Method for preventing gastritis using amylin or amylin agonists

The present invention relates to formulations suitable for parenteral administration, wherein the formulations comprise an amylin or an amylin agonist, or a salt thereof. The invention further relates to the use of an amylin or an amylin agonist for the preparation of a pharmaceutical composition for treating or preventing gastritis in a subject, comprising administering to said subject a therapeutically effective amount of an amylin or an amylin agonist, wherein said amylin agonist is not a calcitonin and said amylin or amylin agonist is not administered intracerebroventricularrly.

Fig. 1

Mean Injury Score (Percent of Value Observed with Vehicle Alone)

ED_{50} = 0.039 µg
Description

[0001] This application is a continuation-in-part of U.S. Serial No. 08/851,965, filed May 6, 1997 the contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to methods for treating or preventing gastritis or gastric injury by administering an amylin or an amylin agonist. The present invention also relates to the treatment of pain, fever, inflammation, arthritis, hypercoagulability, or other conditions for which a non-steroidal anti-inflammatory drug would be indicated, comprising administering an amylin or an amylin agonist in conjunction with a non-steroidal anti-inflammatory drug. Pharmaceutical compositions comprising an amylin or an amylin agonist and a non-steroidal anti-inflammatory agent are also described by the present invention.

BACKGROUND

[0003] All publications and other materials including patents and patent applications used to illuminate the specification are hereby incorporated by reference.

Amylin


[0005] Amylin is a 37 amino acid protein hormone. It was isolated, purified and chemically characterized as the major component of amyloid deposits in the islets of pancreases of human Type II diabetics (Cooper et al., Proc. Natl. Acad. Sci., USA 84:8628-8632 (1987)). The amylin molecule has two important post-translational modifications: the C-terminus is amidated, yielding tyrosinamide as the 37th amino acid residue, and the cysteines in positions 2 and 7 are cross-linked to form a cystine residue and, thus, an N-terminal loop. The sequence of the open reading frame of the human amylin gene shows the presence of the Lys-Arg dibasic amino acid proteolytic cleavage signal, prior to the N-terminal codon for Lys, and the Gly prior to the Lys-Arg proteolytic signal at the C-terminal position, a typical sequence for amidation for protein amidating enzyme, PAM (Cooper et al., Biochm. Biophys. Acta, 1014:247-258 (1989)). Amylin is the subject of United Kingdom patent application Serial No. 8709871, filed April 27, 1997, and corresponding United States Patent No. 5,367,052, issued November 22, 1994.

[0006] In Type 1 diabetes, amylin has been shown to be deficient, and combined replacement with insulin has been proposed as a preferred treatment over insulin alone in all forms of diabetes. The use of amylin and other amylin agonists for the treatment of diabetes mellitus is the subject of United States Patent No. 5,175,145, issued December 29, 1992. Pharmaceutical compositions containing amylin and amylin plus insulin are described in United States Patent No. 5,124,314, issued June 23, 1992.

[0007] Amylin is primarily synthesized in pancreatic beta cells and is secreted in response to nutrient stimuli such as glucose and arginine. Nutrient secretagogues such as glucose and arginine, stimulate release of amylin as well as insulin. The molar amylin:insulin ratio of the secreted proteins varies between preparations from about 0.01 to 0.4, but appears not to vary much with acute stimuli in any one preparation. However, during prolonged stimulation by elevated glucose, the amylin:insulin ratio can progressively increase (Gedulin et al, Biochem. Biophys. Res. Commun., 180(1):782-789 (1991)). Thus, amylin and insulin are not always secreted in a constant ratio.

[0008] It has been discovered and reported that certain actions of amylin are similar to non-metabolic actions of CGRP and calcitonin; however, the metabolic actions of amylin discovered during investigations of this recently identified protein appear to reflect its primary biologic role. At least some of these metabolic actions are mimicked by CGRP, albeit at doses which are markedly vasodilatory (see, e.g., Leighton et al., Nature, 335:632-635 (1988)) ; Molina et. al., Diabetes, 39:260-265 (1990) .

[0009] The first discovered action of amylin was the reduction of insulin-stimulated incorporation of glucose into glycogen in rat skeletal muscle (Leighton et al., Nature, 335:632-635 (1988)); the muscle was made "insulin-resistant." Subsequent work with rat soleus muscle ex-vivo and in vitro has indicated that amylin reduces glycogen synthase activity, promotes conversion of glycogen phosphorylase from the inactive b form to the active a form, promotes net loss of glycogen (in the presence or absence of insulin), increases glucose-6-phosphate levels, and can increase lactate output (see, e.g., Deems et al., Biochem. Biophys. Res. Commun., 181 (1) :116-120 (1991)) ; Young et al., FEBS Letts, 281(1,2):149-151 (1991)). Amylin appears not to affect glucose transport per se (e.g., Pittner et al., FEBS Letts., 365 (1):98-100 (1995)). Studies of amylin and insulin dose-response relations show that amylin acts as a noncompetitive or
functional antagonist of insulin in skeletal muscle (Young et al., Am. J. Physiol., 263(2):E274-E281 (1992)). There is no evidence that amylin interferes with insulin binding to its receptors, or the subsequent activation of insulin receptor tyrosine kinase (Follett et al., Clinical Research, 39(1):39A (1991)); Koopmans et al., Diabetologia, 34:218-224 (1991)).


[0011] While amylin has marked effects on hepatic fuel metabolism in vivo, there is no general agreement as to what amylin actions are seen in isolated hepatocytes or perfused liver. The available data do not support the idea that amylin promotes hepatic glycogenolysis, i.e., it does not act like glucagon (e.g., Stephens et al., Diabetes, 40:395-400 (1991); Gomez-Foix et al., Biochem J., 276:607-610 (1991)). It has been suggested that amylin may act on the liver to promote conversion of lactate to glycogen and to enhance the amount of glucose able to be liberated by glucagon (see Roden et al., Diabetologia, 35:116-120 (1992)). It is most likely that amylin has no direct effect on liver cells. (Pittner, R. A., Eur. J. of Pharm. 325:189-197 (1997)).

[0012] In fat cells, contrary to its action in muscle, amylin has no detectable actions on insulin-stimulated glucose uptake, incorporation of glucose into triglyceride, CO2 production (Cooper et al., Proc. Natl. Acad. Sci., 85:7763-7766 (1988)), epinephrine-stimulated lipolysis, or insulin-inhibition of lipolysis (Lupien and Young, "Diabetes Nutrition and Metabolism - Clinical and Experimental," Vol. 6(1), pages 1318 (February 1993)). Amylin thus exerts tissue-specific effects, with direct action on skeletal muscle, and indirect (via supply of substrate) effects on liver, while adipocytes appear "blind" to the presence or absence of amylin.

[0013] It has also been reported that amylin can have marked effects on secretion of insulin (Young et al., Mol. Cell. Endocrinol., 84:R1-R5 (1992)). Other workers, however, have been unable to detect effects of amylin on isolated β-cells, on isolated islets, or in the whole animal (see Broderick et al., Biochem. Biophys. Res. Commun., 177:932-938 (1991) and references therein).

[0014] Amylin or amylin agonists potently inhibit gastric emptying in rats (Young et al., Diabetologia 38(6):642-648 (1995)), dogs (Brown et al., Diabetes 43 (Suppl 1):172A (1994)) and humans (Macdonald et al., Diabetologia 38(Suppl 1):A32 (abstract 118)(1995)). Gastric emptying is reportedly accelerated in amylin-deficient type 1 diabetic BB rats (Young et al., Diabetologia, supra; Nowak et al., J. Lab. Clin. Med., 123(1):110-6 (1994)) and in rats treated with the selective amylin antagonist, AC187 (Gedulin et al., Diabetologia, 38 (Suppl 1) :A244 (1995)). Methods for reducing gastric motility and slowing gastric emptying by blocking the administration of an amylin agonist (including amylin) are the subject of United States Patent Application Serial No. 08/118,381, filed September 7, 1993, and United States Patent Application Serial No. 08/302,069, filed September 7, 1994 (and corresponding PCT application, Publication No. WO 95/07098, published March 16, 1995). The effect of amylin on gastric emptying appears to be physiological (operative at concentrations that normally circulate). Supraphysiological levels of amylin have also reportedly been studied with regard to the inhibition of gastric acid secretion (Guidobono, F., et al., Peptides 15:699-702 (1995)) and in regard to protection from gastritis. (Guidobono et al., Brit. J. Pharm 120:581-86 (1997)). The latter authors reported that subcutaneous injections of amylin had no effect on ethanol- or indomethacin-induced gastritis in rats, although intracerebroventricular injections did have an effect. The same authors also concluded that any gastroprotective effects of amylin were distinct from effects to inhibit acid secretion.

[0015] Non-metabolic actions of amylin include vasodilator effects which may be mediated by interaction with CGRP receptors. Reported in vivo tests suggest that amylin is at least about 100 to 1000 times less potent than CGRP as a vasodilator (Brain et al., Eur. J. Pharmacol., 183:2221 (1990); Wang et al., FEBS Letts., 291:195-198 (1991)). The effect of amylin on regional hemodynamic actions, including renal blood flow, in conscious rats has been reported (Gardiner et al., Diabetes, 40:948-951 (1991)). The authors noted that infusion of rat amylin was associated with greater renal vasodilation and less mesenteric vasoconstriction than is seen with infusion of human α-CGRP. They concluded that, by promoting renal hyperemia to a greater extent than did α-CGRP, rat amylin could cause less marked stimulation of the renin-angiotensin system, and thus, less secondary angiotensin II-mediated vasoconstriction. It was also noted, however, that during coninfusion of human α-8-37CGRP and rat amylin, renal and mesenteric vaso-constrictions were unmasked, presumably due to unopposed vasoconstrictor effects of angiotensin II, and that this finding is similar to that seen during coninfusion of human α-CGRP and human α-8-37CGRP (id. at 951).

[0016] Injected into the brain, or administered peripherally, amylin has been reported to suppress food intake, e.g., Chance et al., Brain Res., 539:352-354 (1991)), an action shared with CGRP and calcitonin. Amylin has also been reported to have effects both on isolated osteoclasts where it caused cell quiescence, and in vivo where it was reported to lower plasma calcium by up to 20% in rats, in rabbits, and in humans with Paget's disease (see, e.g., Zaidi et al., Trends in Endocrinol. and Metab., 4:255-259 (1993)). From the available data, amylin seems to be less potent than
human calcitonin for these actions. Interestingly, it was reported that amylin appeared to increase osteoclast cAMP production but not to increase cytosolic Ca2+, while calcitonin does both (Alam et al., Biochem. Biophys. Res. Commun., 179(1):134-139 (1991)). It was suggested, though not established, that calcitonin may act via two receptor types and that amylin may interact with one of these.

[0017] It has also been discovered that, surprisingly in view of its previously described renal vasodilator and other properties, amylin markedly increases plasma renin activity in intact rats when given subcutaneously in a manner that avoids any disturbance of blood pressure. This latter point is important because lowered blood pressure is a strong stimulus to renin release. Amylin antagonists, such as amylin receptor antagonists, including those selective for amylin receptors compared to CGRP and/or calcitonin receptors, can be used to block the amylin-evoked rise of plasma renin activity. The use of amylin antagonists to treat renin-related disorders is described in United States Patent No. 5,376,638, issued December 27, 1994.

[0018] It has also been found that amylin and amylin agonists have an analgesic effect; methods for treating pain comprising the administration of an amylin or an amylin agonist with or without a narcotic analgesic or other pain relief agent are described in U.S. Patent No. 5,677,279, issued October 14, 1997.

[0019] In normal humans, fasting amylin levels from 1 to 10pM and post-prandial or post-glucose levels of 5 to 20pM have been reported (e.g., Hartter et al., Diabetologia, 34:52-54 (1991); Sanke et al., Diabetologia, 34:129-132 (1991); Koda et al., The Lancet, 339:1179-1180 (1992)). In obese, insulin-resistant individuals, post-food amylin levels can go higher, reaching up to about 50pM. For comparison, the values for fasting and post-prandial insulin are 20 to 50uM, and 100 to 300 pM respectively in healthy people, with perhaps 3-to 4-fold higher levels in insulin-resistant people. In Type 1 diabetes, where beta cells are destroyed, amylin levels are at or below the levels of detection and do not rise in response to glucose (Koda et al., The Lancet, 339:1179-1180 (1992)). In normal mice and rats, basal amylin levels have been reported from 30 to 100 pM, while values up to 600 pM have been measured in certain insulin-resistant, diabetic strains of rodents (e.g., Huang et al., Hypertension, 19:1-101-1-109 (1991); Gill et al., Life Sciences, 48:703-710 (1991)).

Gastritis

[0020] Gastritis is inflammation of the gastric mucosa. The condition does not reflect a single disease. Rather, it is common within a group of disorders that have inflammatory changes in the gastric mucosa, but that may have different clinical features, histologic characteristics and pathogenesis. The two principal forms of gastritis, which constitute different clinical entities, are acute gastritis and chronic gastritis. Harrison's Principles of Internal Medicine (Wilson et al., eds., 12th ed. 1991, McGraw-Hill, Inc.) at pages 1244-1248.

[0021] The principal, and certainly the most dramatic, form of acute gastritis is acute hemorrhagic gastritis, which is also referred to as acute erosive gastritis. These terms reflect the bleeding for the gastric mucosa almost invariably found in this form of gastritis and the characteristic loss of integrity of the gastric mucosa (erosion) that accompanies the inflammatory lesion. Erosive gastritis has been estimated to occur in up to 80 to 90 percent of critically ill hospitalized patients. It is most often found in patients in medical or surgical intensive care units with severe trauma, major surgery, hepatic, renal or respiratory failure, shock, massive burns or severe infections with septicemia. Id.

[0022] Various agents are known to injure the gastric mucosa. These include aspirin and other non steroidal anti-inflammatory drugs or agents (NSAIDs), bile acids, pancreatic enzymes and ethanol. These agents disrupt the gastric mucosal barrier, which under normal conditions impedes the back-diffusion of hydrogen ions from the gastric lumen to the mucosa (despite and against an enormous H+ concentration gradient). The most common and very important cause of drug-associated acute erosive gastritis is ingestion of aspirin or other NSAIDs. These drugs inhibit gastric mucosal cyclooxygenase activity, thereby reducing the synthesis and tissue levels of endogenous mucosal prostaglandins, which appear to play important roles in mucosal defense. This reduction in tissue prostaglandins is thought to be a principal, but perhaps not the exclusive, mechanism by which aspirin and other NSAIDs damage the gastric mucosa. Id.

[0023] The two major forms of chronic gastritis have been classified as type A and B based on their distributions in the gastric mucosa coupled with some implications regarding their pathogenesis. Type A gastritis is the less common form of chronic gastritis; it characteristically involves the body and fundus of the stomach with relative sparing of the antrum. Type B gastritis is the much more common form of chronic gastritis. In younger patients, type B gastritis principally involves the antrum, whereas in older patients the entire stomach is affected. Id.

Non-Steroidal Anti-Inflammatory Drugs

[0024] Non-steroidal anti-inflammatory drugs or agents (NSAIDS) are useful analgesics, however, they have the adverse property of inducing various gastric effects in a large fraction of patients; such gastric effects include gastritis, gastric ulcer, epigastric distress, nausea, vomiting, and hemorrhage. (Woodbury, D.M. and Fingl, E. Analgesic-antipyretics, anti-inflammatory agents, and drugs employed in the therapy of gout, in The Pharmacological Basis of Therapeutics (Goodman, L.S., and Gilman, A., eds.) 325-43 (1975)). The most common side effect of NSAIDS is a propensity to induce
gastric or intestinal ulceration that can sometimes be accompanied by anemia from the resultant blood loss. Patients who use NSAIDs on a chronic basis have about three times greater relative risk for serious adverse gastrointestinal events compared to nonusers (Gabriel et al., "Risk for serious gastrointestinal complications related to the use of non-steroidal antiinflammatory drugs. A meta analysis," Ann. Intern Med. 115:1117-1125 (1991)). Gastric damage by these agents can be brought about by at least two distinct mechanisms: diffusion of acid into the gastric mucosa which induces tissue damage; and interference with the biosynthesis of gastric prostaglandins which serve as cytoprotective agents in the gastric mucosa. These side effects are particularly a problem in patients that must continually ingest NSAIDs, such as in patients with chronic inflammatory conditions, such as rheumatoid arthritis. NSAIDs include salicylates; paraaminophenol derivatives, such as acetominophen; indomethacin; sulindac; etodolac; fenamates; telmetin; ketorolac; diclofenac; propionic derivatives, such as ibuprofen, naproxen, naproxen sodium, fenoprofen, ketoprofen, flurbiprofen and oxaprozin; piroxicam; pyrazolon derivatives, such as phenylbutazone; and apazone. Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Chapter 27 (9th ed.), McGraw-Hill 1996.

SUMMARY OF THE INVENTION

[0025] We have discovered that, unexpectedly, amylins and amylin agonists have gastroprotective properties and can prevent the induction of gastritis, and thus treat or prevent gastric injury, such as gastric ulcers, when administered to a subject. The term "amylin" is understood to include compounds such as those defined by Young and Cooper in U.S. Patent 5,234,906, issued August 10, 1993 for "Hyperglycemic Compositions," the contents of which are hereby incorporated by reference. For example, the term includes human amylin and species variations of it, referred to as amylin and secreted from the beta cells of the pancreas. "Amylin agonist" is also a term known in the art. The term refers to compounds which mimic effects of amylin. Amylin agonists include "amylin agonist analogues" which are derivatives of amylin which act as amylin agonists. Amylin agonists may act by binding to or otherwise directly or indirectly interacting with an amylin receptor or other receptor with which amylin itself may interact to elicit biological effects of amylin. In addition to those amylin agonists described herein, other useful amylin agonists are identified in U.S. Patent No. 5,686,411, issued November 11, 1997, the disclosure of which is hereby incorporated by this reference.

[0026] Thus, in a first aspect of the invention, a method is provided for treating or preventing gastritis or gastric ulceration in a subject, comprising administering to said subject a therapeutically effective amount of an amylin or an amylin agonist, wherein said amylin agonist is not a calcitonin and said amylin or amylin agonist is not administered intra-cerebroventricularly. By "calcitonin" is meant human peptide hormone calcitonin and species variations of it, such as rat calcitonin, salmon calcitonin and eel calcitonin. In one embodiment, said gastritis or gastric ulceration is associated with the administration of a non-steroidal anti-inflammatory drug.

[0027] In the methods of the present invention, the gastroprotective effects of amylins and amylin agonists will reduce the propensity of NSAIDS to cause gastritis and ulceration.

[0028] Thus, in another aspect of the invention, a method is provided for treating or preventing a condition for which an NSAID would be indicated comprising administering to a subject a therapeutically effective amount of an amylin or an amylin agonist, wherein said amylin agonist is not a calcitonin and said amylin or amylin agonist is not administered intra-cerebroventricularly, and a therapeutically effective amount of a non-steroidal anti-inflammatory agent. Preferably, said non-steroidal anti-inflammatory agent is selected from the group consisting of salicylate, acetaminophen, phenacetin, naproxen, phenylbutazone, indomethacin, ibuprofen, sulndac, etodolac, fenamates, telmetin, ketorolac, diclofenac, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam and apazone.

[0029] According to the methods of the present invention, the amylin or amylin agonist is administered, for example, orally, intravenously, subcutaneously, nasally, pulmonarily, transdermally, or buccally. Intravenous and subcutaneous administration are presently preferred.

[0030] The subject may be any animal, preferably a mammal, and more preferably a human.

[0031] In other aspects of the present invention, a pharmaceutical composition is provided comprising (1) an amylin or an amylin agonist or a pharmaceutically acceptable salt thereof, wherein said amylin agonist is not a calcitonin, and (2) a non-steroidal anti-inflammatory agent in a pharmaceutically acceptable carrier and dose.

[0032] Preferably said non-steroidal anti-inflammatory agent is selected from the group consisting of salicylate, acetaminophen, phenacetin, naproxen, phenylbutazone, indomethacin, ibuprofen, sulndac, etodolac, fenamates, telmetin, ketorolac, diclofenac, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam and apazone.

[0033] In preferred embodiments of the present invention, the amylin agonist is 25,28,29Pro-h-amylin, also known as pramlintide. Pramlintide is described and claimed in United States Patent No. 5,686,411, issued November 11, 1997.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The invention will be further described with reference to the accompanying drawing in which:
Fig. 1 shows the effect of subcutaneous doses of rat amylin to reduce the gastric injury induced by gavage of ethanol into rats.

DETAILED DESCRIPTION OF THE INVENTION

Amylin agonists may be identified by activity in the gastroprotection assays described below. These compounds may also be assessed by receptor binding and gastric emptying assays described below.

The nomenclature of various amylin agonist compounds useful in the present invention can be used to indicate both the peptide that the sequence is based on and the modifications made to any basic peptide amylin sequence, such as human amylin. An amino acid preceded by a superscript number indicates that the named amino acid replaces the amino acid normally present at the amino acid position of the superscript in the basic amino acid sequence. For example, "^{18}Arg^{25,28}Pro-h-amylin" refers to a peptide based on the sequence of "h-amylin" or "human-amylin" having the following changes: Arg replacing His at residue 18, Pro replacing Ala at residue 25 and Pro replacing Ser at residue 28. The term "des-^{1}Lys-h-amylin" refers to a peptide based on the sequence of human amylin, with the first, or N-terminal, amino acid deleted.

Amylin agonists include the following amylin agonist analogues:

i) An agonist analogue of amylin having the amino acid sequence:

\[
{A_1}, {X}_{1}-{A_{2}}-{N_{3}}-{H_{4}}-{T_{5}}-{A_{6}}-{T_{7}}-{X}_{8}\]

\[
{B_1}, {A_{2}}-{N_{3}}-{H_{4}}-{T_{5}}-{A_{6}}-{T_{7}}-{X}_{8}, {Y}_{9}\]

\[
{I_{10}}, {G_{11}}-{A_{12}}-{T_{13}}-{Y_{14}}-{Z_{15}}\]

wherein

- \(A_1\) is Lys, Ala, Ser or hydrogen;
- \(B_1\) is Ala, Ser or Thr;
- \(C_1\) is Val, Leu or Ile;
- \(D_1\) is His or Arg;
- \(E_1\) is Ser or Thr;
- \(F_1\) is Ser, Thr, Gln or Asn;
- \(G_1\) is Asn, Gln or His;
- \(H_1\) is Phe, Leu or Tyr;
- \(I_1\) is Ile, Val, Ala or Leu;
- \(J_1\) is Ser, Pro or Thr;
- \(K_1\) is Asn, Asp or Gln;

X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, aryloxy or aralkyloxy; and provided that when \(A_1\) is Lys, \(B_1\) is Ala, \(C_1\) is Val, \(D_1\) is Arg, \(E_1\) is Ser, \(F_1\) is Ser, \(G_1\) is Asn, \(H_1\) is Leu, \(I_1\) is Val, \(J_1\) is Pro, and \(K_1\) is Asn; then one or more of \(A_1\) to \(K_1\) is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, aryloxy or aralkyloxy.

ii) An agonist analogue of amylin having the amino acid sequence:

\[
{A_1}, {X}_{1}-{A_{2}}-{N_{3}}-{H_{4}}-{T_{5}}-{A_{6}}-{T_{7}}-{X}_{8}\]

\[
{B_1}, {A_{2}}-{N_{3}}-{H_{4}}-{T_{5}}-{A_{6}}-{T_{7}}-{X}_{8}, {Y}_{9}\]

\[
{I_{10}}, {G_{11}}-{A_{12}}-{T_{13}}-{Y_{14}}-{Z_{15}}\]

wherein

- \(A_1\) is Lys, Ala, Ser or hydrogen;
- \(B_1\) is Ala, Ser or Thr;
- \(C_1\) is Val, Leu or Ile;
- \(D_1\) is His or Arg;
- \(E_1\) is Ser or Thr;
- \(F_1\) is Ser, Thr, Gln or Asn;
G₁ is Asn, Gln or His;
H₁ is Phe, Leu or Tyr;
I₁ is Ile, Val, Ala or Leu;
J₁ is Ser, Pro, Leu, Ile or Thr;
K₁ is Asn, Asp or Gln;

X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; and provided than when

(a) A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Val, J₁ is Pro and K₁ is Asn; or
(b) A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is His, E₁ is Ser, F₁ is Asn, G₁ is Asn, H₁ is Leu, I₁ is Val, J₁ is Ser and K₁ is Asn;

then one or more of A₁ to K₁ is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, alkyloxy, aryloxy or aralkyloxy.

iii) An agonist analogue of amylin having the amino acid sequence:

\[ 1A₁-X-Asn-Thr-5Ala-Thr-Y-Ala-Thr-10Gln-Arg-Leu- \\
B₁-Asn-15Phe-Leu-C₁-D₁-E₁-20F₁-G₁-Asn-H₁-Gly-25I₁-J₁- \\
Leu-Pro-Pro-30Thr-K₁-Val-Gly-Ser-35Asn-Thr-Tyr-Z \]

wherein

A₁ is Lys, Ala, Ser or hydrogen;
B₁ is Ala, Ser or Thr;
C₁ is Val, Leu or Ile;
D₁ is His or Arg;
E₁ is Ser or Thr;
F₁ is Ser, Thr, Gln or Asn;
G₁ is Asn, Gln or His;
H₁ is Phe, Leu or Tyr;
I₁ is Ala or Pro;
J₁ is Ile, Val, Ala or Leu;
K₁ is Asn, Asp or Gin; X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; and provided that when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Pro, J₁ is Val and K₁ is Asn; then one or more of A₁ to K₁ is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy.

iv) An agonist analogue of amylin having the amino acid sequence:

\[ 1A₁-X-Asn-Thr-5Ala-Thr-Y-Ala-Thr-10Gln-Arg-Leu- \\
B₁-Asn-15Phe-Leu-C₁-D₁-E₁-20F₁-G₁-Asn-H₁-Gly-25I₁-J₁- \\
Leu-Pro-Pro-30Thr-K₁-Val-Gly-Ser-35Asn-Thr-Tyr-Z \]

wherein

A₁ is Lys, Ala, Ser or hydrogen;
B₁ is Ala, Ser or Thr;
C₁ is Val, Leu or Ile;
D₁ is His or Arg;
E₁ is Ser or Thr;
F₁ is Ser, Thr, Gln or Asn;
G₁ is Asn, Gln or His;
H₁ is Phe, Leu or Tyr;
I₁ is Ile, Val, Ala or Leu;
J₁ is Asn, Asp or Gln; X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, arylx or aralkyloxy; and provided that when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Val and J₁ is Asn; then one or more of A₁ to K₁ is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, arylx or aralkyloxy.

[0038] Preferred amylin agonist compounds, des″-1Lys-h-amylin, 25.28.29Pro-h-amylin, 18Arg25.28,29Pro-h-amylin, and des″-1Lys18Arg25.28,29Pro-h-amylin, all show amylin-activity in vivo in treated test animals. In addition to having activities characteristic of amylin, certain preferred compounds have also been found to possess more desirable solubility and stability characteristics when compared to human amylin. These preferred compounds include 25Pro26Val28,29Pro-h-amylin, 25.28,29Pro-h-amylin, and 18Arg25.28,29Pro-h-amylin.

[0039] The methods of the present invention employ an amylin or an amylin agonist, for example, amylin receptor agonists such as 18Arg25.28,29Pro-h-amylin, des″-1Lys18Arg25.28,29Pro-h-amylin, 18Arg25.28,29Pro-h-amylin, des″-1Lys18Arg25.28,29Pro-h-amylin, 25.28,29Pro-h-amylin, and 25Pro26Val28,29Pro-h-amylin. Examples of other amylin agonists include:

23Leu25Pro26Val28,29Pro-h-amylin;
23Leu25Pro26Val28Pro-h-amylin;
des″-1Lys25Pro26Val28Pro-h-amylin;
18Arg25Pro26Val28Pro-h-amylin;
19Arg25Leu25.28,29Pro-h-amylin;
19Arg25Leu25.28Pro-h-amylin;
17Ile23Leu25.28,29Pro-h-amylin;
17Ile25.28,29Pro-h-amylin;
des″-1Lys17Ile23Leu25.28,29Pro-h-amylin;
17Ile18Arg21His23Leu26Ala28Leu29Pro31Asp-h-amylin;
17Ile18Arg21His23Leu26Ala29Pro31Asp-h-amylin;
des″-1Lys17Ile18Arg21His23Leu26Ala28,29Pro31Asp-h-amylin;
17Ile18Arg21His23Leu25Pro26Ala28,29Pro31Asp-h-amylin.

[0040] Still further amylin agonists, including amylin agonist analogues, are disclosed, and methods for making and using amylin agonists are further specified, in commonly owned U.S. Patent No. 5,686,411, issued November 11, 1997 which has been incorporated by reference.

[0041] The activity of amylin agonists may be evaluated using certain biological assays described herein. The receptor binding assay can identify both candidate amylin agonists and antagonists and can be used to evaluate binding, while the rat gastric-emptying assay can be used to distinguish between amylin agonists and antagonists. Preferably, agonist compounds exhibit activity in the receptor binding assay on the order of less than about 1 to 5 nM, preferably less than about 1 nM and more preferably less than about 50 pM. In the in vivo rat gastric emptying assay these compounds preferably show ED₅₀ values on the order of less than about 100 to 1000 μg/rat.

[0042] The receptor binding assay is described in United States Patent No. 5,264,372, issued November 23, 1993, the disclosure of which has been incorporated by reference. The receptor binding assay is a competition assay which measures the ability of compounds to bind specifically to membrane-bound amylin receptors. A preferred source of the membrane preparations used in the assay is the basal forebrain which comprises membranes from the nucleus accumbens and surrounding regions. Compounds being assayed compete for binding to these receptor preparations with 125I Bolton Hunter rat amylin. Competition curves, wherein the amount bound (B) is plotted as a function of the log of the concentration of ligand are analyzed by computer, using analyses by nonlinear regression to a 4-parameter logistic equation (Inplot program; GraphPAD Software, San Diego, California) or the ALLFIT program of DeLean et al. (ALLFIT, Version 2.7 (NIH, Bethesda, MD 20892)), Munson, P. and Rodbard, D., Anal. Biochem. 107:220-239 (1980).

[0043] Amylins or amylin agonists can be identified, evaluated, or screened by their effects on gastric emptying using
the methods described in U.S. Application Serial No. 08/118,381, filed September 7, 1993, and U.S. Application Serial No. 08/302,069, filed September 7, 1994 (corresponding to PCT Application, Publication No. WO 95/07098), the disclosures of which are hereby incorporated by reference, or other art-known or equivalent methods for determining gastric motility. One such method for use in identifying or evaluating the ability of a compound to slow gastric motility, comprises:
(a) bringing together a test sample and a test system, said test sample comprising one or more test compounds, and said test system comprising a system for evaluating gastric motility, said system being characterized in that it exhibits, for example, elevated plasma label in response to the intragastric introduction to said system of that label; and, (b) determining the presence or amount of a rise in plasma label in said system. Positive and/or negative controls may be used as well. Optionally, a predetermined amount of amylin antagonist (e.g., 8-32 salmon calcitonin) may be added to the test system.

[0044] Amylin agonists such as those described above are prepared using standard solid phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. Typically, an α-N-carbamoyl protected amino acid and an amino acid attached to the growing peptide chain on a resin are coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidinone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the presence of a base such as diisopropylethylamine. The α-N-carbamoyl protecting group is removed from the resulting peptide-resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired N-protected amino acid to be added to the peptide chain. Suitable N-protecting groups are well known in the art, with t-butyloxycarbonyl (Boc) and fluorenylmethoxy carbonyl (Fmoc) being preferred herein.

[0045] The solvents, amino acid derivatives and 4-methylbenzhydryl-amine resin used in the peptide synthesizer are purchased from Applied Biosystems Inc. (Foster City, CA), unless otherwise indicated. The side-chain protected amino acids are purchased from Applied Biosystems, Inc. and include the following: Boc-Arg(Mts), Fmoc-Arg(Pmc), Boc-Thr (Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl), Fmoc-Ser(t-Bu), Boc-Tyr(Bzl), Fmoc-Tyr(t-Bu), Boc-Lys(CI-Z), Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-Asn(Trt), and Fmoc-Gln(Trt). Boc-His(BOM) is purchased from Applied Biosystems, Inc. or Bachem Inc. (Torrance, CA). Anisole, methylsulfide, phenol, ethanedithiol, and thioanisole are obtained from Aldrich Chemical Company (Milwaukee, WI). Air Products and Chemicals (Allentown, PA) supplies HF. Ethyl ether, acetic acid and methanol are purchased from Fisher Scientific (Pittsburgh, PA).

[0046] Solid phase peptide synthesis is carried out with an automatic peptide synthesizer (Model 430A, Applied Biosystems Inc., Foster City, CA) using the NMP/HOBt (Option 1) system and TBOc or Fmoc chemistry (see, Applied Biosystems User’s Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70). Applied Biosystems, Inc., Foster City, CA) with capping. Boc-peptide-resins are cleaved with HF (-5˚C to 0˚C, 1 hour). The peptide is extracted from the resin with alternating water and acetic acid, and the filtrates are lyophilized. The Fmoc-peptide-resins are cleaved according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc., 1990, pp. 6-12). Some peptides are also assembled using an Advanced Chem Tech Synthesizer (Model MPS 350, Louisville, Kentucky). Peptides are purified by RP-HPLC (preparative and analytical) using a Waters Delta Prep 3000 system. A C4, C8 or C18 preparative column (10 μ, 2.2 x 25 cm; Vydac, Hesperia, CA) is used to isolate peptides, and purity is determined using a C4, C8 or C18 analytical column (5 μ, 0.46 x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1% TFA/CH3CN) are delivered to the analytical column at a flowrate of 1.0 ml/min and to the preparative column at 15 ml/min. Amino acid analyses are performed on the Waters Pico Tag system and processed using the Maxima program. The peptides are hydrolyzed by vapor-phase hydrolysis (115˚C, 20-24 h). Hydrolysates are derivatized and analyzed by standard methods (Cohen, S.A., Meys, M., and Tarrin, T.L. (1989), The Pico Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis, pp. 11-52, Millipore Corporation, Milford, MA). Fast atom bombardment analysis is carried out by M-Scan, Incorporated (West Chester, PA). Mass calibration is performed using cesium iodide or cesium iodide/glycerol. Plasma desorption ionization analysis using time of flight detection is carried out on an Applied Biosystems Bio-ion 20 mass spectrometer.


[0048] The compounds referenced above form salts with various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H2SO4, H3PO4, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid and camphorsulfonic acid. Salts prepared with bases include ammonium salts, alkali metal salts, e.g. sodium and potassium salts, and alkali earth salts, e.g. calcium and magnesium salts. Acetate, hydrochloride, and trifluoroacetate salts are preferred. The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

[0049] Compositions useful in the invention may conveniently be provided in the form of formulations suitable for
parenteral (including, intravenous, intramuscular and subcutaneous) or nasal or transdermal, and/or suitably encapsulated or otherwise prepared by another known methods for oral administration. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., Remington’s Pharmaceutical Sciences by E.W. Martin. See, also Wang, Y.J. and Hanson, M.A. “Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers,” Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988). Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. Preferably, they are dissolved in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 4.3 to 7.4. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to stabilize the formulation, such as pH buffering agents. Usefull buffers include for example, sodium acetate/acetic acid buffers. A form of repository or “depot” slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

Preferably, these parenteral dosage forms are prepared according to the U.S. Provisional Patent Application Serial No. 60/035,140 filed January 8, 1997, entitled “Parenteral, Liquid Formulations for Amylin Agonist Peptides,” and U.S. Patent Application Serial No. 09/005,262, filed January 8, 1998, entitled “Formulations for Amylin Agonist Peptides,” the contents of which are incorporated herein by this reference, and include approximately 0.01 to 0.5 w/v%, respectively, of an amylin and/or an amylin agonist in an aqueous system along with approximately 0.2 to 0.5 w/v% of an acetate, phosphate, citrate or glutamate buffer to obtain a pH of the final composition of approximately 3.0 to 6.0 (more preferably 3.0 to 5.5), as well as approximately 1.0 to 10 w/v% of a carbohydrate or polyhydric alcohol tonifier in an aqueous continuous phase. Approximately 0.005 to 1.0 w/v% of an antimicrobial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl and propyl parabens and phenol is also present in the preferred formulation of product designed to allow the patient to withdraw multiple doses. A stabilizer is not required in this formulation. A sufficient amount of water for injection is used to obtain the desired concentration of solution. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, must maintain the overall stability of the amylin, or an amylin agonist. The liquid formulation should be isotonic. Most preferably, in the amylin and/or amylin agonist formulation for parenteral administration, the polyhydric alcohol is mannitol, the buffer is an acetate buffer, the preservative is approximately 0.1 to 0.3 w/v of m-cresol, and the pH is approximately 3.7 to 4.3.

The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

If desired, solutions of the above compositions may be thickened with a thickening agent such as methyl cellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic surfactant (such as a Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

Compositions useful in the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control toxicity.

For use by the physician, the compositions will be provided in dosage unit form containing an amount of an amylin or amylin agonist, for example, an amylin agonist with an NSAID which will be effective in one or multiple doses to control pain, inflammation, body temperature, blood coagulability, or other targeted biological response at the selected level. Therapeutically effective amounts of an amylin or amylin agonist are those that will alleviate the targeted symptom, or achieve the desired level of control. As will be recognized by those in the field, an effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the patient’s physical condition, the action to be obtained and other factors.

The therapeutically effective daily dose of amylin or amylin agonist, for the treatment of gastritis and ulcers including h-amylin, Arg25,28Pro-h-amylin, des-1Lys18Arg25,28Pro-h-amylin, Arg25,28,29Pro-h-amylin, des-1Lys18Arg25,28,29Pro-h-amylin, and Pro25Pro26Val28,29Pro-h-amylin, will typically be in the range of 0.01 μg/kg/day to about 10 μg/kg/day, preferably between about 0.05 μg/kg/day to about 6.0 μg/kg/day, more preferably between about 1-6 μg/kg/day and even more preferably between about 0.5 μg/kg/day to about 4.0 μg/kg/day administered in single or divided doses.

The effective daily dose of amylin or amylin agonist in combination with an NSAID including h-amylin, Arg25,28Pro-h-amylin, des-1Lys18Arg25,28Pro-h-amylin, Arg25,28,29Pro-h-amylin, des-1Lys18Arg25,28,29Pro-h-amylin, and Pro25Pro26Val28,29Pro-h-amylin, will typically be in the range of 0.01 μg/kg/day to about 10 μg/kg/day, preferably between about 0.05 μg/kg/day to about 6.0 μg/kg/day more preferably between about 1-6 μg/kg/day and even more preferably between about 0.5 μg/kg/day to about 4.0 μg/kg/day administered in single or divided doses. For these indications, the effective daily dose of the NSAID would depend on...
the agent used, and is comparable to the doses when NSAIDs are used alone. For example, daily doses for salicylate (aspirin) are 150mg - 3.5g per day, for phenylbutazone 100mg - 600 mg per day, for indomethacin 50mg - 200mg per day, and for acetaminophen 3g - 6g per day.

[0057] The effective daily dose of amylin or amylin agonist to reduce the adverse gastric effects of the administration of an NSAID, including h-amylin, \(18\text{Arg}^{25,28}\text{Pro-h-amylin}, \text{ des-}^{1}\text{Lys}^{18}\text{Arg}^{25,28}\text{Pro-h-amylin}, \text{18Arg}^{25,28,29}\text{Pro-h-amylin}, \text{ des-}^{1}\text{Lys}^{25,28,29}\text{Pro-h-amylin}, \text{ and } ^{25}\text{Pro}^{26}\text{Val}^{28,29}\text{Pro-h-amylin}, \) will typically be in the range of 0.01 \(\mu\)g/kg/day to about 10 \(\mu\)g/kg/day, preferably between about 0.05 \(\mu\)g/kg/day to about 6.0 \(\mu\)g/kg/day more preferably between about 1.6 \(\mu\)g/kg/day and even more preferably between about 0.5 \(\mu\)g/kg/day to about 4.0 \(\mu\)g/kg/day administered in single or divided doses. For these indications, the effective daily dose of the NSAID would depend on the agent used, and is comparable to the doses when NSAIDs are used alone. For example, daily doses for salicylate (aspirin) are 150mg - 3.5g per day, for phenylbutazone 100mg - 600mg per day, for indomethacin 50mg - 200mg per day, and for acetaminophen 3g - 6g per day.

[0058] The exact dose to be administered for each indication is determined by the attending clinician and is dependent upon where the particular compound lies within the above quoted range, as well as upon the age, weight and condition of the individual. Those of skill in the art will recognize that other non-daily doses may also be administered. Administration should begin at the first sign of symptoms in the case of gastritis or ulcers or at the time it is determined that the subject should begin NSAID therapy. Administration is preferably by intravenous, subcutaneous or intramuscular injection. Administration may also, for example, be nasally, transdermally or buccally. Orally active compounds may be taken as such, or converted to a salt, ester or other derivative. The effective daily dose of an NSAID, including h-amylin, \(18\text{Arg}^{25,28}\text{Pro-h-amylin}, \text{ des-}^{1}\text{Lys}^{18}\text{Arg}^{25,28}\text{Pro-h-amylin}, \text{18Arg}^{25,28,29}\text{Pro-h-amylin}, \text{ des-}^{1}\text{Lys}^{25,28,29}\text{Pro-h-amylin}, \text{ and } ^{25}\text{Pro}^{26}\text{Val}^{28,29}\text{Pro-h-amylin}, \) will typically be in the range of 0.01 \(\mu\)g/kg/day to about 4.0 \(\mu\)g/kg/day administered in single or divided doses. For these indications, the effective daily dose of the NSAID would depend on the agent used, and is comparable to the doses when NSAIDs are used alone. For example, daily doses for salicylate (aspirin) are 150mg - 3.5g per day, for phenylbutazone 100mg - 600mg per day, for indomethacin 50mg - 200mg per day, and for acetaminophen 3g - 6g per day.

[0059] The following Examples are illustrative, but not limiting of the methods of the present invention. Other suitable amylin and amylin agonists that may be adapted for use in the claimed methods are also appropriate and are within the spirit and scope of the invention.

EXAMPLE 1

Gastroprotective Properties of Amylin

[0060] The gastroprotective properties of amylin in an animal model for gastritis -- the ethanol gavaged rat -- are described in this example.

[0061] The effect of amylin on the induction of experimental mucosal damage in rats by gavage of 1 ml absolute ethanol was examined. Mucosal damage was scored between 0 (no damage) and 5 (100% of stomach covered by hyperemia and ulceration) by investigators blinded to the treatment. Rat amylin in saline was injected subcutaneously into fasted conscious male Harlan Sprague Dawley rats at doses of 0, 0.001, 0.01, 0.1, 0.3, 1, 3 or 10 \(\mu\)g (n=12, 5, 5, 5, 9, 9, 5, 6 respectively) 5 min before gavage. Mucosal damage, calculated as percent of scores in the saline-treated controls were, with the above rising subcutaneous doses, respectively: 100.0 \(\pm\) 8.3%, 95.3 \(\pm\) 15.2%, 76.6 \(\pm\) 13.8%, 70.1 \(\pm\) 10.7%, 33.9 \(\pm\) 7.7%, **35.6 \(\pm\) 11.5%, **32.9 \(\pm\) 8.3%, **(P<0.05, **P < 0.001 vs saline control). That is, amylin reduced the injury score by up to 67%, as observed with the 10 \(\mu\)g dose. The ED50 for the gastroprotective effect of amylin in this experimental system was 0.036 \(\mu\)g/rat \(\pm\) 0.4 log units. The 50% gastroprotective dose of rat amylin (0.036 \(\mu\)g/rat) was predicted to increase circulating amylin concentrations by 1.8-4.0 pm. This prediction was obtained by applying the published relationship between injected subcutaneous dose and peak plasma concentration in rats. Young, A. A. et al., Drug Devel. Res. 37:231-48 (1996). A change in plasma concentration of amylin of 1.8 \(\mu\)g/rat was predicted to increase circulating amylin concentrations by 1.8-4.0 pm. This prediction was obtained by applying the published relationship between injected subcutaneous dose and peak plasma concentration in rats. Young, A. A. et al., Drug Devel. Res. 37:231-48 (1996). A change in plasma concentration of amylin of 1.8 pm is within the range of fluctuations reported to occur in normal rodents, indicating that endogenous circulating amylin is likely to exert a tonic gastroprotective effect. Mimicking this physiological effect is unlikely to result in unwanted side effects, as is often the case with administration of unphysiological xenobiotics. The absence of side effects enhances the utility of amylin agonists used for the purposes and in the manner specified herein.

EXAMPLE 2

Preparation of \(25,28,29\text{Pro-h-amylin}\)

[0062] Solid phase synthesis of \(25,28,29\text{Pro-h-amylin}\) using methylbenzhydrylamine anchor-bond resin and N\(^-\text{Boc}/\text{benzylside chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulphide and anisole. The \(25,28,29\text{Pro-h-amylin}\) was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)\(^+\)=3,949.
**EXAMPLE 3**

Preparation of $^{18}$Arg$_{25,28,29}$Pro-h-Amylin

[0063] Solid phase synthesis of $^{18}$Arg$_{25,28,29}$Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N$_4$-Boc/benzylside chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide] amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The $^{18}$Arg$_{25,28,29}$Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)$^+=$3,971.

**EXAMPLE 4**

Preparation of $^{18}$Arg$_{25,28}$Pro-h-Amylin

[0064] Solid phase synthesis of $^{18}$Arg$_{25,28}$Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N$_4$-Boc/benzylside chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide] amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The $^{18}$Arg$_{25,28}$Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)$^+=$3,959.

**EXAMPLE 5**

Receptor Binding Assay

[0065] Evaluation of the binding of compounds to amylin receptors was carried out as follows. $^{125}$I-rat amylin (Bolton-Hunter labeled at the N-terminal lysine) was purchased from Amersham Corporation (Arlington Heights, IL). Specific activities at time of use ranged from 1950 to 2000 Ci/mmol. Unlabeled peptides were obtained from BACHEM Inc. (Torrance, CA) and Peninsula Laboratories (Belmont, CA).

[0066] Male Sprague-Dawley rats (200-250) grams were sacrificed by decapitation. Brains were removed to cold phosphate-buffered saline (PBS). From the ventral surface, cuts were made rostral to the hypothalamus, bounded laterally by the olfactory tracts and extending at a 45˚ angle medially from these tracts. This basal forebrain tissue, containing the nucleus accumbens and surrounding regions, was weighed and homogenized in ice-cold 20 mM HEPES buffer (20 mM HEPES acid, pH adjusted to 7.4 with NaOH at 23˚C). Membranes were washed three times in fresh buffer by centrifugation for 15 minutes at 48,000 x g. The final membrane pellet was resuspended in 20 mM HEPES buffer containing 0.2 mM phenylmethylsulfonyl fluoride (PMSF).

[0067] To measure $^{125}$I-amylin binding, membranes from 4 mg original wet weight of tissue were incubated with $^{125}$I-amylins at 12-16 pM in 20 mM HEPES buffer containing 0.5 mg/ml bacitracin, 0.5 mg/ml bovine serum albumin, and 0.2 mM PMSF. Solutions were incubated for 60 minutes at 23˚C. Incubations were terminated by filtration through GF/B glass fiber filters (Whatman Inc., Clifton, NJ) which had been presoaked for 4 hours in 0.3% polylethyleneimine in order to reduce nonspecific binding of radiolabeled peptides. Filters were washed immediately before filtration with 5 ml cold PBS, and immediately after filtration with 15 ml cold PBS. Filters were removed and radioactivity assessed in a gamma-counter at a counting efficiency of 77%. Competition curves were generated by measuring binding in the presence of $10^{-12}$ to $10^{-6}$ M unlabeled test compound and were analyzed by nonlinear regression using a 4-parameter logistic equation (Inplot program; GraphPAD Software, San Diego).

[0068] In this assay, purified human amylin binds to its receptor at a measured IC$_{50}$ of about 50 pM. Results for test compounds are set forth in Table I, showing that each of the compounds has significant receptor binding activity.

| TABLE I |
|---|---|
| EC$_{50}$(nM) | Receptor Binding Assay IC$_{50}$(pM) |
| 1) $^{28}$Pro-h-Amylin | 15.0 |
| 2) $^{25}$Pro$^{26}$Val$^{28,29}$Pro-h-Amylin | 18.0 |
| 3) $^{2,7}$Cyclo-$^{[2}$Asp, $^{7}$Lys]$^{\cdot}$h-Amylin | 310.0 |
| 4) $^{2-37}$h-Amylin | 236.0 |
EXAMPLE 6

PHENOL RED GASTRIC EMPTYING ASSAY

[0069] Gastric emptying was measured using a modification (Plourde et al., Life Sci. 53:857-862 (1993)) of the original method of Scarpignato et al. (Arch. Int. Pharmacodyn. Ther. 246:286-295 (1980)). Briefly, conscious rats received by gavage, 1.5 mL of an acoloric gel containing 1.5% methyl cellulose (M-0262, Sigma Chemical Co., St. Louis, MO) and 0.05% phenol red indicator. Twenty minutes after gavage, rats were anesthetized using 5% halothane, the stomach exposed and clamped at the pyloric and lower esophageal sphincters using artery forceps, removed and opened into an alkaline solution which was made up to a fixed volume. Stomach content was derived from the intensity of the phenol red in the alkaline solution, measured by absorbance at a wavelength of 560 nm. In most experiments, the stomach was clear. In other experiments, particulate gastric contents were centrifuged to clear the solution for absorbance measurements. Where the diluted gastric contents remained turbid, the spectroscopic absorbance due to phenol red was derived as the difference between that present in alkaline vs acetified diluent. In separate experiments on 7 rats, the stomach and small intestine were both excised and opened into an alkaline solution. The quantity of phenol red that could be recovered from the upper gastrointestinal tract within 29 minutes of gavage was 89±4%; dye which appeared to bind irrecoverably to the gut luminal surface may have accounted for the balance. To compensate for this small loss, percent of stomach contents remaining after 20 minutes were expressed as a fraction of the gastric contents recovered from control rats sacrificed immediately after gavage in the same experiment. Percent gastric emptying contents remaining = (absorbance at 20 min)/(absorbance at 0 min). Dose response curves for gastric emptying were fitted to a 4-parameter logistic model using a least-squares iterative routine (ALLFIT, v2.7, NIH, Bethesda, MD) to derive ED_{50}s. Since ED_{50} is log-normally distributed, it is expressed ± standard error of the logarithm. Pairwise comparisons were performed using one-way analysis of variance and the Student-Newman-Keuls multiple comparisons test (Instat v2.0, GraphPad Software, San Diego, CA) using P < 0.05 as the level of significance.

[0070] In dose response studies, rat amylin (Bachem, Torrance, CA) dissolved in 0.15M saline, was administered as a 0.1 mL subcutaneous bolus in doses of 0, 0.01, 0.1, 1, 10 or 100 μg 5 minutes before gavage in Harlan Sprague Dawley (non-diabetic) rats fasted 20 hours and diabetic BB rats fasted 6 hours. When subcutaneous amylin injections were given 5 minutes before gavage with phenol red indicator, there was a dose-dependent suppression of gastric emptying (data not shown). Suppression of gastric emptying was complete in normal HSD rats administered 1 μg of amylin, and in diabetic rats administered 10μg (P = 0.22, 0.14). The ED_{50} for inhibition of gastric emptying in normal rats was 0.43 μg (0.60 nmol/kg) ± 0.19 log units, and was 2.2μ (2.3 nmol/kg) ± 0.18 log units in diabetic rats.

EXAMPLE 7

TRITIATED GLUCOSE GASTRIC EMPTYING ASSAY

[0071] Conscious, non-fasted, Harlan Sprague Dawley rats were restrained by the tail, the tip of which was anesthetized using 2% lidocaine. Tritium in plasma separated from tail blood collected 0, 15, 30, 60, 90 and 120 minutes after gavage was detected in a beta counter. Rats were injected subcutaneously with 0.1 mL saline containing 0, 0.1, 0.3, 1, 10 or 100 μg of rat amylin 1 minute before gavage (n=8,7,5,5,5, respectively). After gavage of saline pre-injected rats with tritiated glucose, plasma tritium increased rapidly (t 1/2 of about 8 minutes) to an asymptote that slowly declined. Subcutaneous injection with amylin dose-dependently slowed and/or delayed the absorption of the label. Plasma tritium
activity was integrated over 30 minutes to obtain the areas under the curve plotted as a function of amylin dose. The 
ED$_{50}$ derived from the logistic fit was 0.35 $\mu$g of amylin.

Claims

1. A formulation suitable for parenteral administration, said formulation comprising an amylin or an amylin agonist, or a salt thereof.

2. The formulation according to Claim 1, wherein said amylin or amylin agonist is selected from the group consisting of

i) an agonist analogue of amylin having the amino acid sequence:

\[^{1}A_{1}-X-Asn^{5}Ala-Thr-Y-Ala-Thr^{10}Gln-Arg-Leu-\]
\[^{15}B_{1}-Asn^{15}Phe-Leu-C_{1}-D_{1}-E_{1}^{20}F_{1}-G_{1}-Asn-H_{1}-Gly^{25}Pro-I_{1},\]
\[Leu-Pro-J_{1}^{30}Thr-K_{1}^{35}Val-Gly-Ser-^{35}Asn-Thr-Tyr-Z\]

wherein

\[A_{1}\] is Lys, Ala, Ser or hydrogen;

\[B_{1}\] is Ala, Ser or Thr;

\[C_{1}\] is Val, Leu or Ile;

\[D_{1}\] is His or Arg;

\[E_{1}\] is Ser or Thr;

\[F_{1}\] is Ser, Thr, Gln or Asn;

\[G_{1}\] is Asn, Gin or His;

\[H_{1}\] is Phe, Leu or Tyr;

\[I_{1}\] is Ile, Val, Ala or Leu;

\[J_{1}\] is Ser, Pro or Thr;

\[K_{1}\] is Asn, Asp or Gln;

\[X\] and \[Y\] are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and \[Z\] is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, aryloxy or aralkoxy; and provided that when \[A_{1}\] is Lys, \[B_{1}\] is Ala, \[C_{1}\] is Val, \[D_{1}\] is Arg, \[E_{1}\] is Ser, \[F_{1}\] is Ser, \[G_{1}\] is Asn, \[H_{1}\] is Leu, \[I_{1}\] is Val, \[J_{1}\] is Pro, and \[K_{1}\] is Asn; then one or more of \[A_{1}\] to \[K_{1}\] is a D-amino acid and \[Z\] is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, aryloxy or aralkoxy;

ii) an agonist analogue of amylin having the amino acid sequence:

\[^{1}A_{1}-X-Asn^{5}Ala-Thr-Y-Ala-Thr^{10}Gln-Arg-Leu-\]
\[^{15}B_{1}-Asn^{15}Phe-Leu-C_{1}-D_{1}-E_{1}^{20}F_{1}-G_{1}-Asn-H_{1}-Gly^{25}Pro-I_{1},\]
\[Leu-Pro-J_{1}^{30}Thr-K_{1}^{35}Val-Gly-Ser-^{35}Asn-Thr-Tyr-Z\]

wherein

\[A_{1}\] is Lys, Ala, Ser or hydrogen;

\[B_{1}\] is Ala, Ser or Thr;

\[C_{1}\] is Val, Leu or Ile;

\[D_{1}\] is His or Arg;

\[E_{1}\] is Ser or Thr;

\[F_{1}\] is Ser, Thr, Gln or Asn;

\[G_{1}\] is Asn, Gin or His;

\[H_{1}\] is Phe, Leu or Tyr;

\[I_{1}\] is Ile, Val, Ala or Leu;

\[J_{1}\] is Ser, Pro or Thr;

\[K_{1}\] is Asn, Asp or Gln;

\[X\] and \[Y\] are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and \[Z\] is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkoxy, aryloxy or aralkoxy; and provided than when

(a) \[A_{1}\] is Lys, \[B_{1}\] is Ala, \[C_{1}\] is Val, \[D_{1}\] is Arg, \[E_{1}\] is Ser, \[F_{1}\] is Ser, \[G_{1}\] is Asn, \[H_{1}\] is Leu, \[I_{1}\] is Val, \[J_{1}\] is Pro and \[K_{1}\] is Asn; or
(b) A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is His, E₁ is Ser, F₁ is Asn, G₁ is Asn, H₁ is Leu, I₁ is Val, J₁ is Ser and K₁ is Asn; then one or more of A₁ to K₁ is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy;

iii) an agonist analogue of amylin having the amino acid sequence:

\[
\begin{align*}
\text{A₁-X-Asn-Thr-} & \text{5Ala-Thr-Y-Ala-Thr-10Gln-Arg-Leu-B₁-Asn-} \\
& \text{15Phe-Leu-C₁-D₁-E₁-} \text{20F₁-G₁-Asn-H₁-Gly-25I₁-J₁-Leu-Pro-} \\
& \text{30Thr-K₁-Val-Gly-Ser-} \text{35Asn-Thr-Tyr-} \text{Z}
\end{align*}
\]

wherein

- A₁ is Lys, Ala, Ser or hydrogen;
- B₁ is Ala, Ser or Thr;
- C₁ is Val, Leu or Ile;
- D₁ is His or Arg;
- E₁ is Ser or Thr;
- F₁ is Ser, Thr, Gln or Asn;
- G₁ is Asn, Gln or His;
- H₁ is Phe, Leu or Tyr;
- I₁ is Ala or Pro;
- J₁ is Ile, Val, Ala or Leu;
- K₁ is Asn, Asp or Gln;

X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said intramolecular linkage comprises a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; and provided that when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Pro, J₁ is Val and K₁ is Asn; then one or more of A₁ to K₁ is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy

or from the group consisting of

\[
\begin{align*}
\text{18Arg} & \text{25,28Pro-h-amylin, des-1Lys18Arg25,28Pro-h-amylin,} \\
& \text{18Arg25,28,29Pro-h-amylin, des-1Lys18Arg25,28,29Pro-h-amylin,} \\
& \text{25,28,29Pro-h-amylin, des-1Lys25,28,29Pro-h-amylin,} \\
& \text{25,28,29Pro-h-amylin, des-1Lys25,28,29Pro-h-amylin,} \\
& \text{25,28,29Pro-h-amylin, des-1Lys25,28,29Pro-h-amylin,} \\
& \text{25,28,29Pro-h-amylin, des-1Lys25,28,29Pro-h-amylin,} \\
& \text{17Ile25,28,29Pro-h-amylin, des-1Lys17Ile25,28,29Pro-h-amylin,} \\
& \text{17Ile18Arg21His23Leu26Ala28Leu29Pro31Asp-h-amylin,} \\
& \text{17Ile18Arg21His23Leu26Val29Pro-h-amylin,} \\
& \text{17Ile18Arg21His23Leu26Val29Pro-h-amylin,} \\
& \text{17Ile18Arg21His23Leu26Val29Pro-h-amylin,}
\end{align*}
\]

3. The formulation according to any of Claims 1-2, said formulation further comprising an isotonic buffer solution at a pH of about 4.3 to 7.4.

4. The formulation according to any of Claims 1-2, said formulation further comprising a pH buffering agent.

5. The formulation according to Claim 4, wherein said pH buffering agent is a sodium acetate/acetic acid buffer.

6. The formulation according to any of Claims 1-2, said formulation further comprising a buffer selected from the group consisting of acetate, phosphate, citrate and glutamate, said formulation have a final pH of approximately 3.0 to 6.0.

7. The formulation according to any of Claims 1-2, said formulation further comprising a carbohydrate or polyhydric alcohol tonicifier in an aqueous continuous phase.
8. The formulation according to any of Claims 1-2, said formulation further comprising an antimicrobial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol.

9. The formulation according to any of Claims 1-2, said formulation further comprising mannitol, an acetate buffer, 0.1 to 0.3 w/v of m-cresol, and having pH approximately 3.7 to 4.3.

10. The formulation according to any of Claims 1-2, said formulation further comprising a thickening agent.

11. The formulation according to any of Claims 1-2, said formulation prepared in emulsified form.

12. The formulation according to any of Claims 1-2, said formulation further comprising a non-steroidal anti-inflammatory drug.

13. The formulation according to Claim 12, wherein said non-steroidal anti-inflammatory agent is selected from the group consisting of salicylate, phenylbutazone, indomethacin, acetaminophen, phenacetin, naproxen, ibuprofen, sulindac, etodolac, fenamates, telmetin, ketorolac, diclofenac, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam and apazone.

14. Use of an amylin or an amylin agonist for the preparation of a pharmaceutical composition for treating or preventing gastritis in a subject, comprising administering to said subject a therapeutically effective amount of an amylin or an amylin agonist, wherein said amylin agonist is not a calcitonin and said amylin or amylin agonist is not administered intracerebroventricularly.

15. Use of an amylin or an amylin agonist for the preparation of a pharmaceutical composition for treating or preventing gastric ulceration in a subject, comprising administering to said subject a therapeutically effective amount of an amylin or an amylin agonist, wherein said amylin agonist is not a calcitonin and said amylin or amylin agonist is not administered intracerebroventricularly.

16. Use of an amylin or an amylin agonist for the preparation of a pharmaceutical composition for treating or preventing a condition for which a non-steroidal anti-inflammatory agent is indicated, comprising administering to subject a therapeutically effective amount of an amylin or an amylin agonist, wherein said amylin agonist is not a calcitonin, and said amylin or amylin agonist is not administered intracerebroventricularly, and a therapeutically effective amount of a non-steroidal anti-inflammatory agent.

17. The use according to any of claims 14-16, wherein said subject is human.

18. The use according to any of claims 14-16, wherein said amylin or amylin agonist is administered by a route selected from the group consisting of subcutaneous, intravenous, nasal, oral, pulmonary, transdermal, and buccal administration.

19. The use according to any of claims 14-16 wherein said amylin agonist is selected from the group consisting of

- 18Arg25,28Pro-h-amylin, des-1Lys18Arg25,28Pro-h-amylin,
- 18Arg25,28Pro-h-amylin, des-1Lys18Arg25,28Pro-h-amylin,
- 25Pro26Val28,29Pro-h-amylin,
- Leu25Pro26Val28,29Pro-h-amylin,
- 25Leu25Pro26Val28,29Pro-h-amylin, des-1Lys25Pro26Val28,29Pro-h-amylin,
- Arg23Leu25Pro26Val28,29Pro-h-amylin, 18Arg23Leu25Pro26Val28,29Pro-h-amylin,
- 13Thr21His23Leu26Ala28Leu29Pro31Asp-h-amylin, des-1Lys13Thr21His23Leu26Ala28Leu29Pro31Asp-h-amylin,
- 13Thr21His23Leu26Ala29Pro31Asp-h-amylin, des-1Lys13Thr21His23Leu26Ala28Leu29Pro31Asp-h-amylin,
- 13Thr21His23Leu26Ala29Pro31Asp-h-amylin, and
- 13Thr18Arg21His23Leu26Ala28Pro31Asp-h-amylin.

20. The use according to any of claims 14-16, wherein said amylin agonist is 25,28,29Pro-h-amylin.
21. The use according claim 14, wherein said gastritis is associated with the administration of a non-steroidal anti-inflammatory agent.

22. The use according to claim 15 wherein said gastric ulceration is associated with the administration of a non-steroidal anti-inflammatory agent.

23. The use according to claim 16 wherein said non-steroidal anti-inflammatory agent is selected from the group consisting of salicylate, phenylbutazone, indomethacin, acetaminophen, phenacetin, naproxen, ibuprofen, sulandac, etodolac, fenamates, telmetin, ketoralac, diclofenac, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam and apazone.

24. A pharmaceutical composition comprising (a) an amylin or an amylin agonist, or a pharmaceutically acceptable salt thereof, wherein said amylin agonist is not a calcitonin, and (b) a non-steroidal anti-inflammatory drug, in a pharmaceutically acceptable carrier and dose.

25. The pharmaceutical composition according to claim 24, wherein said non-steroidal anti-inflammatory agent is selected from the group consisting of salicylate, phenylbutazone, indomethacin, acetaminophen, phenacetin, naproxen, ibuprofen, sulandac, etodolac, fenamates, telmetin, ketoralac, diclofenac, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam and apazone.
Fig. 1

ED$_{50}$ = 0.036 µg

MEAN INJURY SCORE (PERCENT OF VALUE OBSERVED WITH vehicle ALONE)
REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 08851965 B [0001]
- GB 8709871 A [0005]
- US 5367052 A [0005]
- US 5175145 A [0006]
- US 5124314 A [0006]
- US 5264372 A [0010] [0042]
- US 11838193 A [0014] [0043]
- US 30206994 A [0014] [0043]
- WO 9507098 A [0014] [0043]
- US 5376638 A [0017]
- US 5677279 A [0018]
- US 5234906 A [0025]
- US 5686411 A [0025] [0033] [0040]
- US 03514097 P [0050]
- US 00526298 A [0050]

Non-patent literature cited in the description

- LUPIN; YOUNG. Diabetes Nutrition and Metabolism - Clinical and Experimental, February 1993, vol. 6 (1), 1318 [0012]
• HARTTER. Diabetologia, 1991, vol. 34, 52-54 [0019]
• KODA et al. The Lancet, 1992, vol. 339, 1179-1180 [0019] [0019]
• GILL et al. Life Sciences, 1991, vol. 48, 703-710 [0019]
• Analgesic-antipyretics, anti-inflammatory agents, and drugs employed in the therapy of gout. WOOD-BURY, D.M ; FINGL, E. The Pharmacological Basis of Therapeutics. 1975, 325-43 [0024]
• DELEAN. ALLFIT, Version 2.7 [0042]
• Introduction to Cleavage Techniques. Applied Biosystems, Inc, 1990, 6-12 [0046]
• Remington’s Pharmaceutical Sciences [0049]