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[54] **AMYLIN AGONIST PEPTIDES AND USES THEREFOR**

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Related U.S. Application Data

[63] Continuation of Ser. No. 794,266, Nov. 19, 1991, abandoned, which is a continuation-in-part of Ser. No. 667,040, Mar. 8, 1991, abandoned.

[51] **Int. Cl.**[6] **A61K 38/16**; A61K 38/28; C07K 14/00

[52] **U.S. Cl.** **514/12**; 514/2; 514/4; 514/866; 530/324

[58] **Field of Search** 530/324; 514/2, 514/4, 806, 12

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[57] **ABSTRACT**

Agonist analogues of amylin and related pharmaceutical compositions, and methods of treatment of diabetes and other insulin-requiring states, as well as methods of treatment of hypoglycemia, are provided.

45 Claims, 3 Drawing Sheets

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FIGURE 1

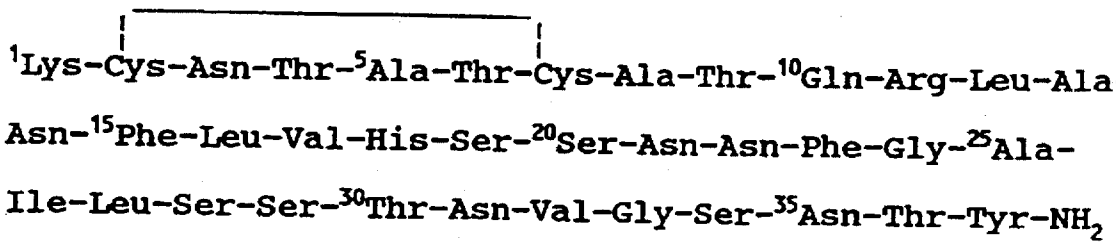

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FIGURE 2

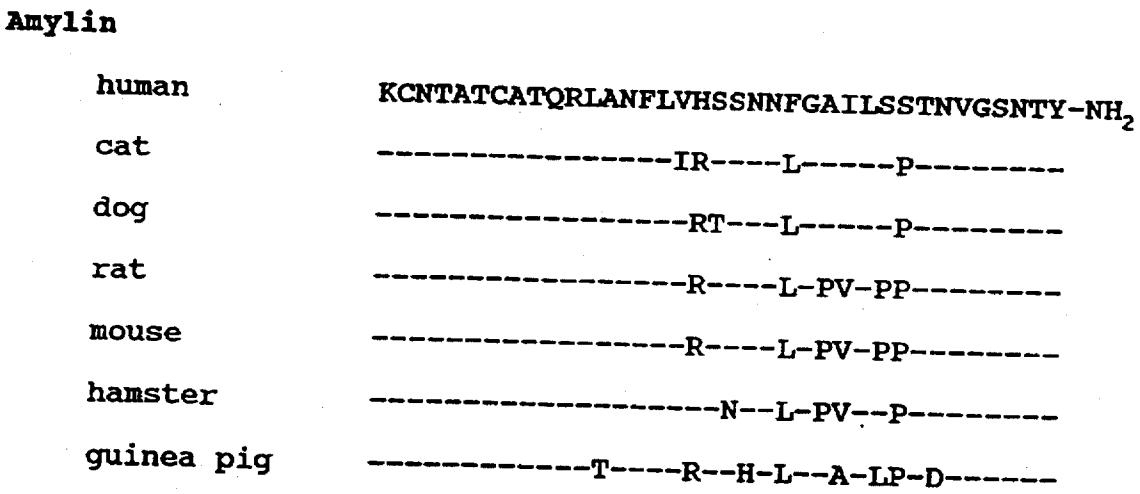

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FIGURE 3

$^{1}A_1$-X-Asn-Thr-^{5}Ala-Thr-Y-Ala-Thr-^{10}Gln-Arg-Leu-

B_1-Asn-^{15}Phe-Leu-C_1-D_1-E_1-$^{20}F_1$-G_1-Asn-H_1-Gly-$^{25}I_1$-J_1-

Leu-K_1-L_1-^{30}Thr-M_1-Val-Gly-Ser-^{35}Asn-Thr-Tyr-Z

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AMYLIN AGONIST PEPTIDES AND USES THEREFOR

This is a continuation of application Ser. No. 07/794,266 filed on Nov. 19, 1991, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/667,040 filed Mar. 8, 1991 (abandoned), which is hereby incorporated by reference.

BACKGROUND

1. Field of the Invention

The field of the invention is medicine, particularly the treatment and prevention of hypoglycemic conditions and other conditions in which enhanced amylin action is of benefit, including insulin-requiring states such as diabetes mellitus. More specifically, the invention relates to the preparation and use of agonist analogues of the peptide hormone amylin.

2. Description of Related Art and Introduction to the Invention

Diabetes mellitus is a serious metabolic disease that is defined by the presence of chronically elevated levels of blood glucose (hyperglycemia). This state of hyperglycemia is the result of a relative or absolute lack of activity of the peptide hormone, insulin. Insulin is produced and secreted by the β cells of the pancreas. Insulin is reported to promote glucose utilization, protein synthesis, and the formation and storage of neutral lipids. Glucose, the principal source of carbohydrate energy, is stored in the body as glycogen, a form of polymerized glucose, which may be converted back into glucose to meet metabolism requirements. Under normal conditions, insulin is secreted at both a basal rate and at enhanced rates following glucose stimulation, all to maintain metabolic homeostasis by the conversion of glucose into glycogen.

The term diabetes mellitus encompasses several different hyperglycemic states. These states include Type 1 (insulin-dependent diabetes mellitus or IDDM) and Type 2 (non-insulin-dependent diabetes mellitus or NIDDM) diabetes. The hyperglycemia present in individuals with Type I diabetes is associated with deficient, reduced, or nonexistent levels of insulin which are insufficient to maintain blood glucose levels within the physiological range. Treatment of Type 1 diabetes involves administration of replacement doses of insulin, generally by the parenteral route. The hyperglycemia present in individuals with Type II diabetes is initially associated with normal or elevated levels of insulin; however, these individuals are unable to maintain metabolic homeostasis due to a state of insulin resistance in peripheral tissues and liver and, as the disease advances, due to a progressive deterioration of the pancreatic β cells which are responsible for the secretion of insulin. Thus, initial therapy of Type 2 diabetes may be based on diet and lifestyle changes augmented by therapy with oral hypoglycemic agents such as sulfonylureas. Insulin therapy is often required, however, especially in the latter stages of the disease, in attempting to produce some control of hyperglycemia and minimize complications of the disease. Thus, many Type 2 diabetics ultimately require insulin in order to survive.

Amyloid is the name given to extracellular deposits of β sheet protein filaments. Deposits of amyloid material have been reported to be found in pancreas of patients with Type 2 diabetes mellitus. Other studies have indicated that the degree of amyloid depositions increases with the degree of hyperglycemia in humans and the severity of Type 2 diabetes. Chemical analysis of pancreatic amyloid led to the surprising and unexpected discovery of the peptide hormone, amylin. Clark, A., et al., *Lancet* ii: 231–234 (1987). This peptide was discovered to be comprised of 37 amino acids, none of which are acidic residues, to have a disulfide linkage between the cysteine residues at positions 2 and 7, and to be C-terminally amidated. Amylin is the major protein constituent of the amyloid which is reported to be found in the pancreatic Islets of Langerhans in patients with type 2 diabetes mellitus.

It has been reported that the presence of both the intramolecular cystine bridge and the carboxy terminal amide group in the peptide structure of the synthetic molecule yield the greatest biological activity to inhibit glycogen synthesis in skeletal muscle. E.g., Cooper, G. J. S., et al., *Proc. Natl. Acad. Sci.* (U.S.A.) 84:8628–8632 (1987); Cooper G. J. S., et al., in *Diabetes* 1988, ed. Larkins, R., Zimmet, P. & Chisholm, D. (Elsevier, Amsterdam), pp. 493–496 (1989). The amino acid sequence of amylin (see FIG. 1) has 46% homology with human calcitonin gene related peptide 2 (CGRP-2).

One report states that a limited segment of the amylin molecule, residues 20–29, is a potential contributor toward amyloid fibril formation in the islets of Langerhans in Type 2 diabetes mellitus. Glenner et al., *Biochem. Biophys. Res Commun.* 155:608–614 (1988). It has also been reported that amino acid sequence differences between amylins from certain mammalian species occur in this region, and further investigation has focused on identifying residues linked to amyloid formation. Westermark et al., *Proc. Natl. Acad. Sci. (USA)* 87: 5036–5040 (1990). The study of Westermark et al. reported attempts to synthesize various 20–29 amino acid segments of amylin sequences from different species followed by a comparison of their ability to form amyloid fibrils. It was proposed that the residues 25–29 of human amylin were the most strongly amyloidogenic and that the proline-for-serine substitution in position 28, as in several rodent species, significantly inhibited fibril formation in the studied decapeptides.

Amylin is a complex peptide, and the synthesis of bioactive preparations of amylin is laborious. Amylin has also been found to have limited solubility and limited stability in solution. We have found that rat amylin has a higher solubility and stability in solution than human amylin. This may be due in some measure, although this is not known, to the different aggregation properties of the amylins from different species. Only the human, non-human primate, and cat species of amylin have been reported to aggregate to form islet amyloid in vivo. The sequences of amylin now reported to have been isolated from a number of species are set forth in FIG. 2.

In Type I diabetes, amylin levels are severely reduced or are nonexistent when compared to normal controls. In the disease state of Type I diabetes mellitus, the β-cells, which are the producers of insulin and amylin, have been destroyed by an autoimmune process. Amylin has been proposed to be useful in the treatment of diabetes mellitus and hypoglycemia, including insulin-induced hypoglycemia. It has also been proposed that the co-administration of insulin with amylin is a superior therapy to the existing administration of insulin alone, and that coadministration of amylin with glucagon for the treatment of hypoglycemia is a superior therapy to the existing administration of glucagon alone. It would be useful to provide, for such purposes and others, less complicated compounds that have the activities of native human amylin, as well as compounds which may show enhanced solubility and/or stability over native human amylin. Such compounds are described and claimed herein.

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