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(71) Applicant: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK–2880 Bagsvaerd (DK).

(72) Inventors: KNUDSEN, Liselotte, Bjerre; Valby Langgade 49A, 1. tv., DK–2500 Valby (DK). HUUSFELDT, Per, Olaf; Applebys Plads 27, 5. mf., DK–1411 Copenhagen K (DK). NIELSEN, Per, Franklin; Dalsø Park 59, DK–3500 Værløse (DK). KAARSHOLM, Niels, C.; Clausholmvej 38, DK–2720 Vanløse (DK). OLSEN, Helle, Birk; Skolelodden 23, DK–3450 Allerød (DK). BJØRN, Søren, Erik; Marie Grubbes Allé 47, DK–2800 Lyngby (DK).

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(57) Abstract

The present invention relates to a pharmaceutical composition comprising a GLP–1 derivative of improved solubility and/or stability, and to a method for improving the solubility and/or stability of GLP–1 or a fragment or an analogue thereof.

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GLP-1 DERIVATIVES WITH HELIX-CONTENT EXCEEDING 25 %, FORMING PARTIALLY STRUCTURED MICELLAR-LIKE AGGREGATES

Field of the invention

The present invention relates to a pharmaceutical composition comprising a GLP-1 derivative of improved solubility and/or stability, and to a method for improving the solubility and/or stability of GLP-1 or a fragment or an analogue thereof.

Background of the invention

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 (1) 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) (2). Furthermore, as an insulinotropic hormone, GIP was found to be almost ineffective in NIDDM (2). The other incretin hormone, GLP-1 is the most potent insulinotropic substance known (3). 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 (4), 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 (5). The glu-

cagon gene is processed differently in the pancreas and in the intestine. In the pancreas (9), 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 (10, 11); 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 (9). 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 (8). The following products are formed and secreted in parallel: 1) glicentin, corresponding to PG (1-69), with the glucagon sequence occupying residues Nos. 33-61 (12); 2) GLP-1(7-36)amide (PG (78-107))amide (13), not as originally believed PG (72-107)amide or 108, which is inactive). Small amounts of C-terminally glycine-extended but equally bioactive GLP-1(7-37), (PG (78-108)) are also formed (14); 3) intervening peptide-2 (PG (111-122)amide) (15); and 4) GLP-2 (PG (126-158)) (15, 16). A fraction of glicentin is cleaved further into GRPP (PG (1-30)) and oxyntomodulin (PG (33-69)) (17, 18). Of these peptides, GLP-1, has the most conspicuous biological activities.

The amino acid sequence of GLP-1 is given *i.a.* by Schmidt *et al.* (*Diabetologia* **28** 704-707 (1985). Although the interesting pharmacological properties of GLP-1(7-37) and analogues thereof have attracted much attention in recent years only little is known about the structure of these molecules. The secondary structure of GLP-1 in micelles has been described by Thorton *et al.* (*Biochemistry* **33** 3532-3539 (1994)), but in normal solution, GLP-1 is considered a very flexible molecule. Surprisingly, we found that derivatisation of this relatively small and very flexible molecule resulted in compounds whose plasma profile were highly protracted and still had retained activity (PCT application No. DK97/00340).

While much attention has been focused on the pharmacological properties of acylated GLP-1 derivatives, 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. Accordingly, it is one object

of the present invention to provide pharmaceutical solutions comprising GLP-1 derivatives with improved solubility and stability.

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