



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/605, A61K 38/26	A1	(11) International Publication Number: WO 99/43708 (43) International Publication Date: 2 September 1999 (02.09.99)
(21) International Application Number: PCT/DK99/00086 (22) International Filing Date: 25 February 1999 (25.02.99) (30) Priority Data: 0274/98 27 February 1998 (27.02.98) DK 60/084,357 5 May 1998 (05.05.98) US (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): KNUDSEN, Liselotte, Bjerre [DK/DK]; Valby Langgade 49A, 1. tv., DK-2500 Valby (DK). HUUSFELDT, Per, Olaf [DK/DK]; Applebys Plads 27,5. mf., DK-1411 Copenhagen K (DK). NIELSEN, Per, Franklin [DK/DK]; Dalsø Park 59, DK-3500 Værløse (DK). MADSEN, Kjeld [DK/DK]; Nyvestergårdsvej 3, DK-3500 Værløse (DK). (74) Common Representative: NOVO NORDISK A/S; Novo Allé, DK-2880 Bagsvaerd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: GLP-1 DERIVATIVES OF GLP-1 AND EXENDIN WITH PROTRACTED PROFILE OF ACTION (57) Abstract The present invention relates to derivatives exendin and of GLP-1(7-C), wherein C is 35 or 36, which derivatives have just one lipophilic substituent which is attached to the C-terminal amino acid residue.		

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GLP-1 DERIVATIVES OF GLP-1 AND EXENDIN WITH PROTRACTED PROFILE OF ACTION

FIELD OF THE INVENTION

5 The present invention relates to novel derivatives of human glucagon-like peptide-1 (GLP-1) and fragments thereof and analogues of such fragments which have a protracted profile of action and to methods of making and using them. The invention furthermore relates to novel derivatives of exendin and the uses of such derivatives.

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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. When native peptides or analogues thereof are used in therapy it is generally found that they have a high clearance. A high clearance of a therapeutic agent is inconvenient in cases where it is desired to maintain a high blood level thereof over a prolonged period of time since repeated administrations will then be necessary. Examples of peptides which have a high clearance are: ACTH, corticotropin-releasing factor, angiotensin, calcitonin, insulin, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, insulin-like growth factor-1, insulin-like growth factor-2, gastric inhibitory peptide, growth hormone-releasing factor, pituitary adenylate cyclase activating peptide, secretin, enterogastrin, somatostatin, somatotropin, somatomedin, parathyroid hormone, thrombopoietin, erythropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, vasopressin, oxytocin, opioids and analogues thereof, superoxide dismutase, interferon, asparaginase, arginase, arginine deaminase, adenosine deaminase and ribonuclease. In some cases it is possible to influence the release profile of peptides by applying suitable pharmaceutical compositions, but this approach has various shortcomings and is not generally applicable.

30 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 insulinotro-

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pic, 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
5 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 insuli-
10 notropic 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.

15 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 glucagon 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
20 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
25 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),
30 (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.

35 Being secreted in parallel with glicentin/enteroglucagon, it follows that the many studies of enteroglucagon secretion (6, 7) to some extent also apply to GLP-1 secretion, but GLP-1 is metabolised more quickly with a plasma half-life in humans of 2 min (19). Carbohydrate or fat-rich meals stimulate secretion (20), presumably as a result of direct interaction of yet

unabsorbed nutrients with the microvilli of the open-type L-cells of the gut mucosa. Endocrine or neural mechanisms promoting GLP-1 secretion may exist but have not yet been demonstrated in humans.

5 The incretin function of GLP-1(29-31) has been clearly illustrated in experiments with the
GLP-1 receptor antagonist, exendin 9-39, which dramatically reduces the incretin effect eli-
cited by oral glucose in rats (21, 22). The hormone interacts directly with the β -cells via the
GLP-1 receptor (23) which belongs to the glucagon/VIP/calcitonin family of G-protein-
coupled 7-transmembrane spanning receptors. The importance of the GLP-1 receptor in re-
10 gulating insulin secretion was illustrated in recent experiments in which a targeted disruption
of the GLP-1 receptor gene was carried out in mice. Animals homozygous for the disruption
had greatly deteriorated glucose tolerance and fasting hyperglycaemia, and even hete-
rozygous animals were glucose intolerant (24). The signal transduction mechanism (25) pri-
marily involves activation of adenylate cyclase, but elevations of intracellular Ca^{2+} are also
15 essential (25, 26). The action of the hormone is best described as a potentiation of glucose
stimulated insulin release (25), but the mechanism that couples glucose and GLP-1 stimula-
tion is not known. It may involve a calcium-induced calcium release (26, 27). As already
mentioned, the insulintropic action of GLP-1 is preserved in diabetic β -cells. The relation of
the latter to its ability to convey "glucose competence" to isolated insulin-secreting cells (26,
20 28), which respond poorly to glucose or GLP-1 alone, but fully to a combination of the two, is
also not known. Equally importantly, however, the hormone also potently inhibits glucagon
secretion (29). The mechanism is not known, but seems to be paracrine, via neighbouring
insulin or somatostatin cells (25). Also the glucagonostatic action is glucose-dependent, so
that the inhibitory effect decreases as blood glucose decreases. Because of this dual effect,
25 if the plasma GLP-1 concentrations increase either by increased secretion or by exogenous
infusion the molar ratio of insulin to glucagon in the blood that reaches the liver via the portal
circulation is greatly increased, whereby hepatic glucose production decreases (30). As a
result blood glucose concentrations decrease. Because of the glucose dependency of the
insulintropic and glucagonostatic actions, the glucose lowering effect is self-limiting, and the
30 hormone, therefore, does not cause hypoglycaemia regardless of dose (31). The effects are
preserved in patients with diabetes mellitus (32), in whom infusions of slightly supraphysio-
logical doses of GLP-1 may completely normalise blood glucose values in spite of poor meta-
bolic control and secondary failure to sulphonylurea (33). The importance of the glucagono-
static effect is illustrated by the finding that GLP-1 also lowers blood glucose in type-1 diabe-
35 tic patients without residual β -cell secretory capacity (34).

In addition to its effects on the pancreatic islets, GLP-1 has powerful actions on the gastro-
intestinal tract. Infused in physiological amounts, GLP-1 potently inhibits pentagastrin-

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