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Title

Physically stable formulation of modified GLP-1

Background

Peptides are widely used in medical practice, and since they can be produced by recombinant DNA technology it can be expected that their importance will increase also in the years to come.

The hormones regulating insulin secretion belong to the so-called enteroinsular axis, designating a group of hormones, released from the gastrointestinal mucosa in response to the presence and absorption of nutrients in the gut, which promote an early and potentiated release of insulin. The enhancing effect on insulin secretion, the so-called incretin effect, is probably essential for a normal glucose tolerance. Many of the gastrointestinal hormones, including gastrin and secretin (cholecystokinin is not insulinotropic in man), are insulinotropic, but the only physiologically important ones, those that are responsible for the incretin effect, are the glucose-dependent insulinotropic polypeptide, GIP, and glucagon-like peptide-1 (GLP-1). Because of its insulinotropic effect, GIP, isolated in 1973 immediately attracted considerable interest among diabetologists. However, numerous investigations carried out during the following years clearly indicated that a defective secretion of GIP was not involved in the pathogenesis of insulin dependent diabetes mellitus (IDDM) or non insulin-dependent diabetes mellitus (NIDDM). Furthermore, as an insulinotropic hormone, GIP was found to be almost ineffective in NIDDM. The other incretin hormone, GLP-1 is the most potent insulinotropic substance known. Unlike GIP, it is surprisingly effective in stimulating insulin secretion in NIDDM patients. In addition, and in contrast to the other insulinotropic hormones (perhaps with the exception of secretin) it also potently inhibits glucagon secretion. Because of these actions it has pronounced blood glucose lowering effects particularly in patients with NIDDM.

GLP-1, a product of the proglucagon, is one of the youngest members of the secretin-VIP family of peptides, but is already established as an important gut hormone with regulatory function in glucose metabolism and gastrointestinal secretion and metabolism. The glucagon gene is processed differently in the pancreas and in the intestine. In the pancreas, the processing leads to the formation and parallel secretion of 1) glucagon itself, occupying positions 33-61 of proglucagon (PG); 2) an N-terminal peptide of 30 amino acids (PG (1-30)) often called glicentin-related pancreatic peptide, GRPP; 3) a hexapeptide corresponding to PG (64-69); 4) and, finally, the so-called major proglucagon fragment (PG (72-158)), in which the two glucagon-like sequences are buried. Glucagon seems to be the only biologically active product. In contrast, in the intestinal mucosa, it is glucagon that is buried in a larger molecule, while the two glucagon-like peptides are formed separately.

While much attention has been focused on the pharmacological properties of acylated GLP-1 compounds, hitherto little is known about their physico-chemical and solution structural properties. Such knowledge is a prerequisite for rational handling during e.g. production, purification and formulation work and is eventually important for understanding of the structural basis for the protraction mechanism.

GLP-1 and analogues of GLP-1 and fragments thereof are potentially useful *i.a.* in the treatment of type 1 and type 2 diabetes. However, solubility limitations and the low stability against the actions of endogenous diaminopeptidyl peptidase limits the usefulness of these compounds, and thus there still is a need for improvements in this field.

In WO 99/43341 are disclosed certain pharmaceutical formulations comprising GLP-1 having a lipophilic substituent. All of the disclosed formulations are maintained at pH 7.4.

In WO 00/37098 are disclosed shelf-stable formulations comprising GLP-1, a preservative, and a tonicity modifier, at pH 8.2 to 8.8. It is specifically stated that maintaining pH in a range of about 8.2 to about 8.8 unexpectedly improves the chemical stability of the formulation. In addition it is stated that the concentration of the GLP-1 molecule also plays a role in the stability of the formulations. In this respect it is stated that a GLP-1 concentration equal to or greater than 1mg/ml was physically unstable.

Summary of the invention

Human GLP-1 is a 37 amino acid residue peptide originating from preproglucagon which is synthesised *i.a.* in the L-cells in the distal ileum, in the pancreas and in the brain. Processing of preproglucagon to give GLP-1(7-36)amide, GLP-1(7-37) and GLP-2 occurs mainly in the L-cells. A simple system is used to describe fragments and analogues of this peptide. Thus, for example, Gly^{8}-GLP-1(7-37) (or Gly8GLP-1(7-37)) designates a fragment of GLP-1 formally derived from GLP-1 by deleting the amino acid residues Nos. 1 to 6 and substituting the naturally occurring amino acid residue in position 8 (Ala) by Gly. Similarly, $Lys^{34}(N^{\varepsilon}$-tetradecanoyl)-GLP-1(7-37) designates GLP-1(7-37) wherein the ε-amino group of the Lys residue in position 34 has been tetradecanoylated. For convenience the amino acid sequence of GLP-1 (7-37) is given below, wherein the N-terminal His is no. 7 and the C-terminal Gly is no. 37:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-
Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly.

Where reference in this text is made to C-terminally extended GLP-1 analogues, the amino acid residue in position 38 is Arg unless otherwise indicated, the optional amino acid residue in

position 39 is also Arg unless otherwise indicated and the optional amino acid residue in position 40 is Asp unless otherwise indicated. Also, if a C-terminally extended analogue extends to position 41, 42, 43, 44 or 45, the amino acid sequence of this extension is as in the corresponding sequence in human preproglucagon unless otherwise indicated.

We have discovered that certain modified GLP-1 or analogues thereof when formulated in aqueous solution together with a buffer, are physically stable at high concentrations of the modified GLP-1 or analogues thereof, when kept in the pH range from about 7 to about 10. The present formulations are physically stable within a given shelf life period at the recommended storage temperature (typically 2-3 years at 2-8°C). Furthermore, the present formulations are physically stable during in-use (typically 1 month at accelerated temperatures e.g. 25°C or 37°C). The formulations of the invention are also chemically stable thus rendering them shelf-stable and suitable for invasive (eg. injection, subcutaneous injection, intramuscular, intraveneous or infusion) as well as non-invasive (eg nasal or pulmonary, transdermal or transmucosal e.g. buccal) means of administration. When the inventive formulation comprising a GLP-1 compound was compared to the same formulation comprising GLP-1(7-37) substituted for the GLP-1 compound, the physical stability was increased considerably, and typically the shelf-life was increased from a few seconds to several months in the tests used.

In one aspect the invention relates to a pharmaceutical formulation comprising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10;

provided that if an isotonic agent is present and pH is 7.4 then mannitol or NaCl is not the isotonic agent.

In another aspect the invention relates to a pharmaceutical formulation comprising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml or above, and wherein said formulation has a pH from 7.0 to 10.

In a further aspect the invention relates to a pharmaceutical formulation comprising a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof, wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10.

In a further aspect the invention relates to a method of preparing a physically stable pharmaceutical formulation of a GLP-1 compound wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof, wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, comprising preparation of a formulation containing the GLP-1 compound, and a buffer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml or above, and wherein said formulation has a pH from 7.0 to 10.

In a further aspect the invention relates to a method of preparing a physically stable pharmaceutical formulation of a GLP-1 compound wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof, wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, comprising preparation of a formulation containing the GLP-1 compound, and a buffer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10.

In a further aspect the invention relates to a method of preparing a physically stable pharmaceutical formulation of a GLP-1 compound wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof, wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, comprising preparation of a formulation containing the GLP-1 compound, and a buffer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10; provided that if an isotonic agent is present and pH is 7.4 then mannitol or NaCl is not the isotonic agent.

In one embodiment of the invention the pharmaceutical formulation is an aqueous formulation. Such formulation is typically a solution or a suspension. In a further embodiment of the invention the pharmaceutical formulation is an aqueous solution.

In a further aspect the invention relates to a pharmaceutical formulation comprising an aqueous solution of a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml or above, and wherein said formulation has a pH from 7.0 to 10.

In a further aspect the invention relates to a pharmaceutical formulation comprising an aqueous solution of a GLP-1 compound, and a buffer, wherein said GLP-1 compound is GLP-1(7-37) or an analogue thereof, wherein an amino acid residue of the parent peptide has a lipophilic substituent attached optionally via a spacer, wherein said GLP-1 compound is present in a concentration from 1 mg/ml to 100 mg/ml, and wherein said formulation has a pH from 7.0 to 10.

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