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4.7H20, and 1.4 mM KH_2PO_4.

[0108] The GLP-2 analog, as a powdered peptide, is added to the working PBS solution as required to generate formulations having the desired peptide concentrations. For example, to generate a PBS solution of GLP-2 analog at 130 mg/l, 5.2 mg of GLP-2 analog is dissolved in 40 ml of PBS to yield a GLP-2 concentration of 130 μg/ml, 0.5 ml is injected twice daily.

[0109] To generate the gelatin-based GLP-2 analog formulations, a gelatin solution was first prepared by dissolving 12 grams of gelatin (Sigma, G-8150 Lot #54H07241 Type A from Porcine skin [9000-70-8]~300 Bloom) in 100 ml distilled water. The gelatin solution was then autoclaved, warmed at 37° C., and the GLP-2 peptide previously dissolved in phosphate buffered saline as described above was then added to achieve specific, desired peptide concentrations. For instance, to generate a gelatin-based PBS solution of the GLP-2 at a concentration of 13 mg/l, 10 ml of a PBS solution prepared with 5.2 mg of GLP-2 was diluted with 30 ml of the 20% working gelatin solution as first described above. The solution was mixed by gentle pipeting, to yield a final solution of 130 mg/l GLP-2 in PBS/15% gelatin.

EXAMPLE 3

Assay for Resistance to DiPeptidyl Peptidase IV

[0110] The following peptides were tested for resistance to dipeptidyl peptidase IV (DPP-IV): a control peptide, rGLP-2; the [D-Ala^2]rGLP-2 analog; and the [Gly^2]rGLP-2 analog. To perform the assay, 2.5 microliters (μl) of a solution of human placental DPP-IV (Calbiochem, La Jolla, CA, cat. # 317624) containing 0.125 milliunits (mU) of enzyme in 50% glycerol, 10 mM Tris, pH 7.8, EDTA and 0.02% NaN_3 was added to 50 μl of a solution of the test peptide prepared at a concentration of 0.2 mg/ml in PBS at pH 7.4. The mixture was incubated at 37° C. in a circulating water bath for 24 hours. The incubation was quenched by the addition of 50 μl of a solution of diprotin A prepared at a concentration of 4 mg/ml in PBS. Each peptide was tested in duplicate.

[0111] Each sample was analyzed by reverse-phase (RP) HPLC as follows: 90 μl of the quenched incubation mixture was injected onto a Rainin Dynamax 300 Å, C18, 5 micron, 4.6×250 millimeter column. The samples were eluted with 0.1% trifluoroacetic acid (TFA) in water modified with 0.1% acetonitrile using a linear gradient and a flow rate of 1 ml per minute. Sample components were detected at 214 nanometers (nm). The extent of cleavage was measured by relative integration of the peak corresponding to the cleavage product compared to that of the remaining undigested parent peptide. The cleavage product of the control peptide, rGLP-2(1-33), which should be rGLP-2(3-33), was confirmed to have result from cleavage between residues Ala^2 and Asp^3 by comparison of the retention time of this component to that of a synthetic peptide standard, rGLP-2(3-33), and by collection of the product from the HPLC and analysis by mass spectrometry.

[0112] After the 24 hour incubation, 22% of the control peptide, rGLP-2, was cleaved by DPP-IV. No cleavage products were detected for the peptides [D-Ala^2]rGLP-2 and [Gly^2]rGLP-2 after 24 hours.

EXAMPLE 4

GLP-2 analog assessment

[0113] Recipients were CD1 mice obtained from Charles River Laboratory (Ontario, Canada). The CD1 mice were aged-matched females at time of injection (n=3-4 per group), 6 weeks of age, unless otherwise specified. The animals were allowed a minimum of 24 hours to acclimatize to the laboratory facility before the initiation of each experiment. Animals were identified by ear punch. The mice were not restricted by diet or activity during the experiments. The light/dark cycle was 12 hours, between 6 pm to 6 am. Controls were age-and sex-matched (n=3-4) animals. Mice were injected subcutaneously, twice a day (b.i.d.), with 2.5 μg peptide in a total volume of 0.5 cc of PBS and were monitored daily in the laboratory facility. Animals were sacrificed 10 or 14 days after injection, and were fasted at least 20 hours before sacrifice.

[0114] The mice were anaesthetised with $C0_2$ and exsanguinated by cardiac puncture. Blood was collected in 75 μl of TED (Trasysol; EDTA (5000 KIU/ml: 1.2 mg/ml; Diprotin-A), and the blood was centrifuged at 14 k×g for 5 minutes and the plasma was stored at −70 prior to analysis. The small bowel was removed from the peritoneal cavity, from pylorus to cecum, cleaned weighed and measured. For comparative purpose, sections from each animal were obtained from the identical anatomical position. Fragments each measuring 1.5-2.0 cm in length were obtained 8±2 cm, 18±2 cm, 32±2 cm from pylorus for histomorphometry representing proximal jejunum, distal jejunum and distal ileum. Each small bowel fragment was opened longitudi-

nally on its antimesenteric border in a tissue block and then placed on 10% formalin (vol./vol.) overnight, then transferred to 70% ETOH.

[0115] Percentage change in small bowel weight was calculated by dividing the mean change in bowel weight of analog treated mice, relative to mice treated with vehicle only, by the mean bowel weight of mice treated with vehicle only, and multiplying this figure by 100.

[0116] Results of intestinotrophic activity assessment are shown in Table 1:

TABLE 1

#	GLP-2 analog (1-33 unless noted)	% Increase in small bowel weight
1	rGLP-2	45
2	[Tyr^1]rGLP-2	37
3	[D-Ala^2]rGLP-2	86
4	[Gly^2]rGLP-2	70
5	[Val^2]rGLP-2	65
6	[Gly^2]hGLP-2	59
7	[Gly^2Ala^{20}]hGLP-2	46
8	[Gly^2Ala^{10}]hGLP-2	33
9	[Ala^1Gly^2]hGLP-2	23
10	[Gly^2Ala^3]hGLP-2	18
11	[Gly^2Ala^4]hGLP-2	36
12	[Glu^3]rGLP-2	33
13	[Ala^4]rGLP-2	30
14	[$Tyr^9Ser^{10}Lys^{11}Tyr^{12}$(desIle^{13})] hGLP-2	41
15	[Leu^{10}]rGLP-2	26
16	[$Nleu^{10}$]rGLP-2	52
17	[Met SO_2^{10}]rGLP-2	8
18	[Lys^{20}]rGLP-2	62
19	[$Val^{23}Gln^{24}$]hGLP-2	36
20	Amidated C-term	23
21	[Gly^2Ala^{24}]hGLP-2	67
22	[Gly^2Ala^8]hGLP-2	33
23	[Gly^2Ala^{11}]hGLP-2	42
24	[Gly^2Ala^{21}]hGLP-2	35
25	[Gly^2Ala^9]hGLP-2	31
26	[Gly^2Ala^{16}]hGLP-2	36
27	[Gly^2Ala^{17}]hGLP-2	36
28	[Gly^2Ala^{28}]hGLP-2	31
29	[Gly^2Ala^5]hGLP-2	38
30	[Gly^2Ala^{31}]hGLP-2	28
31	[Gly^2Ala^{27}]hGLP-2	22
32	[Gly^2Ala^{12}]hGLP-2	18
33	[Gly^2Ala^{13}]hGLP-2	18
34	[Gly^2Ala^7]hGLP-2	22
35	[Gly^2Ala^6]hGLP-2	18

[0117] It can be readily seen from the above table that analogs of mammalian GLP-2 molecules can have enhanced intestinotrophic activity. Furthermore, it is clear that depending on the substitution made, various levels of intestinotrophic activity are manifest. For example, [Gly^2]rGLP-2 has a greatly enhanced intestinotrphic activity compared to the naturally occuring molecule; [Gly^2]hGLP-2 has a substantially increased intestinotrophic activity compared with rGLP-2 and (Leu^{10}]rGLP-2 has less than a 50% increase in intestinotrophic activity.

[0118] The following experiments were conducted to confirm that the increase in bowel weight seen in experimental animals treated with DPP-IV resistant analogs of GLP-2, compared with animals treated with wild-type GLP-2, was attributable in part to the DPP-IV resistant nature of the molecules. This experiment took advantage of the availability of a DPP-IV deficient rat strain, Fisher 334 DPP-IV- rats. The effect of rGLP-2 injections on small bowel weight in the Fisher DPP-IV deficient rats was compared to that in normal Sprague-Dawley rats.

[0119] In the following experiments the Sprague-Dawley rats were injected s.c. twice daily with rGLP-2 in the Gelatin formulation. The Fisher rats were injected s.c. twice daily with GLP-2 peptides (both rGLP-2, and rGLP-2 analogs) in PBS.

[0120] Normal Sprague-Dawley rats treated with rGLP-2, 2.5 μg b.i.d. or 25 μg b.i.d., showed no % change in small bowel weight compared to control animals given vehicle alone. However, Fisher 334 DPP-IV-rats (DPP-IV deficient animals) demonstrated approximately a 40-50% increase in % change in small bowel weight when treated with 20 μg b.i.d. rGLP-2 compared to animals treated with vehicle only. Moreover, DPP-IV deficient animals treated with 20 μg [Gly^2]rGLP-2 showed a 50-60% increase in % change in small bowel weight compared with animals given vector alone. Further, Fisher 344 wild-type rats showed 75-85% increase in small bowel weight, over control animals given vehicle alone, when treated with 20 μg b.i.d. of [Gly^2]rGLP-2.

[0121] The above results strongly indicate that GLP-2 is inactivated in vivo in normal rats by cleavage of the two N-terminal residues by a DPP-IV-like enzyme. Furthermore, these results demonstrate that a GLP-2 analog modified so as to be resistant to cleavage by DPP-IV caused a substantial increase in bowel weight in normal rats, presumably as a result of increased GPL-2 half-life in vivo.

[0122] As the DPP-IV cleavage site is conserved in all known naturally occurring forms of GLP-2 it seems likely that in mammals DPP-IV cleavage is an important, and probably the primary mechanism whereby GLP-2 is inactivated in vivo. Importantly, GLP-2 analogs resistant to DPP-IV cleavage have enhanced intestinotrophic activity compared to native form.

EXAMPLE 5

[0123] Experiments assessing the small bowel inducing activity of various analogs was repeated as described above for Example 4. In these experiments, the small bowel inducing activity of each analog in mice was calculated relative to that of native rat GLP-2(1-33) (expressed as 100% of activity). Resistance of analogs to DPP-IV cleavage was performed as described above in Example 3. Results are tabulated below in Table 2.

TABLE 2

Identification	Description of Sequence ALE-0109	% Activity Rel. to rGLP-2	% Cleaved by DPP-IV
ratGLP-2(1-33)	Native rat sequence	100%	58%
ratGLP-2(4-33)	N-3; 3 N-terminal res. removed	6%	
Ac-ratGLP-2(1-33)	N-Acetyl	44%	0%
anglerfish GLP-1	Native anglerfish GLP-1	7%	
[Arg-1]ratGLP-2(-1-33)	Arg added to N-terminus	11%	0%
ratGLP-2(1-30)	C-3; 3 C-terminal res. removed	23%	

TABLE 2-continued

Identification	Description of Sequence ALE-0109	% Activity Rel. to rGLP-2	% Cleaved by DPP-IV
ratGLP-2(1-25)	C-8; 8 C-terminal res. removed	8%	
ratGLP-2(1-33)amide	C-terminal amide	56%	
[Arg-2,Arg-1]ratGLP-2 (2-33)	Arg—Arg added to N-terminus	43%	0%
[DAla2]ratGLP-2(1-33)	Ala2–>D-Ala2	210%	0%
[Gly2]ratGLP-2(1-33)	Ala2–>Gly2	196%	0%
[Tyr1]ratGLP-2(1-33)	His1–>Tyr1	81%	100%
[Ala4]ratGLP-2(1-33)	Gly4–>Ala4	59%	72%
[Glu3]ratGLP-2(1-33)	Asp3–>Glu3	65%	50%
[Leu10]ratGLP-2(1-33)	Met10–>Leu10	51%	
[Nle10]ratGLP-2(1-33)	Met10–>Nle10	100%	
[Lys20]ratGLP-2(1-33)	Arg20–>Lys20	120%	
[Ser2,Gln3]hGLP-2(1-33)	Ala2–>Ser2, Asp3–>Glu3	11%	0%
[Val23,Gln24]hGLP-2 (1-33)	Ile23–>Val23, Asn24–>Gln24	82%	
[Val2]GLP-2(1-33)	Ala2–>Val2, TFA	145%	0%
Degu GLP-2	Native degu sequence	70%	
[Tyr9,Ser10,Lys11,Tyr12, des-Ilel3]hGLP-2(1-33)	Glu9–>Tyr9, Met10–>Ser10, Asn11–>Lys11, Thr12–>Tyr12, and deletion of Ile13	94%	
[Gly2,Ala8]hGLP-2(1-33)	Ala2–>Gly2, Asp8–>Ala8	120%	
[Gly2,Ala11]hGLP-2(1-33)	Ala2–>Gly2, Asn11–>Ala11	150%	
[Gly2,Ala21]hGLP-2(1-33)	Ala2–>Gly2, Asp21–>Ala21	125%	
[Gly2,Ala24]hGLP-2(1-33)	Ala2–>Gly2, Asn24–>Ala24	139%	0%
[Gly2,Ala25]hGLP-2(1-33)	Ala2–>Gly2, Trp25–>Ala25	18%	
[Gly2,Ala26]hGLP-2(1-33)	Ala2–>Gly2, Leu26–>Ala26	28%	
[Gly2,Ala27]hGLP-2(1-33)	Ala2–>Gly2, Ile27–>Ala27	82%	
[Gly2,Ala9]hGLP-2(1-33)	Ala2–>Gly2, Glu9–>Ala9	112%	
[Gly2,Ala10]hGLP-2(1-33)	Ala2–>Gly2, Met10–>Ala10	82%	
[Gly2,Ala12]hGLP-2(1-33)	Ala2–>Gly2, Thr12–>Ala12	67%	
[Gly2,Ala13]hGLP-2(1-33)	Ala2–>Gly2, Ile13–>Ala13	67%	
[Gly2,Ala14]hGLP-2(1-33)	Ala2–>Gly2, Leu14–>Ala14	30%	
[Gly2,Ala16]hGLP-2(1-33)	Ala2–>Gly2, Asn16–>Ala16	130%	
[Gly2,Ala17]hGLP-2(1-33)	Ala2–>Gly2, Leu17–>Ala17	130%	
[Gly2,Ala20]hGLP-2(1-33)	Ala2–>Gly2, Arg20–>Ala20	105%	
[Gly2,Ala23]hGLP-2(1-33)	Ala2–>Gly2, Ile23–>Ala23	24%	
[Gly2,Ala28]hGLP-2(1-33)	Ala2–>Gly2, Gln28–>Ala28	130%	
[Gly2,Ala30]hGLP-2(1-33)	Ala2–>Gly2, Lys30–>Ala30	24%	
[Gly2,Ala31]hGLP-2(1-33)	Ala2–>Gly2, Ile31–>Ala31	100%	
[Gly2]hGLP-2(1-33)	Ala2–>Gly2	300%	0%
[Ala1,Gly2]hGLP-2(1-33)	His1–>Ala1, Ala2–>Gly2	53%	0%
[Gly2,Ala3]hGLP-2(1-33)	Ala2–>Gly2, Asp3–>Ala3	44%	0%
[Gly2,Ala4]hGLP-2(1-33)	Ala2–>Gly2, Gly4–>Ala4	100%	0%
[Gly2,Ala5]hGLP-2(1-33)	Ala2–>Gly2, Ser5–>Ala5	136%	0%

TABLE 2-continued

Identification	Description of Sequence ALE-0109	% Activity Rel. to rGLP-2	% Cleaved by DPP-IV
[Gly2,Ala6]hGLP-2(1-33)	Ala2–>Gly2, Phe6–>Ala6	67%	
[Gly2,Ala7]hGLP-2(1-33)	Ala2–>Gly2, Ser7–>Ala7	82%	
[Pro1]hGLP-2(1-33)	His1–>Pro1	12%	0%
[Met(O)10]ratGLP-2(1-33)	Met10 sulfoxide	18%	
[Gln20]hGLP-2(1-33)	Arg20–>Gln20	80%	
[Asp1]hGLP-2(1-33)	His1–>Asp1	60%	0%
[tBuGly2]hGLP-2	Ala2–>t-butyl-Gly2	123%	0%
[Tyr34]hGLP-2(1-34)	Addition of C-terminal Tyr	135%	
[Asp2]hGLP-2(1-33)	Ala2–>Asp2	83%	0%
[Glu2]hGLP-2(1-33)	Ala2–>Glu2	93%	0%
[Phe2]hGLP-2(1-33)	Ala2–>Phe2	50%	0%
[His2]hGLP-2(1-33)	Ala2–>His2	0%	0%
[Ile2]hGLP-2(1-33)	Ala2–>Ile2	90%	0%
[Lys2]hGLP-2(1-33)	Ala2–>Lys2	90%	0%
[Met2]hGLP-2(1-33)	Ala2–>Met2	170%	0%
[Asn2]hGLP-2(1-33)	Ala2–>Asn2	67%	0%
[Pro2]hGLP-2(1-33)	Ala2–>Pro2	50%	16%
[Gln2]hGLP-2(1-33)	Ala2–>Gln2	100%	0%
[Ser2]hGLP-2(1-33)	Ala2–>Ser2	167%	0%
[Thr2]hGLP-2(1-33)	Ala2–>Thr2	117%	0%
[Val2]hGLP-2(1-33)	Ala2–>Val2	180%	13%
[Tyr2]hGLP-2(1-33)	Ala2–>Tyr2	57%	0%
[D-Ala2]hGLP-2(1-33)	Ala2–>D-Ala2	130%	0%
hGLP-2(1-33)	Native human sequence	100%	60%
[desNH2Tyr1]hGLP-2 (1-33)	His1–>desamino-Tyr1	125%	15%
[Thr5]hGLP-2(1-33)	Ser5–>Thr5	73%	
[Ser16,Arg17,Arg18] hGLP-2(1-33)	Asn16–>Ser16, Leu17–>Arg17, Ala18–>Arg18	59%	
[Asn33]hGLP-2(1-33)	Asp33–>Asn33	91%	
[Pen2]hGLP-2(1-33)	Ala2–>L-penicillamine2	58%	20%
[bAla2]hGLP-2(1-33)	Ala2–>beta-Ala2	118%	0%
[aAbu2]hGLP-2(1-33)	Ala2–>alpha-aminobutyric acid2	84%	49%
[Nval2]hGLP-2(1-33)	Ala2–>Nval2 (norvaline)	111%	0%
[PhGly2]hGLP-2(1-33)	Ala2–>L-phenyl-Gly2	37%	0%
[Agm34]hGLP-2(1-34)	Addition of C-terminal agmatine (Agm is des-COOH Arg)	97%	
[Arg30]hGLP-2(1-33)	Lys30–>Arg30	103%	59%
[Pro3]hGLP-2(1-33)	Asp3–>Pro3	140%	0%
[Ala5, Ala7]hGLP-2(1-33)	Ser5–>Ala5, Ser7–>Ala7	108%	77%
[Tyr1, Gly2]hGLP-2(1-33)	His1–>Tyr1, Ala2–>Gly2	241%	0%
[Glu33]hGLP-2(1-33)	Asp33–>Glu33	121%	55%
[Phe25]hGLP-2(1-33)	Trp25–>Phe25	114%	54%
[Tyr25]hGLP-2(1-33)	Trp25–>Tyr25	64%	48%
[Gly2, Tyr34]hGLP-2(1-34)	Ala2–>Gly2 + C-terminal Tyr	297%	0%
[Aib2]hGLP-2(1-33)	Ala2–>aminoisobutyric acid2	202%	0%
[Gly2, Arg34]hGLP-2 (1-34)	Ala2–>Gly2 + C-terminal Arg	~250%	0%

EQUIVALENTS

[0124] The foregoing written specification is sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-described means for carrying out the invention which are obvious to those skilled in the field of molecular biology, protein chemistry, medicine or related fields are intended to be within the scope of the following claims.

What is claimed is:

1. A GLP-2 analog which is characterized by intestinotrophic activity and which conforms to Formula 1:

R1-(Y1)m-X1-X2-X3-X4-Ser5-Phe6-Ser7-Asp8-(P1)-Leu14-Asp15-Asn16-Leu17- Ala18-X19-X20-Asp21-Phe22-(P2)-Trp25-Leu26-Ile27-Gln-28-Thr29-Lys30-(P3)-(Y2)n-R2, wherein

X1 is His or Tyr

X2 is Ala or an Ala-replacement amino acid conferring on said analog resistance to DPP-IV enzyme;

X3 is Pro, HPro, Asp or Glu;

X4 is Gly or Ala;

P1 is Glu-X10-Asn-Thr-Ile or Tyr-Ser-Lys-Tyr;

X10 is Met or an oxidatively stable Met-replacement amino acid;

X19 is Ala or Thr;

X20 is Arg, Lys, His or Ala;

P2 is Ile-Asn, Ile-Ala or Val-Gln;

P3 is a covalent bond, or is Ile, Ile-Thr or Ile-Thr-Asn;

R1 is H or an N-terminal blocking group;

R2 is OH or a C-terminal blocking group;

Y1 is one or two basic amino acids selected from the group Arg, Lys, and His;

Y2 is one or two basic amino acids selected from the group Arg, Lys, and His; and

m and n, independently, are 0 or 1; and

wherein at least one of X1, X2, X3, X4, P1, X10, X19, X20, P2 and P3 is other than a wild type, mammalian GLP-2 residue.

2. The GLP-2 analog according to claim 1, wherein said analog incorporates at least one amino acid substitution selected from:

a) incorporation at X2 of an amino acid which renders said intestinotrophic GLP-2 analog resistant to cleavage by human DPP-IV enzyme;

b) incorporation at X10 of an oxidatively stable, Met-replacement amino acid; and

c) incorporation at X20 of a basic amino acid selected from His or Lys.

3. The GLP-2 analog according to claim 2, wherein X2 is substituted with an amino acid which confers on said analog resistance to digestion by DPP-IV enzyme.

4. The GLP-2 analog according to claim 3, wherein X2 is selected from the group consisting of D-hPr, D-Pro, D-Ala, Gly, Val, Glu, Lys, Arg, Leu, and Ile.

5. The GLP-2 analog according to claim 3 which incorporates at least one amino acid substitution at the following positions: X1, X3, X4, X10, X19, X20 and P2.

6. The GLP-2 analog according to claim 2, wherein X10 is substituted with an amino acid chosen from the group consisting of Val, Ile, Asn, Glx, Tyr, Phe, Leu, Nle, Ala, Ser, and Gly.

7. The GLP-2 analog according to claim 1 selected from the group consisting of: [Tyr^{1}]rGLP-2; [Ala^{4}]rGLP-2; [$Val^{23}Gln^{24}$]hGLP-2 and [Asn^{33}]hGLP-2(1-33).

8. A pharmaceutical composition comprising a therapeutically effective amount of a GLP-2 analog according to claim 1 and a pharmaceutically acceptable carrier.

9. A method for promoting growth of small bowel tissue in a patient in need thereof, comprising the step of delivering to the patient the pharmaceutical composition of claim 8.

10. A method for treating a gastrointestinal disease, wherein the method comprises administering to a patient having the gastrointestinal disease a therapeutically effective amount of a GLP-2 analog according to claim 1, together with a pharmaceutically acceptable carrier to reduce a pathological effect or symptom of the gastrointestinal disease.

11. The method of claim 10, wherein the gastrointestinal disease is selected from the group consisting of ulcers, digestion disorders, malabsorption syndromes, short-gut syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue, tropical sprue, hypogammaglobulinemic sprue, enteritis, regional enteritis (Crohn's disease), small intestinal damage due to toxic or other chemotherapeutic agents, and short bowel syndrome.

12. An analog of a human GLP-2 peptide, wherein the GLP-2 analog comprises at least one amino acid substitution selected from the group consisting of:

a) incorporation at position X2 and/or X3 of a replacement amino acid which renders the analog resistant to cleavage by DPP-IV enzyme;

b) incorporation at position X10 of a replacement amino acid which is oxidatively stable; and

c) incorporation at position X20 of a replacement amino acid other than Arg.

13. The GLP-2 analog according to claim 12 which incorporates an amino acid substitution at position X2, and wherein the amino acid substituted at position X2 is chosen from the group consisting of D-hPr, D-Pro, D-Ala, Gly, Val, Glu, Lys, Arg, Leu, and Ile.

14. The GLP-2 analog of claim 13, wherein the analog is selected from the group consisting of:

[Gly^{2}]hGLP-2;

[D-$Ala^{2}Thr^{19}$]hGLP-2;

[$Gly^{2}Thr^{19}$]hGLP-2;

[$Ala^{1}Gly^{2}$]hGLP-2;

[$Gly^{2}Ala^{3}$]hGLP-2;

[$Gly^{2}Ala^{4}$]hGLP-2;

[$Gly^{2}Ala^{5}$]hGLP-2;

[$Gly^{2}Ala^{6}$]hGLP-2;

[$Gly^{2}Ala^{7}$]hGLP-2;

[$Gly^{2}Ala^{8}$]hGLP-2;

[$Gly^{2}Ala^{9}$]hGLP-2;

[$Gly^{2}Ala^{10}$]hGLP-2;

[$Gly^{2}Ala^{11}$]hGLP-2;

[$Gly^{2}Ala^{12}$]hGLP-2;

[$Gly^{2}Ala^{13}$]hGLP-2;

[$Gly^{2}Ala^{16}$]hGLP-2;

[Gly^{2}Ala^{17}]hGLP-2;

[Val^{2}Thr^{19}]hGLP-2;

[Gly^{2}Ala^{20}]hGLP-2

[Gly^{2}Ala^{12}]hGLP-2;

[Gly^{2}Ala^{24}]hGLP-2;

[Gly^{2}Ala^{27}]hGLP-2;

[Gly^{2}Ala^{28}]hGLP-2; and

[Gly^{2}Ala^{31}]hGLP-2.

15. The GLP-2 analog according to claim 12 which incorporates an amino acid substitution at position X10, wherein the amino acid substituted at position X10 is chosen from the group consisting of Val, Ile, Asn, Glx, Tyr, Phe, Leu, Nle, Ala, Ser, and Gly.

16. The GLP-2 analog according to claim 15, wherein the analog is selected from the group consisting of [Ser^{10}] hGLP-2(1-33); [Nle^{10}]hGLP-2(1-33); [Ala^{10}]hGLP-2(1-33); [Leu^{10}]rGLP-2(1-33); [Nle^{10}]ratGLP-2(1-33); [Gly^{2}Ala^{10}]hGLP-2(1-33); [Met())^{10}]ratGLP-2(1-33) and [Tyr^{9}Ser^{10}Lys^{11}Tyr^{12} (desIle^{13})]hGLP-2(1-33).

17. The GLP-2 analog according to claim 12 wherein the nalog is selected from the group consisting of [Pro^{3}]hGLP-2; [HPr^{3}]hGLP-2; [Glu^{3}Thr^{19}]hGLP-2; and [Thr^{19}Lys^{20}]hGLP-2.

18. The GLP-2 analog of claim 12, wherein the analog is elected from the group consisting of:

[Ser^{2},Gln^{3}]hGLP-2(1-33);

[Gly^{2}, Ala^{24}]hGLP-2(1-33);

[Gly^{2}, Ala^{26}]hGLP-2(1-33);

[Gly^{2}, Ala^{14}]hGLP-2(1-33);

[Gly^{2}, Ala^{23}]hGLP-2(1-33);

[Gly^{2}, Ala^{30}]hGLP-2(1-33);

[Tyr^{1}, Gly^{2}]hGLP-2(1-33);

[Gly^{2}, Arg^{34}]hGLP-2(1-34);

[Gly^{2}, Tyr^{34}]hGLP-2(1-34);

[tBuGly^{2}]hGLP-2;

[Asp^{2}]hGLP-2 (1-33);

[Glu^{2}]hGLP-2 (1-33);

[Phe^{2}]hGLP-2(1-33);

[His^{2}]hGLP-2(1-33) ;

[Ile^{2}]hGLP-2(1-33);

[Lys^{2}]hGLP-2 (1-33);

[Met^{2}]hGLP-2(1-33);

[Asn^{2}]hGLP-2(1-33);

[Pro^{2}]hGLP-2(1-33)

[Gln^{2}]hGLP-2 (1-33);

[Ser^{2}]hGLP-2 (1-33);

[Thr^{2}]hGLP-2(1-33);

[Val^{2}]hGLP-2(1-33);

[Tyr^{2}]hGLP-2(1-33);

[D-Ala^{2}]hGLP-2 (1-33);

[Pen^{2}]hGLP-2(1-33);

[bAla^{2}]hGLP-2(1-33);

[aAbu^{2}]hGLP-2 (1-33);

[Nval^{2}]hGLP-2 (1-33);

[PhGly^{2}]hGLP-2(1-33); and

[Aib^{2}]hGLP-2(1-33).

19. A pharmaceutical composition comprising a therapeutically effective amount of a GLP-2 analog according to claim 12, and a pharmaceutically acceptable carrier.

20. A method for promoting growth of small bowel tissue in a patient in need thereof, comprising the step of delivering to the patient the pharmaceutical composition of claim 19.

21. A method for treating a gastrointestinal disease, wherein the method comprises administering to a patient having the gastrointestinal disease a therapeutically effective amount of a GLP-2 analog according to claim 12, together with a pharmaceutically acceptable carrier to reduce a pathological effect or symptom of the gastrointestinal disease.

22. The method of claim 21, wherein the gastrointestinal disease is selected from the group consisting of ulcers, digestion disorders, malabsorption syndromes, short-gut syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue, tropical sprue, hypogammaglobulinemic sprue, enteritis, regional enteritis (Crohn's disease), small intestinal damage due to toxic or other chemotherapeutic agents, and short bowel syndrome.

23. A method of identifying intestinotrophic analogs of GLP-2, comprising the steps of:

a) obtaining a GLP-2 analog according to claim 1;

b) treating a mammal with said sing a regimen capable of eliciting an intestinotrophic effect when utilized for rat GLP-2; and

c) determining the effect of said analog on small bowel weight relative to a mock treated control mammal, whereby said intestinotrophic analog of GLP-2 is identified as an analog which elicits an increase in said weight.

24. An intestinotrophic GLP-2 analog selected from the group consisting of:

ratGLP-2(4-33)

Ac-ratGLP-2(1-33);

ratGLP-2(1-30);

ratGLP-2(1-25);

ratGLP-2(1-33)amide;

[Arg-^{2},Arg-^{1}]ratGLP-2 (2-33);

[Pro^{1}]hGLP-2 (1-33);

[Gln^{20}]hGLP-2(1-33);

[Asp^{1}]hGLP-2 (1-33);

[Tyr^{34}]hGLP-2(1-34);

[desNH2Tyr^{1}]hGLP-2 (1-33);

[Thr^{5}]hGLP-2(1-33);

[ser^{16}, Arg^{17},Arg^{18}]hGLP-2(1-33);

[Agm^{34}]hGLP-2(1-34);

[Arg^{30}]hGLP-2(1-33);

[Ala^{5}, Ala^{7}]hGLP-2 (1-33);

[Glu^{33})hGLP-2(1-33);

[Phe^{25}]hGLP-2(1-33); and

[Tyr^{25}]hGLP-2(1-33).

25. A pharmaceutical composition comprising a therapeutically effective amount of a GLP-2 analog according to claim 24, and a pharmaceutically acceptable carrier.

26. A method for promoting growth of small bowel tissue in a patient in need thereof, comprising the step of delivering to the patient the pharmaceutical composition of claim 25.

27. A method for treating a gastrointestinal disease, wherein the method comprises administering to a patient having the gastrointestinal disease a therapeutically effective amount of a GLP-2 analog according to claim 24, together with a pharmaceutically acceptable carrier to reduce a pathological effect or symptom of the gastrointestinal disease.

28. The method of claim 27, wherein the gastrointestinal disease is selected from the group consisting of ulcers, digestion disorders, malabsorption syndromes, short-gut syndrome, cul-de-sac syndrome, inflammatory bowel disease, celiac sprue, tropical sprue, hypogammaglobulinemic sprue, enteritis, regional enteritis (Crohn's disease), small intestinal damage due to toxic or other chemotherapeutic agents, and short bowel syndrome.

29. The peptide [Gly^{2}]hGLP-2.

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