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(54) Title: GLUCAGON-LIKE PEPTIDE-1 ANALOGS

(57) Abstract: Disclosed are glucagon-like peptide-1 (GLP-1) compounds with modifications at one or more of the following positions: 11, 12, 16, 22, 23, 24, 25, 27, 30, 33, 34, 35, 36, or 37. Methods of treating these GLP-1 compounds are also disclosed.

GLUCAGON-LIKE PEPTIDE-1 ANALOGS

This application claims the benefit of U.S. Provisional Application Number 60/212,171, filed June 16, 2000 and U.S. Provisional Application Number 60/240,349, filed October 13, 2000.

Glucagon-Like Peptide 1 (GLP-1) is a 37 amino acid peptide that is secreted by the L-cells of the intestine in response to food ingestion. It has been found to stimulate insulin secretion (insulinotropic action), thereby causing glucose uptake by cells and decreased serum glucose levels (*see*, e g., Mojsov, S., *Int. J. Peptide Protein Research*, 40:333-343 (1992)). However, GLP-1(1-37) is poorly active and attention has been focused on truncated analogs, referred to as GLP compounds, which are biologically much more potent than GLP-1. Examples include GLP-1(7-37), GLP-1(7-36)NH_2, Gly^8-GLP-1(7-37)OH and Ser^{34}-GLP-1(7-37)OH. Because of their ability to stimulate insulin secretion, GLP compounds show great promise as agents for the treatment of diabetes, obesity, and related conditions.

GLP-1 compounds can exist in at least two different forms. The first form is physiologically active and dissolves readily in aqueous solution at physiological pH (7.4). In contrast, the second form has little or no insulinotropic activity and is substantially insoluble in water at pH 7.4. Unfortunately, the inactive form is readily produced when aqueous GLP-1 solutions are agitated, exposed to hydrophobic surfaces or have large air/water

interfaces. The tendency to convert to the insoluble form considerably complicates the production of commercial quantities of active GLP-1 compounds; mixing operations or continuous movement through a pump are common operations in bulk manufacturing processes and these operations cause the agitation, air/water interfaces and/or contact with hydrophobic surfaces that results in the insoluble form. Conversion to the inactive form may also occur during storage or after administration to a patient, further complicating the use of these compounds as drugs. Therefore, there is a great need for biologically active GLP-1 analogs which convert less readily to the insoluble form than currently available GLP-1 compounds.

It has now been found that a number of GLP-1 analogs with modifications at one or more of the following positions: 11, 12, 16, 22, 23, 24, 26, 27, 30, 33, 34, 35, 36 or 37, show markedly decreased propensity to aggregate compared with GLP-1(7-37)OH.

Many of these analogs retain GLP-1 receptor activation that is comparable and in some cases greater than known GLP-1 compounds such as GLP-1(7-37)OH and Val^{8}-GLP-1(7-37)OH. For example, the aggregation time of Val^{8}-Glu^{22}-GLP(7-37)OH is over twenty fold greater and its GLP-1 receptor activation is about 25% greater than GLP-1(7-37)OH. Based on these discoveries, novel GLP-1 compounds and methods of treatment using the novel GLP-1 compounds are disclosed herein.

One embodiment of the present invention is a polypeptide having the amino acid sequence of formula I (SEQ ID NO: 1):

$His-Xaa_8-Glu-Gly-Xaa_{11}-Xaa_{12}-Thr-Ser-Asp-Xaa_{16}-Ser-Ser-Tyr-Leu-Glu-Xaa_{22}-Xaa_{23}-Xaa_{24}-Ala-Xaa_{26}-Xaa_{27}-Phe-Ile-Ala-Xaa_{31}-Leu-Xaa_{33}-Xaa_{34}-Xaa_{35}-Xaa_{36}-R$

formula I (SEQ ID NO: 1)

wherein:

Xaa_8 is: Gly, Ala, Val, Leu, Ile, Ser, or Thr;

Xaa_{11} is: Asp, Glu, Arg, Thr, Ala, Lys, or His;

Xaa_{12} is: His, Trp, Phe, or Tyr;

Xaa_{16} is: Leu, Ser, Thr, Trp, His, Phe, Asp, Val, Glu, or Ala;

Xaa_{22} is: Gly, Asp, Glu, Gln, Asn, Lys, Arg, Cys, or Cysteic Acid;

Xaa_{23} is: His, Asp, Lys, Glu, or Gln;

Xaa_{24} is: Glu, His, Ala, or Lys;

Xaa_{26} is: Asp, Lys, Glu, or His;

Xaa_{27} is: Ala, Glu, His, Phe, Tyr, Trp, Arg, or Lys;

Xaa_{30} is: Ala, Glu, Asp, Ser, or His;

Xaa_{33} is: Asp, Arg, Val, Lys, Ala, Gly, or Glu;

Xaa_{34} is: Glu, Lys, or Asp;

Xaa_{35} is: Thr, Ser, Lys, Arg, Trp, Tyr, Phe, Asp, Gly, Pro, His, or Glu;

Xaa_{36} is: Arg, Glu, or His;

R is: Lys, Arg, Thr, Ser, Glu, Asp, Trp, Tyr, Phe, His, $-NH_2$, Gly, Gly-Pro, or Gly-Pro-NH_2, or is deleted.

provided that the polypeptide does not have the sequence of GLP-1(7-37)OH or GLP-1(7-36)-NH_2 and provided that the polypeptide is not Gly^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-36)NH_2, Val^8-GLP-1(7-37)OH, Val^8-GLP-1(7-36)NH_2, Leu^8-GLP-1(7-37)OH, Leu^8-GLP-1(7-36)NH_2, Ile^8-GLP-1(7-37)OH, Ile^8-GLP-1(7-36)NH_2, Ser^8-GLP-1(7-37)OH, Ser^8-GLP-1(7-36)NH_2, Thr^8-GLP-1(7-37)OH, or Thr^8-GLP-1(7-36)NH_2, Ala^{11}-Glp-1(7-37)OH, Ala^{11}-Glp-1(7-36)NH_2, Ala^{16}-Glp-1(7-37)OH, Ala^{16}-Glp-

1(7-36)NH_2, Ala^{27}-Glp-1(7-37)OH, Ala^{27}-Glp-1(7-36)NH_2, Glu^{27}-Glp-1(7-37)OH, Glu^{27}-Glp-1(7-36)NH_2, Ala^{33}-Glp-1(7-37)OH, or Ala^{33}-Glp-1(7-36)NH_2.

Another embodiment of the present invention is a polypeptide having the amino acid sequence of formula II (SEQ ID NO: 2):

His-Xaa_8-Glu-Gly-Thr-Xaa_{12}-Thr-Ser-Asp-Xaa_{16}-Ser-Ser-Tyr-Leu-Glu-Xaa_{22}-Xaa_{23}-Ala-Ala-Xaa_{26}-Glu-Phe-Ile-Xaa_{30}-Trp-Leu-Val-Lys-Xaa_{35}-Arg-R

formula II (SEQ ID NO: 2)

wherein:

Xaa_8 is: Gly, Ala, Val, Leu, Ile, Ser, or Thr;

Xaa_{12} is: His, Trp, Phe, or Tyr;

Xaa_{16} is: Leu, Ser, Thr, Trp, His, Phe, Asp, Val, Glu, or Ala;

Xaa_{22} is: Gly, Asp, Glu, Gln, Asn, Lys, Arg, Cys, or Cysteic Acid;

Xaa_{23} is: His, Asp, Lys, Glu, or Gln;

Xaa_{26} is: Asp, Lys, Glu, or His;

Xaa_{30} is: Ala, Glu, Asp, Ser, or His;

Xaa_{35} is: Thr, Ser, Lys, Arg, Trp, Tyr, Phe, Asp, Gly, Pro, His, or Glu;

R is: Lys, Arg, Thr, Ser, Glu, Asp, Trp, Tyr, Phe, His, $-NH_2$, Gly, Gly-Pro, or Gly-Pro-NH_2, or is deleted.

provided that the polypeptide does not have the sequence of GLP-1(7-37)OH or GLP-1(7-36)-NH_2 and provided that the polypeptide is not Gly^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-36)NH_2, Val^8-GLP-1(7-37)OH, Val^8-GLP-1(7-36)NH_2, Leu^8-GLP-1(7-37)OH, Leu^8-GLP-1(7-36)NH_2, Ile^8-GLP-1(7-37)OH, Ile^8-GLP-1(7-36)NH_2, Ser^8-GLP-1(7-37)OH, Ser^8-GLP-1(7-36)NH_2, Thr^8-GLP-1(7-37)OH, Thr^8-GLP-1(7-36)NH_2, Ala^{16}-GLP(7-37)OH, or Ala^{16}-Glp-1(7-36)NH_2.

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