Title: USE OF AMYLIN AND AMYLIN AGONISTS AS CARDIOPROTECTIVE OR MYOPROTECTIVE AGENTS

Abstract: Methods for providing for the use of amylin and amylin agonists as a cardioprotective agent. Compounds of the invention are used to reduce, prevent, or delay the mortality and/or morbidity associated with heart disease. The compounds of the invention are useful for treating or preventing cardiac and/or cardiovascular diseases. Compounds of the invention are useful for increasing or promoting the health of the heart.
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USE OF AMYLIN AND AMYLIN AGONISTS AS CARDIOPROTECTIVE OR MYOPROTECTIVE AGENTS

FIELD OF THE INVENTION

[0001] The present invention relates to the medical field and in particular to the area of heart disease, diseases that affect the heart and cardiovascular system, and the skeletal system.

BACKGROUND OF THE INVENTION

[0002] It has been stated that cardiovascular disease is by far the leading cause of morbidity and mortality in the developed world. By the year 2020, it is predicted that acute coronary occlusion will be the major cause of death in the world. Therefore, despite much time, money and effort that has been expended to understand, treat and prevent various cardiac and cardiovascular diseases, there continues to be a need for greater understanding of the diseases and effective options for treating them.

[0003] Cardiac and cardiovascular diseases include left ventricular hypertrophy, coronary artery disease, essential hypertension, acute hypertensive emergency, cardiomyopathy, heart insufficiency, exercise tolerance, chronic heart failure, arrhythmia, cardiac dysrhythmia, syncopy, atherosclerosis, mild chronic heart failure, angina pectoris, cardiac bypass reocclusion, intermittent claudication (atherosclerosis obliterens), diastolic dysfunction and systolic dysfunction.

[0004] Abnormal Na+/K+-ATPase enzyme, or sodium pump, activity has been postulated to be involved in the pathophysiology of several diseases, including cardiac and cardiovascular diseases, among others. These complex effects may be related to the role of the pump in controlling the cellular ingress of other molecules.

[0005] The Na+/K+-ATPase enzyme is a membrane protein responsible for establishing an electrochemical gradient of Na+ and K+ ions across the plasma membrane of mammalian cells. The ion gradient formed by this enzyme is necessary for the active transport of essential nutrients into the cells, for regulation of osmotic balance and cell volume, and for maintaining the resting membrane potential in excitable cells. The Na+/K+-ATPase enzyme is the only known receptor for cardiac glycosides such as digitalis. The tight conservation of the digitalis binding site over many phyla, among other observations, suggests the existence of endogenous sodium pump inhibitors (SPIs) in mammals as well. These hypothetical mammalian inhibitors would be involved in modulating the activity of the sodium pump, and might be involved in vivo sodium homeostasis.

[0006] There are at least 3 isoforms of each the α- and β-subunits of Na+/K+-ATPase. These are distributed in a tissue-specific manner, and show different functional characteristics (e.g., different
affinities to Na+ or cardiac glycosides). Acute effects of numerous hormones on Na+/K+-ATPase appear to be receptor mediated, and hence dependent upon the existence of the relevant receptors in tissues of interest. Receptor distribution is highly tissue specific, so various tissues may not respond similarly to a particular hormone.

[0007] It is generally understood that transmembrane sodium and potassium gradients across cells represent stored electrochemical potential, the release of which powers impulse propagation in excitable tissues such as nerves and muscles, including heart muscle. This potential is established in an energy-consuming Na+/K+ exchange whereby Na+ is pumped out of cells and K+ is pumped into cells via a transporter, Na+/K+-ATPase.

[0008] Compounds such as calcitonin gene related peptide and intermedin have been purported to have an effect on the heart. Gennari et al. 1990, concludes that CGRP improves myocardial contractility in patients with congestive heart. WO 2004/048547 describes intermedin (AFP-6) and lists its potential use as a hypotensive agent, as a cardioprotective agent, in the regulation of gastrointestinal motility, and in stimulating the release of prolactin, e.g. as an aid in fertilization, lactation, pre-term labor, and the like.

[0009] Amylin

[0010] Human amylin is a 37 amino acid peptide hormone that is co-secreted with insulin from pancreatic β-cells in response to nutrient stimuli. Human amylin has the following amino acid sequence:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-GlnArg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-ne-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr  (SEQ ID NO:1).

[0011] Amylin is believed to regulate gastric emptying, and suppress glucagon secretion and food intake, thus regulating the rate of glucose appearance in the circulation. It appears to complement the actions of insulin, which regulates the rate of glucose disappearance from the circulation and its uptake by peripheral tissues. These actions are supported by experimental findings in rodents and humans, which indicate that amylin complements the effects of insulin in postprandial glucose control by at least three independent mechanisms, all of which affect the rate of glucose appearance.

[0012] First, amylin suppresses postprandial glucagon secretion. Compared to healthy adults, patients with type 1 diabetes have no circulating amylin and patients with type 2 diabetes have diminished postprandial amylin concentrations. Furthermore, infusion of an amylin specific monoclonal antibody, which bound circulating amylin, again resulted in greatly elevated glucagon concentrations relative to controls. Both of these results point to a physiological role of endogenous amylin in the regulation of postprandial glucagon secretion. Second, amylin slows gastrointestinal
motility and gastric emptying. Finally, intrahypothalamic injections of rat amylin were shown to reduce feeding in rats and alter neurotransmitter metabolism in the hypothalamus. In certain studies, food intake was significantly reduced for up to eight hours following the intrahypothalamic injection of rat amylin and rat CGRP.

[0013] In human trials, an amylin analog, pramlintide, has been shown to reduce weight or weight gain. Amylin may be beneficial in treating metabolic conditions such as diabetes and obesity. Amylin may also be used to treat pain, bone disorders, gastritis, to modulate lipids, in particular triglycerides, or to affect body composition such as the preferential loss of fat and sparing of lean tissue.

[0014] It has now surprisingly been found that amylin and its agonists may have a cardioprotective, myoprotective effect, or both.

SUMMARY OF THE INVENTION

[0015] The present invention relates generally to methods for cardioprotection, myoprotection, or both by administration of amylin or amylin agonists. In one general aspect, the present invention describes methods for ameliorating, treating, or preventing cardiac or cardiovascular diseases, or both cardiac and cardiovascular diseases comprising administering a therapeutically effective amount of an amylin or an amylin agonist. In another general aspect, the present invention describes methods for reducing, preventing, or delaying the onset of the mortality, morbidity, or both associated with heart disease comprising administering a therapeutically effective amount of an amylin or an amylin agonist. Methods are provided for the use of amylin and amylin agonists as a cardioprotective agent.

[0016] In yet another general aspect, the present invention describes methods for promoting or increasing the health of the heart comprising administering a therapeutically effective amount of an amylin or an amylin agonist. In still another general aspect, the present invention describes methods for ameliorating, treating, preventing, or delaying the onset of cardiovascular disease or cardiac death by affecting the Na+/K+-ATPase transport system. In still another general aspect, the present invention describes methods for promoting heart health by affecting the Na+/K+-ATPase transport system. In still another aspect, the present invention involves the amylin or amylin agonist stimulating (increasing) the Na+/K+-ATPase transport system. In yet another aspect, the present invention describes methods for ameliorating, treating, preventing or delaying the onset of cardiovascular diseases, or increasing or promoting heart health, by stimulating a Na+/K+-ATPase
transport system comprising administering a therapeutically effective amount of an amylin or an amylin agonist to stimulate (increase) the Na+/K+-ATPase.

[0017] In one embodiment of the above-described methods, an amylin agonist is not a calcitonin gene related peptide (CGRP) or AFP-6 (intermedin) and AFP-6 analogs described in U.S. Provisional Application Serial No. 60/617,468, published as WO2006042242. In another embodiment, the amylin agonist is not a CGRP analog. In another embodiment, the amylin agonist is not a CGRP, CGRP agonist, or CGRP analog described in WO2005070444, WO2005070445 or in US Patent application nos. 10/756,490, 10/756,157, or provisional application no. 60/565,056. In still another embodiment, the amylin agonist is an amylin agonist analog. In still other embodiments, the amylin agonist is a small molecule such as that described in WO 2005/025504, incorporated herein by reference.

[0018] In another embodiment, an amylin or amylin agonist useful in the methods of the present invention is combined with one or more other active ingredients useful in cardioprotection. The compounds may be combined in a unitary dosage form, or in separate dosage forms intended for simultaneous or sequential administration to a patient in need of treatment. The compounds may be chemically joined to create a hybrid compound. In further embodiments the methods of the invention comprise administration of an angiotensin II antagonist, renin inhibitor, calcium channel blocker and/or additional cardioprotective or myoprotective peptide.

[0019] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. All references cited herein are incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 illustrates the effects of amylin on cumulative VF duration.

[0021] Figure 2 illustrates the effects of amylin on cumulative mortality.

[0022] Figure 3 illustrates the effects of amylin agonist on mortality associated with cardiac events in insulin-treated subjects.

DESCRIPTION OF THE INVENTION

[0023] The present invention generally provides methods for cardioprotection, myoprotection, or both cardioprotection and myoprotection by the administration of an amylin or amylin agonist to a subject. In general "cardioprotection" refers to the amelioration, treatment, or prevention of
diseases and conditions of the heart and cardiac tissues, as well as cardiovascular tissues. Without wishing to be bound by theory, it is proposed that amylin, calcitonin, and their agonists may exert a cardioprotective effect or myoprotective effect on cardiac and skeletal muscle by stimulating Na+/K+-ATPase, also referred to as the sodium pump, via amylin receptors or calcitonin-like receptors or both. Na+/K+-ATPase stimulation in cardiocytes, as in skeletal muscle, may restore an ionic milieu less likely to result in potentially fatal dysrhythmia, and may increase cardiac contractility.

[0024] In one aspect of the present invention, heart diseases and conditions include, acute and chronic congestive cardiac failure, abnormal contractility, ischemias, ventricular tachyarrhythmias, myocardial infarction, ventricular ectopic beats (VEBs), ventricular tachycardia (VT), ventricular fibrillation (VF), and arrhythmias.

[0025] As used herein, "myoprotection" refers to the amelioration, treatment, or prevention of heart diseases and conditions associated with the myocardium. In one aspect diseases and conditions associated with the myocardium include abnormal contractility, ventricular tachyarrhythmias, myocardial infarction, ventricular ectopic beats (VEBs), ventricular tachycardia (VT), ventricular fibrillation (VF), and arrhythmias.

[0026] Thus, in one aspect of the present invention, methods for cardioprotection, myoprotection or both are provided. In general, the methods comprise administration of an amylin or an amylin agonist to a subject. In a one aspect, the methods comprise administration of an amylin or an amylin agonist to a subject in need of cardioprotection, myoprotection, or both.

[0027] A "subject" may include any mammal, including humans. A "subject" may also include pets (e.g., dogs, cats, horses), as well as other valuable animals. Subjects who may benefit from the methods of the invention may be those who have or are at risk of having a cardiovascular disease. Further, a subject may be one who is in need of or desirous of experiencing the present methods.

[0028] In one aspect, cardioprotection or myoprotection includes the amelioration, treatment, or prevention of diseases and conditions of the myocardium caused by impaired blood perfusion. In one aspect, the diseases and conditions may be caused by ischemic and reperfusion injury. In this aspect, the methods protect heart cells from damage, necrosis, or apoptosis during ischemic or reperfusion injury. Such methods include the administration of an effective amount of an amylin or amylin agonist to a subject for the protection of heart cells or tissues from such damage.

[0029] The term "effective amount" refers to an amount of a pharmaceutical agent used to treat, ameliorate, prevent, or eliminate the identified condition (e.g., disease or disorder), or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers, antigen levels, or time to a measurable event, such as morbidity or mortality. Therapeutic
effects can include preventing further loss of cardiac myocytes, or improving cardiac myocyte efficiency, or both. Therapeutic effects can also include an improvement in cardiac contractility. Further therapeutic effects can include reduction in physical symptoms of a subject, such as, for example, an increased capacity for physical activity prior to breathlessness. The precise effective amount for a subject will depend upon the subject's body weight, size, and health; the nature and extent of the condition, the desired therapeutic effect; and the therapeutic or combination of therapeutics selected for administration. Effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician.

[0030] In another aspect, the invention is directed to a method of treating cardiovascular and related diseases, for example, hypertension, hypertrophy, arrhythmia, congestive heart failure, myocardial ischemia, heart failure subsequent to myocardial infarction, myocardial infarction, ischemia reperfusion injury, and diseases that arise from thrombotic and prothrombotic states by administering a therapeutically effective amount of an amylin or an amylin agonist.

[0031] In one aspect, cardioprotection refers to a state of reduction, amelioration, or prevention of dysrhythmia in a subject. In another aspect, the methods include a reduction in fatal dysrhythmia in a subject. In yet another aspect, the methods result in the reduction in dysrhythmia in combination with other improvements in cardiac or myocyte function. In one aspect, the methods result in the reduction of dysrhythmia and increases cardiac contractility. In one aspect, dysrhythmia includes premature atrial contractions, premature ventricular contractions, atrial fibrillation, atrial flutter, paroxysmal supraventricular tachycardia, accessory pathway tachycardias, AV nodal reentrant tachycardias, ventricular tachycardia, ventricular fibrillation, long QT syndrome, bradyarrhythmias, sinus node dysfunction and heart block.

[0032] In an embodiment, dysrhythmia is ameliorated or reduced to an amount that is less than about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the amount of dysrhythmia in the absence of amylin or amylin agonist administration. In another embodiment, dysrhythmia can be slightly reduced, moderately reduced, or substantially eliminated, as compared to the dysrhythmia in the absence of administering an amylin or amylin agonist. As used herein, a slight reduction of dysrhythmia refers to dysrhythmia that is decreased by about 25% or less as compared with the occurrence of dysrhythmia in the absence of administering amylin or amylin agonist. A moderate reduction in the occurrence of dysrhythmia refers to an occurrence that decreased is by about 50% or less as compared with the occurrence in the absence of administering an amylin or amylin agonist. A substantial elimination of dysrhythmia occurrence refers to the occurrence that is decreased by about 90% or more as compared with the occurrence in the absence of administering an amylin or amylin agonist.
The detection of dysrhythmias can be detected using any method for dysrhythmia detection available to the skilled clinician, including by monitoring a subject's pulse and by electrocardiogram.

In one aspect of the present invention, the methods protect the heart muscles from damage that may result from impaired blood perfusion. Impaired blood perfusion can result from any cause that results in an impairment of blood perfusion, including, thrombosis, heart failure, myocardial infarction, and reduced contractility.

In another aspect, the methods of the present invention provide for the re-establishment of contractility, reducing the loss of contractility, improving the contractility, or maintaining contractility in the heart muscle of a subject. In one aspect, the cardioprotection is not a result of increased cardiac contractility.

In yet a further aspect, the methods of the present invention result in the amelioration of myocardial infarct magnitude. Infarct magnitude can be measured using any method available to the skill artisan. For example, infarct size can be measured using magnetic resonance imaging (MRI).

In an embodiment, myocardial infarct magnitude is ameliorated or reduced to an amount that is less than about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the amount of myocardial infarct magnitude in the absence of amylin or amylin agonist administration. In another embodiment, myocardial infarct magnitude can be slightly reduced, moderately reduced, or substantially eliminated, as compared to the magnitude of infarct in the absence of administering an amylin or amylin agonist. As used herein, a slight reduction of myocardial infarct magnitude refers to myocardial infarct magnitude that is decreased by about 25% or less as compared with infarct in the absence of administering amylin or amylin agonist. A moderate reduction in myocardial infarct magnitude refers to an infarct magnitude that decreased is by about 50% or less as compared with the magnitude in the absence of administering an amylin or amylin agonist. A substantial elimination of an infarct refers to an infarct magnitude that is decreased by about 90% or more as compared with a magnitude in the absence of administering an amylin or amylin agonist.

Any means available to the skilled art worker can be employed for assessing the degree to which a myocardial infarct is reduced.

In still a further aspect, the methods of the present invention provide for a reduction in mortality or morbidity as a result of heart disease. In one aspect, the methods provide for a reduction in mortality in ischemia and reperfusion events. In an embodiment, mortality or morbidity or both is reduced or ameliorated to an amount that is less than about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the amount of mortality or morbidity in the
absence of amylin or amylin agonist administration. In another embodiment, mortality or morbidity can be slightly reduced, moderately reduced, or substantially eliminated, as compared to the mortality or morbidity in the absence of administering an amylin or amylin agonist. As used herein, a slight reduction of mortality or morbidity refers to mortality or morbidity that is decreased by about 25% or less as compared with infarct in the absence of administering amylin or amylin agonist. A moderate reduction in mortality or morbidity refers to an mortality or morbidity that decreased is by about 50% or less as compared with the mortality or morbidity in the absence of administering an amylin or amylin agonist. A substantial elimination of mortality or morbidity refers to mortality or morbidity that is decreased by about 90% or more as compared with mortality or morbidity in the absence of administering an amylin or amylin agonist.

[0040] The methods of the present disclosure will find use in cardioprotective aspects, as well as in the prevention or treatment of conditions that will benefit from cardioprotection, such as congestive heart failure. Thus, amylin, calcitonin, or their agonists, by acting on heart muscles, may be useful in the treatment of congestive failure and similar conditions that will benefit from cardioprotective and inotropic effects. The benefits of these compounds will also be apparent when they act upon compromised skeletal muscle, such as following ischemia, imminent paralysis from a variety of causes, including energy-deficient states and electrolyte disturbance. Benefits from restoring contractility of skeletal muscle will be especially manifest, for example, when that skeletal muscle is respiratory, when weaning patients from mechanical ventilation, improving rehabilitation in chronic obstructive pulmonary disease (COPD), and in preventing or treating respiratory acidosis (from hypoventilation) including acute respiratory acidosis which can occur upon an abrupt failure of ventilation, and chronic respiratory acidosis which may be secondary to many disorders, including COPD. Chronic respiratory acidosis also may be secondary to obesity hypoventilation syndrome (i.e. Pickwickian syndrome), neuromuscular disorders, such as amyotrophic lateral sclerosis, and severe restrictive ventilatory defects as observed in interstitial fibrosis and thoracic deformities.

[0041] In one aspect, not all inotropic compounds are cardioprotective or myoprotective. For example, although drugs with positive inotropic effects can produce short-term symptomatic improvements in patients with heart failure, their long-term use does not prolong survival in patients. In fact, some positive inotropic agents, such as beta agonists (e.g. dobutamine) have been shown to have no effect or a negative impact survival rates. Dobutamine is a direct-acting inotropic agent whose primary activity results from stimulation of the beta-receptors of the heart, hi one study, long-term use of dobutamine lessened the symptoms of congestive heart failure, but did not prolong survival. Pickworth, *CHn. Pharm.* 11:618-624 (1992). In addition, intermittent

[0042] Other examples of inotropic agents that stimulate contractility, yet do not provide cardioprotection, include the oldest cardiotoxic drug, digitalis. After adjusting for atrial fibrillation and left ventricular failure, one study found that serum digoxin (digitalis) concentration was not significantly related to survival. Taken together with the results of 3 other large, nonrandomized studies of digitalis treatment after acute myocardial infarction, it appeared that digitalis treatment might indeed have adverse effects on survival. Other examples include milrinone.

[0043] In one embodiment, the present invention is directed to amylin or amylin agonists that bind to or act at an amylin and/or calcitonin receptor. It has been reported that the biological actions of amylin family peptide hormones are generally mediated \textit{via} binding to two closely related type II G protein-coupled receptors (GPCRs), the calcitonin receptor (CTR) (of which there are multiple forms known in the art) and the calcitonin receptor like receptor (CRLR). Cloning and functional studies have shown that CGRP, calcitonin, adrenomedullin, and amylin interact with different combinations of CTR or the CRLR and the receptor activity modifying protein (RAMP). Many cells express multiple RAMPs. It is believed that co-expression of RAMPs and either the CTR or CRLR is required to generate functional receptors for calcitonin, CGRP, ADM, and amylin. The RAMP family comprises three members (RAMP1, -2, and -3), which share less then 30\% sequence identity, but have a common topological organization. Co-expression of CRLR and RAMP1 leads to the formation of a receptor for CGRP. Co-expression of CRLR and RAMP2 leads to the formation of a receptor for ADM. Co-expression of CRLR and RAMP3 leads to the formation of a receptor for ADM and CGRP. Co-expression of hCTR2 and RAMP1 leads to the formation of a receptor for amylin and CGRP. Co-expression of hCTR2 and RAMP3 leads to the formation of a receptor for amylin.

[0044] In an embodiment of the present invention, samples and subjects that may be benefited by administration of an amylin or amylin agonist to prevent cardiac damage can be ascertained by the artisan in light of conditions and risk factors related to the sample or subject. In one embodiment samples and subjects of the present invention include those which have experienced, are experiencing or are at risk to experience a condition associated with heart disease. A condition associated with heart disease can be any condition or disorder in which abnormal Na+/K+-ATPase activity is known to occur or thought to be a risk. Conditions associated with abnormal sodium pump activity include, for example, myocardial infarction, ischemia/reperfusion, oxidative stress, advanced glycation endproducts, abnormal cardiac wall tension, sympathetic stimulation, myocarditis, hypertension, and heart transplantation.
In accordance with the methods of the present invention, the amylin or amylin agonist may be administered in any manner known in the art that renders amylin or amylin agonist biologically available to the subject or sample in an effective amount. For example, the amylin or amylin agonist may be administered to a subject via any central or peripheral route known in the art including, but not limited to: oral, parenteral, transdermal, transmucosal, or pulmonary routes. In one embodiment is parenteral administration. Exemplary routes of administration include oral, ocular, rectal, buccal, topical, nasal, ophthalmic, subcutaneous, intramuscular, intravenous, intracerebral, transdermal, and pulmonary. Another embodiment of a route of administration is subcutaneous. Further, the amylin or amylin agonist can be administered to a sample via pouring, pipetting, immersing, injecting, infusing, perfusing, or any other means known in the art. Determination of the appropriate administration method is usually made upon consideration of the condition (e.g., disease or disorder) to be treated, the stage of the condition (e.g., disease or disorder), the comfort of the subject, and other factors known to those of skill in the art.

Administration by the methods of the present invention can be intermittent or continuous, both on an acute and/or chronic basis. Yet another mode of administration of amylin or amylin agonist is continuous. Continuous intravenous or subcutaneous infusion, and continuous transcutaneous infusion are exemplary embodiments of administration for use in the methods of the present invention. Subcutaneous infusions, both acute and chronic, are particularly notable embodiments of administration.

In one embodiment, administration of an amylin or amylin agonist to provide cardioprotection can be a prophylactic treatment, beginning concurrently with the diagnosis of conditions (e.g., disease or disorder) which places a subject at risk of heart diseases, such as for example upon a diagnosis of diabetes. In the alternative, administration of an amylin or amylin agonist to prevent heart diseases or conditions can occur subsequent to occurrence of symptoms associated with heart disease. For example, in subjects suffering from an acute cardiovascular event, administration of an amylin or amylin agonist during the cardiovascular event can limit damage and enhance survival. In subjects who have had a previous cardiovascular event, administration of an amylin or amylin agonist can provide cardioprotective measures that can prevent recurrence and long-term cardiovascular related damage. In subjects naive to an initial cardiovascular event, administration of amylin or amylin agonist can provide protective measures that can prevent or reduce the severity of an initial cardiovascular event, particularly in those in the general population and in subjects who are at high risk due to an adverse cardiovascular risk profile. Such at risk profiles are well-known in the medical arts. For example, those at risk for and in need
of prevention or reduction in severity of a myocardial infarction are known to be those who had
antecedent angina, have peripheral artery disease, had a stroke, or had heart failure.

[0048] Increasing age is perhaps the most notable risk factor for heart failure. Male and female
subjects older than 65 years have a significantly increased incidence of heart failure, and thus
increased need for the treatments discussed herein, with male subjects having an even more
increased risk. Deteriorating left ventricular function is an important marker for identifying those
having increased risk of heart failure. Clinical indicators for deteriorating left ventricular function
include ECG evidence of left ventricular hypertrophy or a rapid resting heart rate, a low or
decreased vital capacity, and radiographic evidence of an enlarged heart. The risk increases when
multiple markers are present. Left ventricular hypertrophy by itself increases the risk of heart failure
two- to threefold. Additional risk factors for heart failure include elevated systolic blood pressure,
increased pulse pressure, angina, a history of myocardial infarction, diabetes, and valvular heart
disease. Five-year mortality for patients with heart failure is reported at 60% to 75%, with African
American men and women having high mortality rates. Those subjects in particular need for
treatment will have a multivariable risk profile that includes age, sex, ECG record, vital capacity,
systolic blood pressure, heart rate, diabetic status, radiographic evidence of cardiomegaly, and the
presence of coronary heart disease (CHD) or murmurs. Those in even more need will have the
multivariable risk profile of heart failure and be predisposed by virtue of the presence of
hypertension, coronary or valve disease, or impaired left ventricular systolic function.

[0049] In yet another embodiment an amylin or amylin agonist will reduce hospital admissions and
the length of hospital stay related to cardiovascular events, such as heart failure, congestive heart
failure, acute hypertensive emergency, cardiac dysrhythmia, angina pectoris, and cardiac bypass
reocclusion.

[0050] For any amylin or amylin agonist, the effective amount can be estimated initially either in
cell culture assays, e.g., in animal models, such as rat or mouse models. An animal model may also
be used to determine the appropriate concentration range and route of administration. Such
information can then be used to determine useful doses and routes for administration in humans.

[0051] Efficacy and toxicity may be determined by standard pharmaceutical procedures in cell
cultures or experimental animals, e.g., ED_{50} (the dose therapeutically effective in 50% of the
population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between
therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio,
ED_{50}/LD_{50}. Pharmaceutical compositions that exhibit large therapeutic indices are one embodiment
of interest. The data obtained from cell culture assays and animal studies may be used in
formulating a range of dosage for human use. The dosage contained in such compositions can be
within a range of circulating concentrations that include an ED$_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0052] As mentioned above, the amylin or amylin agonist may be administered on an acute or chronic basis. An acute administration includes a temporary administration for a period of time before, during and/or after the occurrence of a transient event. An acute administration generally entails an administration that is indicated by a transient event or condition. For example, acute administration may be implicated during an evolving myocardial infarction or during unstable angina. Administration before, during, and/or after a percutaneous cardiac intervention ("PCI") also constitutes an example of an acute administration. In addition, amylin or amylin agonist may be administered acutely before, during and/or after any cardiac surgery, such as open heart surgery, coronary bypass, minimally invasive cardiac surgery, valvuloplasty, or cardiac transplantation. Alternatively, amylin or amylin agonist may also be administered acutely on the basis of congestive heart failure following myocardial infarction or surgery.

[0053] Acute administration before, during, and/or after a particular event may begin at any time before the happening of the event (e.g., such as surgery or transplant) and may continue for any length of time, including for an extended period of time after the event, that is useful to prevent or ameliorate cardiac myocyte apoptosis associated with the event. The duration of an acute administration can be determined by a clinician in light of the risk of cardiac myocyte apoptosis related to the event or condition.

[0054] Amylin agonists for use in the methods include amylin agonist analogs, examples of which are described in US Pat. 5,686,411; US Pat 6,610,824; US Pat 5,998,367; US Pat 6,087,334; international application PCT/US2005/004631; and WO2006052608, all of which are incorporated herein by reference. In certain embodiments, methods of the invention amylin agonists do not include AFP-6 (intermedin) and novel compounds described in US provisional application no. 60/617,468, filed Oct. 8, 2004, published as WO2006042242. In certain embodiments, the amylin agonists do not include calcitonin. In certain embodiments, the amylin agonists do not include salmon calcitonin. In other embodiments, the amylin agonists do not include CGRP. In still other embodiments, amylin agonists do not include analogs of CGRP or calcitonin. Accordingly, it is contemplated that methods of the invention may include a proviso that excludes CGRP or AFP-6 or calcitonin, or their analogs.

[0055] By "amylin" is meant the human peptide hormone referred to as amylin and secreted from the beta cells of the pancreas, and species variations thereof, as described in U.S. Pat. No. 5,234,906, issued Aug. 10, 1993, for "Hyperglycemic Compositions," the contents of which are
hereby incorporated by reference. More particularly, amylin is a 37-amino acid polypeptide hormone normally co-secreted with insulin by pancreatic beta cells in response to nutrient intake (see, e.g., Koda et al., Lancet 339:1179-1180, 1992). In this sense, "amylin," "wild-type amylin," and "native amylin," i.e., unmodified amylin, are used interchangeably. Amylin is also sometimes referred to as "IAPP."

[0056] By "agonist" is meant a compound which elicits a biological activity of amylin, in one embodiment having a potency better than amylin, or within five orders of magnitude (plus or minus) of potency compared to amylin, or 4, 3, 2, or 1 order of magnitude, when evaluated by art-known measures such as receptor binding/competition studies as described herein. Agonists include peptide as well as non-peptide compounds.

[0057] In one embodiment, the term refers to a compound which elicits a biological effect similar to that of native amylin, for example a compound (1) having activity in a food intake, gastric emptying, pancreatic secretion, or weight loss assay (international application PCT/US2005/004631, filed on Feb. 11, 2005, or in WO2006052608, both incorporated by reference) similar to native human reference peptide, and/or (2) which binds specifically in a reference receptor assay or in a competitive binding assay with amylin. In one embodiment, the agonists will bind in such assays with an affinity of less than 1 μM, and in other embodiments can have an affinity of less than 1-5 nM, 500, 100, 50, or 5 pM. Such agonists may comprise a polypeptide comprising an active fragment of amylin or a small chemical molecule. It is, however, contemplated that in certain embodiments of the invention, salmon calcitonin, calcitonin, CGRP, AFP-6, and/or their respective analogs may be excluded from the scope of amylin agonists for use in the methods of the present invention.

[0058] Agonists include amylin analogs and amylin derivatives. By "analog" is meant a peptide whose sequence is derived from that of amylin including insertions, substitutions, extensions, and/or deletions, having at least some amino acid identity to amylin or region of an amylin peptide. Analogs may have at least 50 or 55% amino acid sequence identity with a native amylin, or at least 70%, 80%, 90%, or 95% amino acid sequence identity with a native amylin. In one embodiment, such analogs may comprise conservative or non-conservative amino acid substitutions (including non-natural amino acids and L and D forms). Amylin agonist analogs are analogs as herein described and function as an amylin agonist.

[0059] A "derivative" is defined as a molecule having the amino acid sequence of a native amylin or analog, but additionally having a chemical modification of one or more of its amino acid side groups, α-carbon atoms, terminal amino group, or terminal carboxylic acid group. A chemical modification includes, but is not limited to, adding chemical moieties, creating new bonds, and
removing chemical moieties. Modifications at amino acid side groups include, without limitation, acylation of lysine ε-amino groups, N-alkylation of arginine, histidine, or lysine, alkylation of glutamic or aspartic carboxylic acid groups, and deamidation of glutamine or asparagine. Modifications of the terminal amino include, without limitation, the desamino, N-lower alkyl, N-dilower alkyl, constrained alkyls (e.g. branched, cyclic, fused, adamantyl) and N-acyl modifications. Modifications of the terminal carboxy group include, without limitation, the amide, lower alkyl amide, constrained alkyls (e.g. branched, cyclic, fused, adamantyl) alkyl, dialkyl amide, and lower alkyl ester modifications. Lower alkyl is C1-C4 alkyl. Furthermore, one or more side groups, or terminal groups, may be protected by protective groups known to the ordinarily-skilled synthetic chemist. The α-carbon of an amino acid may be mono- or dimethylated.

Exemplary Amylin Agonists and Analogs.

[0060] Amylin is a 37 amino acid peptide hormone that is co-secreted with insulin from pancreatic beta-cells in response to nutrient stimuli. Human amylin has the following amino acid sequence:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-He-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr (SEQ ID NO:1), although the use of amylin from any species is contemplated.

[0062] Amylin agonists contemplated in the use of the invention include those described in U.S. Patent Nos. 5,686,411, 6,114,304, and 6,410,511, which are herein incorporated by reference in their entirety. Such compounds include those having formula I,

\[ \text{A}_1^{1} \text{-X-Asn-Thr-}^{5} \text{Ala-Thr-Y-}^{10} \text{Ala-Thr-}^{15} \text{Arg-Leu-B}_1^{15} \text{-Asn-}^{20} \text{Phe-Leu-C}_1^{20} \text{-D}_1^{20} \text{-E}_1^{20} \text{-F}_1^{20} \text{-G}_1^{20} \text{-H}_1^{20} \text{-Gly-}^{1} \text{JrLeu-KrLr}^{1} \text{-Thr-MrVal-Gly-Ser-}^{1} \text{Asn-Thr-Tyr} \]

\begin{align*}
\text{wherein A}_1^{1} & \text{ is Lys, Ala, Ser or hydrogen;} \\
\text{B}_1^{1} & \text{ is Ala, Ser or Thr;} \\
\text{C}_1^{1} & \text{ is Val, Leu or He;} \\
\text{D}_1^{1} & \text{ is His or Arg;} \\
\text{E}_1^{1} & \text{ is Ser or Thr;} \\
\text{F}_1^{1} & \text{ is Ser, Thr, Gln or Asn;} \\
\text{G}_1^{1} & \text{ is Asn, Gln or His;} \\
\text{H}_1^{1} & \text{ is Phe, Leu or Tyr;} \\
\text{I}_1^{1} & \text{ is Ala or Pro;} \\
\text{J}_1^{1} & \text{ is He, Val, Ala or Leu;} \\
\text{K}_1^{1} & \text{ is Ser, Pro, Leu, He or Thr;} \\
\text{L}_1^{1} & \text{ is Ser, Pro or Thr;}
\end{align*}
M₁ is Asa, Asp, or Gln;
X and Y are independently selected amino acid residues having side chains which are
chemically bonded to each other to form an intramolecular linkage.
[0063] The C-terminal portion can be amino, alkylamino, dialkylamino, cycloalkylamino,
arylamino, aralkylamino, arylxoy, aryloxy, aralkyloxy or carboxyl.

[0064] Suitable side chains for X and Y include groups derived from alkyl sulfhydryls which may
form disulfide bonds; alkyl acids and alkyl amines which may form cyclic lactams; alkyl aldehydes
or alkyl halides and alkylamines which may condense and be reduced to form an alkyl amine
bridge; or side chains which may be connected to form an alkyl, alkenyl, alkynyl, ether or thioether
bond. Alkyl chains can include lower alkyl groups having from about 1 to about 6 carbon atoms.

[0065] An additional aspect of the present invention is directed to agonist analogues of SEQ ID
NO:2 which are not bridged, and wherein X and Y are independently selected from Ala, Ser, Cys,
Val, Leu and He or alkyl, aryl, or aralkyl esters and ethers of Ser or Cys.

[0066] Exemplary compounds include, but are not limited to des-Lys-h-amylin, 28-Pro-h-amylin,
2525-Pro-h-amylin, 18Arg2528-Pro-h-amylin, and des-Lys18Arg2528-Pro-h-amylin, which all show
amylin activity in vivo in treated test animals, (e.g., provoking marked hyperlactemia followed by
hyperglycemia). In addition to having activities characteristic of amylin, some compounds of the
invention have also been found to possess more desirable solubility and stability characteristics
when compared to human amylin. Examples of these compounds include 25-Pro26Val2829-Pro-h-
amylin, 252829-Pro-h-amylin, and 18Arg2528-Pro-h-amylin.

[0067] Other compounds include 18Arg2528-Pro-h-amylin, des-Lys18Arg2528-Pro-h-amylin,
18Arg252829-Pro-h-amylin, des-Lys18Arg252829-Pro-h-amylin, 252829-Pro-h-amylin, des-Lys
252829-Pro-h-amylin, 25-Pro26Val2829-Pro-h-amylin, 23Leu25-Pro26Val2829-Pro-h-amylin,
21Leu25-Pro26Val28-Pro-h-amylin, des-Lys23Leu25-Pro26Val28-Pro-h-amylin,
18Arg23Leu25-Pro26Val28-Pro-h-amylin, 18Arg23Leu252829-Pro-h-amylin, 18Arg23Leu252829-Pro-h-amylin,
17Leu252829-Pro-h-amylin, 17nLe252829-Pro-h-amylin, des-Lys17nLe252829-Pro-h-amylin,
17He18Arg23Leu28-Pro-h-amylin, 17nLe18Arg23Leu28-Pro-h-amylin, 17nLe18Arg23Leu28-Pro-h-amylin,
13Tr1His23Leu26Ala28-Leu29Pro31Asp-h-amylin, 13Thr23His23Leu26Ala29-Pro31Asp-h-amylin,
13Thr18Arg21His23Leu26Ala29-Pro31Asp-h-amylin, 13Thr18Arg21His23Leu26Ala29-Pro31Asp-h-amylin,
13Thr18Arg21His23Leu2829-Pro31Asp-h-amylin, and 13Thr18Arg21His23Leu25-Pro26Ala2829-Pro31Asp-h-
amylin.

[0068] Useful amylin agonist analogs include those identified in PCT publication WO93 10146
entitled "Amylin Agonist Peptides and Uses Therefor" and related U.S. Patent Nos. 5,686,411,
5,998,367, and 6,610,824 the contents of which are hereby incorporated by reference.
Useful amylin agonists also include analogs of formula $\pi$, $X_1$ - $X_a a_1$ - $X_2$ - $X_a a_2$ - $X_3$ - $X_a a_3$ - $X_4$ - $X_a a_4$ - $X_5$ - $X_a a_5$ - $X_6$ (SEQ ID NO:3) wherein $X_1$ is Lys, Arg or absent; $X_2$ is $X_a a_2$, $X_a a_3$, $X_a a_4$, $X_a a_5$, $X_a a_6$, $X_a a_7$, SerThr, provided that if $X_2$ is Z-$X_a a_8$ SerThr, then $X_1$ and $X_a a_8$ are both absent; $X_3$ is AlaThr, AlaSer, SerMet, GluThr or ValThr; $X_4$ is ArgLeuAla, HisLeuAla, ArgLeuAla, LysHeAla, ArgMetAla, HisMetAla, LysMetAla or ArgLeuThr; $X_5$ is PheLeu, PheHe, PheMet, TyrLeu, TyrHe, TyrMet, TrpMet, TrpHe or TrpMet; $X_6$ is ArgSerSerGlyTyr (SEQ ID NO:5), LysSerSerGlyTyr (SEQ ID NO:6), HisSerSerGlyTyr (SEQ ID NO:7), ProSerSerGlyTyr (SEQ ID NO:8), ArgSerArgGlyTyr (SEQ ID NO:9), ArgThrSerGlyTyr (SEQ ID NO:10), ArgAlaSerGlyTyr (SEQ ID NO:11), AlaSerSerGlyTyr (SEQ ID NO:12), ArgSerAlaGlyTyr (SEQ ID NO:13), HisSerAlaGlyTyr (SEQ ID NO:14), ArgSerGlyTyr (SEQ ID NO:15), ArgSer, LysSer, HisSer, ArgThr, ProSer or Arg; $X_{a1}$ is Cys or absent; $X_{a2}$ is Cys or Ala; $X_{a3}$ is Gln, Ala or Asn; $X_{a4}$ is Asn, Ala or Gln; $X_{a5}$ is Val, Ala, He, Met, Leu, PentylGly, or t-butylGly; $X_{a5}$ is Asn, Gln or Asp; $X_{a7}$ is Thr, Ser, Met, Val, Leu or He; $X_{a8}$ is Ala or Val; $X_{a9}$ is Thr or Ser; $X_{a10}$ is Leu, Val, Met or He; and $Z$ is an alkanoyl group of about 1 to about 8 carbon atoms or absent, and pharmaceutically acceptable salts thereof.

Useful amylin agonists may include analogs of comprising an amino acid sequence of formula $X_a a 1$ X $X_{a2}$ X $X_{a3}$ X $X_{a4}$ X $X_{a5}$ X $X_{a6}$ Y X $X_{a7}$ X $X_{a8}$ X $X_{a9}$ X $X_{a10}$ X $X_{a11}$ X $X_{a12}$ X $X_{a13}$ X $X_{a14}$ X $X_{a15}$ X $X_{a16}$ X $X_{a17}$ X $X_{a18}$ X $X_{a19}$ X $X_{a20}$ X $X_{a21}$ X $X_{a22}$ X $X_{a23}$ X $X_{a24}$ X $X_{a25}$ X $X_{a26}$ X $X_{a27}$ X $X_{a28}$ X $X_{a29}$ X $X_{a30}$ X $X_{a31}$ X $X_{a32}$ (SEQ ID NO:16) wherein $X_{a1}$ is A, C, hC (homoCys), D, E, F, I, L, K, hK (homoLys), R, hR (homoArg), S, Hse(homoSer), T, G, Q, N, M, Y, W, P, Hyp(hydroxyPro), H, V or absent; $X_{a3}$ is A, D, E, N, Q, G, V, R, K, hK, hR, H, I, L, M, or absent; $X_{a4}$ is A, I, L, S, Hse, T, V, M, or absent; $X_{a5}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent; $X_{a6}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent; $X_{a7}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent; $X_{a8}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent; $X_{a9}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent; $X_{a10}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent; and $X_{a11}$ is A, C, hC, D, E, F, I, L, K, hK, hR, H, I, L, M, or absent;
Xaa5 is A, S₅T, Hse, Y, V, I, L₅ or M;
Xaa6 is T, A, S, Hse, Y, V, I, L, or M;
Xaa8 is A, V, I, L, F, or M;
Xaa9 is L, T, S, Hse, V, I, or M;
XaalO is G, H₅Q, K, R₅N₅hK, or hR;
Xaal 1 is K, R, Q, N, hK, hR, or H;
Xaal2 is L, I, V, F, M, W, or Y;
Xaal 3 is A, F₅Y₅N, Q, S₅Hse, or T;
Xaal4 is A₅D₅E₅G₅N₅K₅Q₅R₅H₅hR, or hK;
Xaal 5 is A₅D₅E, F, L, S, Y₅₁, V₅ or M;
Xaal6 is L₅F, M₅V, Y, or r;
Xaal 7 is H, Q, N, S, Hse, T₅ or V;
Xaal 8 is K₅hK, R, hR, H₅u(Cit), or n (Om);
Xaal 9 is F, L₅S, Hse,V, I₅T₅ or absent;
Xaal20 is H₅R₅K₅hR, hK, N, Q, or absent;
Xaal21 is T, S, Hse, V, I, L, Q₅N, or absent;
Xaal22 is F, L, M₅V, Y, or I;
Xaal23 is P or Hyp;
Xaal24 is P₅Hyp, R₅K, hR, hK₅ or H;
Xaal25 is T₅S, Hse, V, I, L, F₅ or Y;
Xaal26 is N₅Q₅D₅ or E;
Xaal27 is T, V, S, F, I, or L;
Xaal28 is G or A;
Xaal29 is S₅Hse, T₅V₅₁, L, or Y;
Xaal30 is E₅G₅K₅N₅D₅, R₅hR₅hK₅H, or Q;
Xaal31 is A₅T₅S, Hse, V₅₁, L₅F, or Y; and
Xaal32 is F₅P₅Y, Hse, S, T₅ or Hyp;

[0071] wherein X and Y are capable of creating a bond and are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage such as disulfide bonds; amide bond; alkyl acids and alkyl amines which may form cyclic lactams; alkyl aldehydes or alkyl halides and alkylamines which may condensed and be reduced to form an alkyl amine or imine bridge; or side chains which may be connected to form an alkyl, alkenyl, alkynyl, ether or thioether bond. Alkyl chains may include lower alkyl groups having from about 1 to about 6 carbon atoms. In certain embodiments, the intramolecular linkage may be a disulfide,
amide, imine, amine, alkyl or alkenyl bond. In certain embodiments, X and Y are independently
selected from Ser, Asp, Glu, Lys, Orn, or Cys. In certain embodiments, X and Y are Cys and Cys. In other embodiments, X and Y are Ser and Ser. In still other embodiments, X and Y are Asp and Lys or Lys and Asp. Other embodiments are described in international application

[0072] Exemplary compounds described with reference to human amylin (SEQ ID NO: 1) and
salmon calcitonin (sCT) CSNLSTCVQKLSQELHKQTYPRKTSN (SEQ ID NO: 17) with modifications at the position(s) indicated include, (1-7 hAmy)(18Arg-8-27 sCT)(33-37 hAmy); (1-7 hAmy)(1118Arg-24Pro-8-27 sCT)(33-37
hAmy); (1-7 hAmy)(18Arg-8-24sCT)(30-37 hAmy); (1-7 hAmy)(llArg,18Arg-8-21 sCT)(27-37
rAmy); (8Val9Leu10Gly--1-15hAmy)(18Arg-16-27sCT)(31-37hAmy); (lAia-1-7 hAmy)(1118Arg~
8-27 sCT)(33-37 hAmy); (3Aia-1-7 hAmy)(n18Arg--8-27 sCT)(33-37 hAmy); (4Aia-1
7 hAmyX1W8Arg-8-27 sCT)(33-37 hAmy); (6Aia-1-7 hAmy)(18Arg-8-27 sCT)(33-37 hAmy);
(2Aia18Arg-1-27 sCT)(33-37 hAmy); (Is0CaP7Ala1U8Arg--5-27 sCT)(33-37 hAmy);
(4Aia1118Arg-1 -27 sCT)(33-37 hAmy); (5Aia1118Arg-l-27 sCT)(33-37 hAmy); (6AiaU18Arg-l-27
sCT)(33-37 hAmy); (1-7 hAmy)(1118Arg-8-27 sCT)(33-37 hAmy); (13Ser14Gln15Glu—1-16
hAmy)(17Arg30Asn32Tyr—17-32 sCT); (3Aia18Arg-1-27 sCT)(33-37 hAmy); (Acetyl-
29Agy1U8 Arg-1-27 sCT)(33-37 hAmy); (Acetyl-27Arg-l-7 hAmy)(1118Arg-8-27 sCT)(33-37
hAmy); (Isocap7Aia10Aib11Lys(For)17Aib18Lys(For)--5-27 sCT)(33-37 hAmy); (Isocap-
7Aia10Aib 5 Lys(For)17Aib18Lys(For)-5-24sCT)(30-37 hAmy); ((Isocap-
7Aia10Aib 14 Lys(For)17Aib18Lys(For)-5-22 sCT)(2829Pro-28-37 hAmy); (Isocap-
7Aia10Aib 14 Lys(For)17Aib18Lys(For)--5-21 sCT)(2829Pro-28-37 hAmy); (1-7
hAmy)(LLQQWQKLLQKLKQ (SEQ ID NO:20)) (2829Pro32Thr-27-37 hAmy); (1-7'
hAmyXLLQQLQKLLQKLKQY (SEQ ID NO:21) (2829Pro32Thr-28-37 hAmy); (6Ser-1-7
hAmy)(1118Arg-8-27 sCT)(33-37 hAmy); (6Val-1-7 hAmy)(1118Arg--8-27 sCT)(33-37 hAmy); (1-
7 riAmy)(1118Arg--8-18 sCT)(2829Pro32Thr-27-37 hAmy); (1-7 hAmy)(11ATg-S-1 7
sCT)(2829Pro32Thr-27-37 hAmy); (1-7 hAmy)(11ATg-S-15sCT)(27Tyr28Pro32Arg32Thr-27-37
hAmy); (1-7 hAmy)(11ATg-S-15sCT)(27Tyr28Pro32Arg32Thr-27-37 hAmy); (1-7 hAmyX 18 Arg-8-
14 sCT)(27Tyr28Pro32Arg32Thr-27-37 hAmy); (1-7 hAmy)(11Lys(For))-8-27 sCT)(33-37 hAmy);
(6D-Thr-1-7 hAmy)(1118Arg)-8-27 sCT)(33-37 hAmy); (Acetyl-1-7 hAmyX 18 LysPEGSO00-S-
27 sCT)(33-37 hAmy); (Acetyl^Aia-1^ hAmy)(11Lys(PEG5000) 18Arg-8-27 sCT)(33-37 hAmy);
(Acetyl^Aia-1^ hAmy)(11Arg 18Lys(PEG5000)-8-27 sCT)(33-37 hAmy); (1-7 hAmyX 18 Arg-8-
21 sCT)(19-27 sCT)(33-37 hAmy); (1-7 hAmy)(1118Arg-8-21 sCT)(18Leu-18-27 sCT)(33-37
hAmy);
hAmy); (l-7hAmy)(8-27sCT)(33-37hAmy); (5Ser-1-7hAmy)(11,18ATg-S-IVsCT)(SS-SVhAnly); (1-12hAmy)(18Arg-13-2VsCT\(\chi\)33-3VhAmy); (1-12hAmy)(18Arg-13-2VsCT)(30-3VhAmy); (5Ser15Glu18Arg-1-18hAmy)(19-24sCT)(30-3VhAmy); (6Hse-1-VhAmy)(11,18Arg-8-2VsCT)(33-3VhAmy); (6Mb-1-VhAmy)(11,18Arg-8-2VsCT)(33-3VhAmy); (6Ahp-1-VhAmy)(11,18Arg-8-2VsCT)(33-3VhAmy); (6Thr(OPO\(\chi\)H2)-1-VhAmy)(11,18ATg-8-2VsCT)(33-3V hAmy); (7Ala\(\alpha\)18Arg-5-27sCT)(33-3V hAmy); (1-7 hAmy)(11,18Orn-18-2VsCT)(33-3V hAmy); (1-7 hAmy)(11,18Cit-8-2VsCT)(33-37 hAmy); (1-7 hAmy)(\(\pi\)11,homoLys-8-27sCT)(33-37 hAmy); (L-Octylglycine-1-VhAmy)(11,18Arg-8-2VsCT)(33-3VhAmy); (N-3,6-dioxaocanoyl-l-V-hAmy)(11,18Arg-8-2VsCT)(33-3VhAmy); (CyClO(l-V)-\(\delta\)Asp7Lys18ATg-1\(\eta\)VsCT)(SS-SV hAmy); (cyclo(2-7)\(\delta\)Asp7Lys-17 hAmy)(11,18Arg-8-27sCT)(33-37 hAmy); (cyclo 2-7 hAmy)(11,18Arg-8-27sCT)(33-37 hAmy); (1-7hAmy)(11,18Arg-8-27sCT)(33-37hAmy-9Anc); (1-7hAmy)(11,18Arg-8-27sCT)(33-3VhAmy-Loctylglycine); (N-isocaproyl-l-7-hAmy)(11,18Arg-8-27sCT)(33-37hAmy); (1-7hAmy)(11,18homoArg-8-27sCT)(33-37hAmy); (1Phe-1-7hAmy)(11,18Arg-8-27sCT)(33-3VhAmy); (1-7hAmy)(11,18Arg-8-24sCT)(32Thr-30-37 hAmy); (1-7 hAmy)(11,18Arg-8-27sCT)(15Glu18Arg-1-18hAmy)(19-24 sCT)(30-3VhAmy); (13Ala14Asp15Phe-1-18hAmy)(19-23sCT)(30-3VhAmy); and (2-18 hAmy)(19-23 sCT)(30-36 hAmy).

[0073] Other useful amylin agonists may include analogs of comprising an amino acid sequence of formula Xaa1 Xaa2 Xaa3 Leu Xaa4 Glu Leu Xaa5 Xaa6 Leu Gln Thr Tyr Pro Arg Thr Asn Xaa7 Z3 [SEQ ID NO 22]

Wherein

(a) Xaa1 is (i) a group having two amino acid residues selected from the group consisting of Leu-Leu, Val-Leu, He-Leu, tert-Leu-Leu, Me-Leu, and Ala-Thr, and N-acylated derivatives thereof; or is (ii) the group Z1-Ser-Thr-Z2-Val-Leu [SEQ ID NO: 23] wherein Z1 is an amino acid residue selected from the group consisting of Leu, Val, He, tert-Leu, Nva, Abu, and Nle or an N-acylated derivative thereof or Z1 is an alkanoyl group; and Z2 is an amino acid residue selected from the group consisting of Ala, Ser, Cys, and Thr;

(b) Xaa2 is an amino acid residue selected from the group consisting Gly, Glu, Asn or Aib;

(c) Xaa3 is an amino acid residue selected from the group consisting of Arg, Orn, Lys, and G-aminomethylated derivatives thereof;

(d) Xaa4 is a group having two or more amino acid residues selected from the group consisting of Ser-Gln, Thr-Gln, Ala-Asn, and Thr-Asn;

(e) Xaa5 is an amino acid residue selected from the group consisting of His, Aib, He, Leu, and Val;

(f) Xaa6 is an amino acid residue selected from the group consisting of Arg, Orn, and Lys and C-aminomethylated derivatives thereof.
(g) Xaa7 is a group having six amino acid residues selected from the group consisting of
(i) Thr-Gly-Ser-Asn-Thr-Tyr [SEQ ID NO: 24];
(ii) Thr-Gly-Ser-Gly-Thr-Pro [SEQ ID NO: 25];
(iii) Val-Gly-Ser-Asn-Thr-Tyr [SEQ ID NO: 26];
(iv) Val-Gly-Ser-Gly-Thr-Pro [SEQ ID NO: 27]; and
(h) Z3 is OH or NH2.

[0074] Peptides useful in the invention, like those above, can be in the acid or amide form.

[0075] Derivatives of the agonists and analogs are also included within the scope of this invention
in which the stereochemistry of individual amino acids may be inverted from (L)/S to (D)/R at one
or more specific sites. Also included within the scope of this invention are the agonists and analogs
modified by glycosylation of Asn, Ser and/or Thr residues. Compounds useful in the methods of
the invention may also be biologically active fragments of the peptides (native, agonist, analog, and
derivative) herein described.

[0076] Agonist and analogs of amylin that contain less peptide character are included within the
scope of this invention. Such peptide mimetics may include, for example, one or more of the
following substitutions for -CO-NH- amide bonds: depsipeptides (—CO—O—), iminomethylenes
(-CH₂-NH-), trans-alkenes (—CH=CH-), beta-enaminonitriles (—C(=CH-CN)-NH—),
thioamides (-CS-NH-), thiomethylene (-S-CH₂ or -CH₂-S), methylenes (—CH₂-C₂-) and
retro-amides (—NH-CO—).

[0077] Compounds of this invention 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, for
example, ammonium salts, alkali metal salts (such as sodium and potassium salts) and alkali earth
salts (such as calcium and magnesium salts). Acetate, hydrochloride, and trifluoroacetate salts are
particularly useful.

[0078] Amylin agonists useful in the invention may also include fragments of amylin and its
analogues as described above as well as those described in EP 289287, the contents of which are
herein incorporated by reference. Amylin agonists analogs may also be compounds having at least
60, 65, 70, 75, 80, 85, 90, 95, or 99% amino acid sequence identity to SEQ ID NO:1, or any of the
amylin analogs specifically described herein having amylin activity. Amylin agonists also include
small molecules and non-peptide molecules, for example those based on small molecule chemistry.

[0079] "Amylin activity" as used herein may include the activities known in the art as described
below or the ability of amylin to modulate Na+/K+-ATPase enzyme efficiency. Desirable amylin
agonists or amylin analogs may have at least one property shared by cardioprotective agents such as bepridil.

[0080] Amylin agonist analogs also include insertions, deletions, extensions, truncations, and/or substitutions in at least one or more amino acid positions of SEQ ID NO:1 or any of the amylin analogs specifically described herein. The number of amino acid insertions, deletions, or substitutions may be at least 5, 10, 15, 20, 25, or 30. Insertions, extensions, or substitutions may be with other natural amino acids, synthetic amino acids, peptidomimetics, or other chemical compounds.

[0081] In general, amylin agonists or amylin agonist analogs are recognized as referring to compounds which, by directly or indirectly interacting or binding with one or more receptors, mimics an action of amylin. They may also be referred to as amylin mimetics.

[0082] Activity as amylin agonists and/or analogs can be confirmed and quantified by performing various screening assays, including the nucleus accumbens receptor binding assay, the soleus muscle assay, a gastric emptying assay, or by the ability to induce hypocalcemia, reduce postprandial hyperglycemia in mammals, or the cardioproteective assays described herein. Methods of testing compounds for amylin activity are known in the art. Exemplary screening methods and assays for testing amylin agonists are described in U.S. Patent Nos. 5,264,372 and 5,686,411, which are incorporated herein by reference.

[0083] The receptor binding assay, a competition assay that measures the ability of compounds to bind specifically to membrane-bound amylin receptors. A very useful 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 $^{125}$I 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, Calif.) or the ALLFIT program of DeLean et al. (ALLFIT, Version 2.7 (NIH, Bethesda, Md. 20892)). Munson and Rodbard, Anal. Biochem. 107:220-239 (1980).

[0084] Assays of biological activity of amylin agonists/analogs in the soleus muscle may be performed using previously described methods (Leighton, B. and Cooper, Nature, 335:632-635 (1988); Cooper, et al., Proc. Natl. Acad. Sci. USA 85:7763-7766 (1988)), in which amylin agonist activity may be assessed by measuring the inhibition of insulin-stimulated glycogen synthesis. In brief, an exemplary method includes soleus muscle strips prepared from 12-h fasted male Wistar rats. The tendons of the muscles are ligated before attachment to stainless steel clips. Muscle strips
are pre-incubated in Erlenmeyer flasks containing 3.5 ml Krebs-Ringer bicarbonate buffer, 7 mM N-2-hydroxyethyl-peperazine-N'-2-ethane-sulphonic acid, pH 7.4, and 5.5 mM pyruvate. Flasks are sealed and gassed continuously with O₂ and CO₂ in the ratio 19:1 (v/v). After pre-incubation of muscles in this medium for 30 min at 37°C in an oscillating water bath, the muscles strips are transferred to similar vials containing identical medium (except pyruvate) with added [U-¹⁴C] glucose (0.5 µCi/ml) and insulin (100 µU/ml). The flasks are sealed and re-gassed for an initial 15 min in a 1-h incubation. At the end of the incubation period, muscles are blotted and rapidly frozen in liquid N₂. The concentration of lactate in the incubation medium can be determined spectrophotometrically and [U-¹⁴C]glucose incorporation in glycogen measured.

Methods of measuring the rate of gastric emptying are disclosed in, for example, Young et al. In a phenol red method, conscious rats receive by gavage an acoloric gel containing methyl cellulose and a phenol red indicator. Twenty minutes after gavage, animals are anesthetized using halothane, the stomach exposed and clamped at the pyloric and lower esophageal sphincters, removed and opened into an alkaline solution. Stomach content may be derived from the intensity of the phenol red in the alkaline solution, measured by absorbance at a wavelength of 560 nm. In a tritiated glucose method, conscious rats are gavaged with tritiated glucose in water. The rats are gently restrained by the tail, the tip of which is anesthetized using lidocaine. Tritium in the plasma separated from tail blood is collected at various timepoints and detected in a beta counter. Test compounds are normally administered about one minute before gavage.

Amylin agonist compounds may exhibit activity in the receptor binding assay on the order of less than about 1 to 5 nM, can be less than about 1 nM and can be less than about 50 pM. In the soleus muscle assay, amylin agonist compounds may show EC₅₀ values on the order of less than about 1 to 10 micromolar. In the gastric emptying assays, in one embodiment the agonist compounds show ED₅₀ values on the order of less than 100 µg/rat.

In one exemplary method of making the compounds, compounds of the invention may be prepared using standard solid-phase peptide synthesis techniques and can further be an automated or semiautomated peptide synthesizer. Typically, using such techniques, 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-
butyloxy carbonyl (tBoc) and fluorenylmethoxycarbonyl (Fmoc) being particularly useful. Other methods of synthesizing or expressing amylin and amylin agonists and purifying them are known to the skilled artisan. For example, chemical and recombinant methods useful to synthesize amylin agonists and hybrids with amylin agonists can be found, for example, in WO2005077072, and other references cited herein.

DOSAGE/FORMULATION

[0088] Compounds useful in the practice of the invention may be administered alone or in combination with pharmaceutically acceptable carriers or excipients, in either single or multiple doses. These pharmaceutical compounds may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in 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).

[0089] In general, the compounds may be formulated into a stable, safe pharmaceutical composition for administration to a patient. Pharmaceutical formulations contemplated for use in the methods of the invention may comprise approximately 0.01 to 6.0% (w/v), can be 0.05 to 1.0%, of the compound, approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer allowing a pH of the final composition of from about 3.0 to about 7.0; approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol tonicifier and, optionally, approximately 0.005 to 1.0% (w/v) of a preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl-, ethyl-, propyl- and butyl- parabens and phenol. Such a preservative is generally included if the formulated peptide is to be included in a multiple use product.

[0090] In a particular embodiment of the present invention, a pharmaceutical formulation of the present invention may contain a range of concentrations of the compound, e.g., between about 0.01% to about 98% w/w, or between about 1 to about 98% w/v, or can be between 80% and 90% w/v, or can be between about 0.01% to about 50% w/v, or in yet other embodiments can be between about 10% to about 25% w/v in this embodiment. A sufficient amount of water for injection may be used to obtain the desired concentration of solution.

[0091] Additional tonicifying agents such as sodium chloride, as well as other known excipients, may also be present, if desired. It one embodiment, however, such excipients will maintain the overall tonicity of the formulations. An excipient may be included in the presently described formulations at various concentrations. For example, an excipient may be included in the
concentration range from about 0.02% to about 20% w/w, can be between about 0.02% and 0.5%
w/w, about 0.02% to about 10% w/w, or about 1% to about 20% w/w. In addition, similar to the
present formulations themselves, an excipient may be included in solid (including powdered),
liquid, semi-solid or gel form.

[0092] The pharmaceutical formulations may be composed in various forms, e.g., solid, semisolid
or liquid. The term "solid", as used herein, is meant to encompass all normal uses of this term
including, for example, powders and lyophilized formulations. The presently described
formulations may be lyophilized.

[0093] The terms buffer, buffer solution and buffered solution, when used with reference to
hydrogen-ion concentration or pH, refer to the ability of a system, particularly an aqueous solution,
to resist a change of pH on adding acid or alkali, or on dilution with a solvent. Characteristic of
buffered solutions, which undergo small changes of pH on addition of acid or base, is the presence
either of a weak acid and a salt of the weak acid, or a weak base and a salt of the weak base. An
example of the former system is acetic acid and sodium acetate. The change of pH is slight as long
as the amount of hydronium or hydroxyl ion added does not exceed the capacity of the buffer
system to neutralize it.

[0094] As described herein, a variety of liquid vehicles are suitable for use in the present peptide
formulations, for example, water or an aqueous/organic solvent mixture or suspension.

[0095] The stability of a peptide formulation of the present invention is enhanced by maintaining
the pH of the formulation in the range of about 3.0 to about 7.0 when in liquid form. In one
embodiment, the pH of the formulation is maintained in the range of about 3.5 to 5.0, or about 3.5
to 6.5, or can be from about 3.7 to 4.3, or about 3.8 to 4.2. A particularly useful pH may be about
4.0. While not seeking to be bound by this theory, it is presently understood that where the pH of
the pharmaceutical formulation exceeds 5.5, chemical degradation of the peptide may be
accelerated such that the shelf life is less than about two years.

[0096] The buffer used in the practice of the present invention is an acetate buffer (which can be at
a final formulation concentration of from about 1-5, e.g., 1.5, to about 60 mM), phosphate buffer
(which can be at a final formulation concentration of from about 1-5, e.g., 1.5, to about 30 mM) or
glutamate buffer (which can be at a final formulation concentration of from about 1-5, e.g., 1.5 to
about 60 mM). A very useful buffer is acetate (particularly at a final formulation concentration of
from about 5 to about 30 mM).

[0097] A stabilizer may be included in the present formulation but, and importantly, is not
necessarily needed. If included, however, a stabilizer useful in the practice of the present invention
is a carbohydrate or a polyhydric alcohol. A suitable stabilizer useful in the practice of the present
invention is approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol. The polyhydric alcohols and carbohydrates share the same feature in their backbones, i.e., -CHOH-CHOH-, which is responsible for stabilizing the proteins. The polyhydric alcohols include such compounds as sorbitol, mannitol, glycerol, and polyethylene glycols (PEGs). These compounds are straight-chain molecules. The carbohydrates, such as mannose, ribose, sucrose, fructose, trehalose, maltose, inositol, and lactose, on the other hand, are cyclic molecules that may contain a keto or aldehyde group. These two classes of compounds have been demonstrated to be effective in stabilizing protein against denaturation caused by elevated temperature and by freeze-thaw or freeze-drying processes. Suitable carbohydrates include: galactose, arabinose, lactose or any other carbohydrate which does not have an adverse affect on a diabetic patient, i.e., the carbohydrate is not metabolized to form unacceptably large concentrations of glucose in the blood. Such carbohydrates are well known in the art as suitable for diabetics. Sucrose and fructose are suitable for use with the compound in non-diabetic subjects (e.g., treating obesity).

[0098] If a stabilizer is included, the compound can be stabilized with a polyhydric alcohol such as sorbitol, mannitol, inositol, glycerol, xylitol, and polypropylene/ethylene glycol copolymer, as well as various polyethylene glycols (PEG) of molecular weight 200, 400, 1450, 3350, 4000, 6000, and 8000). Mannitol is the polyhydric alcohol of one embodiment. Another useful feature of the lyophilized formulations of the present invention is the maintenance of the tonicity of the lyophilized formulations described herein with the same formulation component that serves to maintain their stability. Mannitol is a particularly useful polyhydric alcohol used for this purpose.

[0099] The United States Pharmacopeia (USP) states that anti-microbial agents in bacteriostatic or fungistatic concentrations must be added to preparations contained in multiple dose containers. They must be present in adequate concentration at the time of use to prevent the multiplication of microorganisms inadvertently introduced into the preparation while withdrawing a portion of the contents with a hypodermic needle and syringe, or using other invasive means for delivery, such as pen injectors. Anti-microbial agents should be evaluated to ensure compatibility with all other components of the formula, and their activity should be evaluated in the total formula to ensure that a particular agent that is effective in one formulation is not ineffective in another. It is not uncommon to find that a particular anti-microbial agent will be effective in one formulation but not effective in another formulation.

[00100] A preservative is, in the common pharmaceutical sense, a substance that prevents or inhibits microbial growth and may be added to pharmaceutical formulations for this purpose to avoid consequent spoilage of the formulation by microorganisms. While the amount of the preservative is not great, it may nevertheless affect the overall stability of the peptide.
While the preservative for use in the pharmaceutical compositions can range from 0.005 to 1.0% (w/v), exemplary ranges for each preservative, alone or in combination with others, is: benzyl alcohol (0.1-1.0%), or m-cresol (0.1-0.6%), or phenol (0.1-0.8%) or combination of methyl (0.05-0.25%) and ethyl- or propyl- or butyl- (0.005%-0.03%) parabens. The parabens are lower alkyl esters of para-hydroxybenzoic acid.


Pramlintide, human 25-28 Pro-amylin, does not have a tendency to adsorb onto the glass in a glass container when in a liquid form, therefore, a surfactant is not required to further stabilize the pharmaceutical formulation. However, with regard to compounds which do have such a tendency when in liquid form, a surfactant should be used in their formulation. These formulations may then be lyophilized. Surfactants frequently cause denaturation of protein, both of hydrophobic disruption and by salt bridge separation. Relatively low concentrations of surfactant may exert a potent denaturing activity, because of the strong interactions between surfactant moieties and the reactive sites on proteins. However, judicious use of this interaction can stabilize proteins against interfacial or surface denaturation. Surfactants which could further stabilize the peptide may optionally be present in the range of about 0.001 to 0.3% (w/v) of the total formulation and include polysorbate 80 (i.e., polyoxyethylene(20) sorbitan monoooleate), CHAPS® (i.e., 3-[(3-cholamidopropyl) dimethylammonio] 1-propanesulfonate), Brij® (e.g., Brij 35, which is (polyoxyethylene (23) lauryl ether), poloxamer, or another non-ionic surfactant.

It may also be desirable to add sodium chloride or other salt to adjust the tonicity of the pharmaceutical formulation, depending on the tonicifier selected. However, this is optional and depends on the particular formulation selected. Parenteral formulations can be isotonic or substantially isotonic.

A particularly useful vehicle for parenteral products is water. Water of suitable quality for parenteral administration can be prepared either by distillation or by reverse osmosis. Water for injection is a very useful aqueous vehicle for use in the pharmaceutical injectable formulations.

It is possible that other ingredients may be present in the pharmaceutical formulations. Such additional ingredients may include, e.g., wetting agents, emulsifiers, oils, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatin or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Additionally, polymer solutions, or
mixtures with polymers provide the opportunity for controlled release of the peptide. Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

[00107] Containers are also an integral part of the formulation of an injection and may be considered a component, for there is no container that is totally inert, or does not in some way affect the liquid it contains, particularly if the liquid is aqueous. Therefore, the selection of a container for a particular injection must be based on a consideration of the composition of the container, as well as of the solution, and the treatment to which it will be subjected. Adsorption of the peptide to the glass surface of the vial can also be minimized, if necessary, by use of borosilicate glass, for example, Wheaton Type I borosilicate glass #33 (Wheaton Type 1-33) or its equivalent (Wheaton Glass Co.). Other vendors of similar borosilicate glass vials and cartridges acceptable for manufacture include Kimbel Glass Co., West Co., Bunder Glas GMBH and Forma Vitrum. The biological and chemical properties of the compound may be stabilized by formulation and lyophilization in a Wheaton Type 1-33 borosilicate serum vial to a final concentration of 0.1 mg/ml and 10 mg/ml of the compound in the presence of 5% mannitol, and 0.02% Tween 80.

[00108] In order to permit introduction of a needle from a hypodermic syringe into a multiple-dose vial and provide for resealing as soon as the needle is withdrawn, the open end of each vial can be sealed with a rubber stopper closure held in place by an aluminum band.

[00109] Stoppers for glass vials, such as, West 4416/50, 4416/50 (Teflon faced) and 4406/40, Abbott 5139 or any equivalent stopper can be used as the closure for pharmaceutical for injection. These stops are compatible with the peptide as well as the other components of the formulation. The inventors have also discovered that these stops pass the stopper integrity test when tested using patient use patterns, e.g., the stopper can withstand at least about 100 injections. Alternatively, the peptide can be lyophilized in vials, syringes or cartridges for subsequent reconstitution. Liquid formulations of the present invention can be filled into one or two chambered cartridges, or one or two chamber syringes.


[00111] The manufacturing process for the above liquid formulations generally involves compounding, sterile filtration and filling steps. The compounding procedure involves dissolution of ingredients in a specific order (preservative followed by stabilizer/tonicity agents, buffers and peptide) or dissolving at the same time.
Alternative formulations, e.g., non-parenteral, may not require sterilization. However, if sterilization is desired or necessary, any suitable sterilization process can be used in developing the peptide pharmaceutical formulation of the present invention. Typical sterilization processes include filtration, steam (moist heat), dry heat, gases (e.g., ethylene oxide, formaldehyde, chlorine dioxide, propylene oxide, beta-propiolactone, ozone, chloropicrin, peracetic acid methyl bromide and the like), exposure to a radiation source, and aseptic handling. Filtration is one embodiment of the method of sterilization for liquid formulations of the present invention. The sterile filtration involves filtration through 0.45 µm and 0.22 µm (1 or 2) which may be connected in series. After filtration, the solution is filled into appropriate vials or containers.

The liquid pharmaceutical formulations of the present invention are intended for parenteral administration. Suitable routes of administration include intramuscular, intravenous, subcutaneous, intradermal, intraarticular, intrathecal and the like. The subcutaneous route of administration is one embodiment. Mucosal delivery is another. These routes include, but are not limited to, oral, nasal, sublingual, pulmonary and buccal routes which may include administration of the peptide in liquid, semi-solid or solid form. Administration via these routes requires substantially more peptide to obtain the desired biological effects due to decreased bioavailability compared to parenteral delivery. In addition, parenteral controlled release delivery can be achieved by forming polymeric microcapsules, matrixes, solutions, implants and devices and administering them parenterally or by surgical means. Examples of controlled release formulations are described in U.S. Patent Nos. 6,368,630, 6,379,704, and 5,766,627, which are incorporated herein by reference. These dosage forms may have a lower bioavailability due to entrapment of some of the peptide in the polymer matrix or device. See e.g., U.S. Pat. Nos. 6,379,704, 6,379,703, and 6,296,842.

The compounds may be provided in dosage unit form containing an amount of the compound with or without insulin or glucose (or a source of glucose) that will be effective in one or multiple doses to treat or help in treating the psychiatric disease and/or unwanted side effects of the psychiatric treatment/medication. 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 condition to be treated, and other factors.

However, typical doses may contain from a lower limit of about 1 µg, 5 µg, 10 µg, 50 µg to 100 µg to an upper limit of about 100 µg, 500 µg, 1 mg, 5 mg, 10 mg, 50 mg or 100 mg of the pharmaceutical compound per day. Also contemplated are other dose ranges such as 0.1 µg to 1 mg of the compound per dose. Thus, exemplary doses may be 15, 30, 60, 90, 120, 180, 240, 360, or 500 µg of the compound per dose. The doses per day may be delivered in discrete unit doses or
provided continuously in a 24 hour period, or any portion of that 24 hour period. The number of doses per day may be from 1 to about 4 doses per day, although it could be more. Continuous delivery can be in the form of continuous infusions. Exemplary doses and infusion rates include from 0.005 nmol/kg to about 20 nmol/kg per discrete dose or from about 0.01/pmol/kg/mm to about 10 pmol/kg/min in a continuous infusion. These doses and infusions can be delivered by intravenous administration (i.v.) or subcutaneous administration (s.c). Exemplary total dose/delivery of the pharmaceutical composition given i.v. may be about 2 μg to about 8 mg per day, whereas total dose/delivery of the pharmaceutical composition given s.c may be about 6 μg to about 16 or 24 mg per day.

[00116] In another aspect of the invention, it is also possible to combine an amylin or amylin agonist useful in the methods of the present invention, with one or more other active ingredients useful in cardioprotection. For example, an amylin or amylin agonist may be combined with one or more other compounds, in a unitary dosage form, or in separate dosage forms intended for simultaneous or sequential administration to a patient in need of treatment. When administered sequentially, the combination may be administered in two or more administrations. In an alternative embodiment, it is possible to administer one or more amylin or amylin agonists and one or more additional active ingredients by different routes. The skilled artisan will also recognize that a variety of active ingredients may be administered in combination with amylin or amylin agonists that may act to augment or synergistically enhance the prevention of cardiac cells or tissues.

[00117] The one or more other active ingredients include those useful in cardioprotection, which includes angiotensin II antagonists, renin inhibitors and calcium channel blockers. Angiotensin-π receptor antagonists (or blockers) are anti-hypertensive agents that are selective for the angiotensin II (type 1 receptor). The angiotensin-II receptor antagonists include losartan, valsartan, irbesartan, candesartan, telmisartan, olmesartan, eprosartan, tasosartan and zolarsartan.

In a further embodiment, a diuretic, such as hydrochlorothiazide, is also administered with the amylin or amylin agonist, or in yet a further embodiment, with the AT-II inhibitor. Angiotensin-receptor blockers have been shown to reduce mortality and reduce hospital admissions of subjects at risk for heart failure. For example, candesartan was reported to reduce mortality and hospital admissions in subjects with chronic heart failure. Pfeffer et al., Lancet 362(9386):759-66 (2003).

[00118] Calcium channel blockers include dihydropyridines, phenylalkylamines, and benzodiazipines, including bepridil, nitrendipine, nifedipine, nisoldipine, nimodipine, cilnidipine, aranidipine, manidipine, felodipine, nicardipine, amlodipine, and lacidipine. In a one embodiment, the amylin or amylin agonist is administered in combination with the calcium channel blocker bepridil.
Renin inhibitors inhibit the actions of renin and thus prevent the conversion of angiotensinogen into angiotensin I, to provide cardiovascular benefits. Renin inhibitors include enalkiren, remikiren, 3-alkoxy-4-aryl-piperidines, such as R066-1132 (Hoffmann-La Roche), alkanecarboxamides such as aliskiren (Lindsay and Skrydstrup, J. Org. Chem. 71(13):4766-4777 (2006)), diazabicyclononene and tetrahydropyridine derivatives (see WO2004096804) and azabicyclononene derivatives (see WO2006064484).

In yet another embodiment an amylin or amylin agonist is administered in adjunct therapy with one or more peptides or proteins that provide a cardiovascular benefit such as those that have an inotropic and/or vasodilator effect. Other peptide families useful in adjunct therapy with an amylin or amylin agonist include adrenomedullins, natriuretic peptides, ghrelin, urocortins, intermedins, GLP-I agonists and exendins, and their fragments, analogs and derivatives. Also included are species variations, including, e.g., murine, hamster, chicken, bovine, rat, dog, and frog. The adjunct therapy can include hybrid compounds of the amylin or amylin agonist joined to the peptide or protein. Methods useful for making such hybrid proteins and component peptides useful in such hybrids are discussed for example in WO2005077072. Of particular interest are hybrid proteins having one an amylin-salmon calcitonin-amylin chimera, for example where the chimera is joined (e.g. via a linker) to BNP.

Adrenomedullin (ADM) is a 52 amino acid peptide hormone that has been reported as cardioprotective (Nishikimi et al. Hypertens. Res. Suppl:S121-7 (2003)). See EP0622458B1 and WO2005077072, for example, for exemplary ADM analogs and derivatives. GLP-I (glucagon like peptide-1) includes human GLP-I(l-37), GLP-I(7-37), and GLP-I(7-36)amide (with reference to the full length human GLP-I(l-37)). GLP-I has been reported to improve to improve regional and global LV function after successful reperfusion (Nikolaidis et al., Circulation 109:962-965 (2004)) and to improve outcomes in subjects suffering from acute myocardial infarction who also have impaired glucose tolerance (U.S. Patent 6,747,006). GLP-I analogs and derivatives, particularly those with prolonged serum half-life, are found for example in U. S. Patent 6,268,343. An exemplary GLP-I derivative is liraglutide. Exendins, for example, exendin-3 and exendin-4 (Eng et al., J. Biol. Chem., 265:20259-62 (1990); Eng. et al., J. Biol. Chem., 267:7402-05 (1992)) have been reported as useful to treat subjects suffering from acute coronary syndrome (U. S. Patent 7,056,887) and cardiovascular disease. Exemplary exendin analogs and derivatives are described for example in PCT/US98/16387 filed Aug. 6, 1998 and PCT/US98/24210 filed Nov. 13, 1998. Ghrelin, an endogenous ligand of the growth hormone secretagoge receptor, has also been reported to have beneficial effects on cardiac function (Chang et al. J. Cardiovascular Pharm. 43(2): 165-170 (2004). Intermedin (or AFP-6) is a peptide hormone providing cardiovascular benefit. AFP-6
analogs and derivatives include those described in WO2004048547, WO2006042242 or WO2005077072, for example. Natriuretic peptide hormones are atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and urodilatin. Recombinant human BNP, also known as nesiritide has been approved treatment of subjects with acutely decompensated CHF. Urodilatin is a kidney-derived member of the natriuretic peptide family reported to regulate sodium and water handling in the kidney, as well as a mediator of sodium excretion in patients with congestive heart failure (CHF). Urocorntins have been reported to have cardioprotective effects (Lawrence et al., FASEB Journal 19:831-833 (2005)). Urocorntins include the three human urocorntins: Ucn-1, Ucn-2 and Ucn-3. Ucn-2 or Ucn-3, are particularly useful as vasodilators. These peptide hormones and useful variants thereof can be found, for example, in WO2005077072.

[00122] In one embodiment an amylin and amylin agonists are provided to subjects who are intolerant to other cardioprotective therapy, such as intolerance to angiotensin II antagonists, renin inhibitors, calcium channel blockers, or BNP.

[00123] According to the methods of the invention, an amylin or amylin agonist may be: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by any other combination therapy regimen known in the art. When delivered in alternation therapy, the methods of the invention may comprise administering or delivering the active ingredients sequentially, e.g., in separate solution, emulsion, suspension, tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in simultaneous therapy, effective dosages of two or more active ingredients are administered together. Various sequences of intermittent combination therapy may also be used.

EXAMPLE 1

[00124] In this example, the effects of rat amylin on dysrhythmia and overall mortality were studied in a rat model of reperfusion-induced arrhythmia. The method, which detects antiarrhythmic/antiischemic activity, follows that described by Kane K.A., Parratt J.R. and Williams F.M (Kane, et al., British Journal of Pharmacology, 82(2)349-357 (1984)).

[00125] Male Sprague Dawley® rats (n=12/treatment, 185-215g) were anesthetized with sodium pentobarbital 50 mg/kg i.p. and placed under artificial respiration. They were then prepared to record their electrocardiogram (standard lead π). After a left thoracotomy, they are subjected to 5 minutes of left main coronary artery occlusion followed by reperfusion.
In the 30 minutes following the reperfusion, arrhythmias were recorded by counting the total number of ventricular ectopic beats (VEBs) and the number of VEBs per 5 minutes. Total duration (per 5 minutes) and cumulated duration were also reported for ventricular tachycardia (VT) and for ventricular fibrillation (VF). The incidence (%) of VT and VF was calculated. Mortality was noted (cumulated % survival).

At the end of each experiment, the excised heart was perfused retrogradely with 5 ml 4% formaldehyde in isotonic saline to verify the presence of the ischemic area. The color difference between the normal and the ischemic area was intensifi ed by additional perfusion with 1 ml 70% ethanol. The hearts were then kept in 10% formaldehyde until transport to the sponsor (only upon request).

Rat amylin (Amylin Pharmaceuticals Inc., dissolved in physiological saline: peptide content 87%) and salmon calcitonin (Amylin Pharmaceuticals Inc., dissolved in physiological saline: peptide content 90.5%) were dosed at 300 µg/kg administered i.v. 10 minutes before coronary occlusion and then 10 minutes after. 12 animals were studied per group. The test was performed blind. Bepridil (2 mg/kg i.v., 10 minutes before coronary occlusion) was used as a reference substance.

The positive control, Bepridil, a calcium channel blocker, and rat amylin, each reduced the total duration of ventricular fibrillation, and the overall mortality, especially at earlier time points after ischemia, as shown in Figure 1. Salmon calcitonin did not appear to have a significant cardioprotective effect in this experiment.

In this rodent model, both amylin and bepridil were cardioprotective. Since each agent works through distinctly different mechanisms of action, calcium channel blockers and amylin agonists may act synergistically to reduce the risk of death after myocardial ischemia.

**EXAMPLE 2**

During Phase 3 clinical trials evaluating the safety and efficacy of pramlintide as an adjunct treatment to insulin in the treatment of diabetes, patients with type 2 and type 1 diabetes took pramlintide or placebo with meals (30µg TID to 120µg BID). It was observed that the number of cardiac deaths (from myocardial infarction, myocardial rupture post infarction, cardiac arrest, coronary artery disease and arrhythmias) a total fraction of patients exposed to pramlintide compared to similar patients exposed to placebo differed between the two groups. Of 4415 patients exposed to pramlintide for up to 1 year, 7 suffered cardiac deaths (0.159%). In contrast, of 1504 patients exposed to placebo for up to 1 year, 5 suffered cardiac deaths (0.332%).
Figure 3 provides the mortality rates in insulin-treated patients with type 1 diabetes (upper panel), type 2 diabetes (middle panel), and in all insulin-treated patients (lower panel) treated with pramlintide (at any dose) or placebo. The data in the lower panel are overlaid onto reported values for age-adjusted risk of death for insulin treated patients in a New Zealand study (Brown et al. Diabetes Care 24(1):56 (2001)).

Evaluation of subsequent clinical trials including 5763 pramlintide treated subjects versus 1931 placebo treated subjects, the cardiac death incidence was lower for pramlintide (0.139%; 8/5763) than for placebo (0.363%; 7/1931).

While the foregoing description discloses the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the present invention encompasses all of the usual variations, adaptations, or modifications as being within the scope of the claimed invention.

Additional References


CLAIMS

What is claimed is:

Claim 1. A method for providing cardioprotection, myoprotection, or both in a subject in need thereof, said method comprising:

administering to said subject an amount of an amylin or amylin agonist effective to prevent or ameliorate a heart condition or cardiovascular disease.

Claim 2. The method according to Claim 1, wherein said heart condition or disease is associated with the Na+/K+-ATPase enzyme.

Claim 3. The method according to Claim 1, wherein said heart condition or disease is caused by impaired blood perfusion.

Claim 4. The method according to Claim 1, wherein said heart condition or disease is selected from the group consisting of acute congestive cardiac failure, chronic congestive cardiac failure, abnormal contractility, congestive heart failure, ischemias, ventricular tachyarrhythmias, myocardial infarction, ventricular ectopic beats, ventricular tachycardia, ventricular fibrillation, and arrhythmias.

Claim 5. The method according to Claim 1, wherein said subject has received a heart transplant.

Claim 6. The method according to any one of Claims 1-5, wherein said amylin or amylin agonist is acutely administered to said subject.

Claim 7. The method according to any one of Claims 1-5, wherein said amylin or amylin agonist is chronically administered to said subject.

Claim 8. The method according to any one of Claims 1-7, wherein said amylin agonist is not CGRP, CGRP agonist or CGRP analog.

Claim 9. The method according to any one of Claims 1-7, wherein said amylin agonist is not an AFP-6 or AFP-6 analog.

Claim 10. The method according to any one of Claims 1-9, wherein said amylin or amylin agonist is parenterally administered to said subject.

Claim 11. The method according to any one of Claims 1-7, further comprising administering administration of an angiotensin II antagonist, renin inhibitor, calcium channel blocker and/or cardioprotective or myoprotective peptide to said subject.
Claim 12. A method for treating or preventing myocardial ischemia in a subject in need thereof, said method comprising:
administering to said subject an amount of amylin or amylin agonist effective to treat or prevent myocardial ischemia.

Claim 13. The method according to Claim 12, wherein said subject has congestive heart failure.

Claim 14. The method according to Claim 12, wherein said subject has experienced or is experiencing myocardial infarction.

Claim 15. The method according to Claim 12, wherein said subject has received a heart transplant.

Claim 16. The method according to any one of Claims 12-15, wherein said amylin or amylin agonist is acutely administered to said subject.

Claim 17. The method according to any one of Claims 12-15, wherein said amylin or amylin agonist is chronically administered to said subject.

Claim 18. The method according to any one of Claims 12-15, wherein said amylin agonist is not CGRP, CGRP agonist or CGRP analog.

Claim 19. The method according to any one of Claims 12-15, wherein said amylin agonist is not an AFP-6 or AFP-6 analog.

Claim 20. The method according to any one of Claims 12-19, wherein said amylin or amylin agonist is parenterally administered to said subject.

Claim 21. The method according to any one of Claims 12-15, further comprising administering administration of an angiotensin II antagonist, renin inhibitor, calcium channel blocker and/or cardioprotective or myoprotective peptide to said subject.

Claim 22. A method for improving the sodium pump in a subject in need thereof, said method comprising:
administering to said subject an amount of an amylin or amylin agonist effective to improve the sodium pump.

Claim 23. A method for the treatment or prevention of a condition associated with decreased Na+/K+-ATPase enzyme efficiency in a subject in need thereof, said method comprising:
administering to said subject an amount of amylin or amylin agonist effective to improve Na+/K+-ATPase enzyme efficiency, wherein said condition associated with decreased Na+/K+-ATPase enzyme efficiency is thereby improved.
Claim 24. The method according to Claim 22 or 23, wherein said amylin or amylin agonist is chronically administered to said subject.

Claim 25. The method according to Claim 22 or 23, wherein said amylin or amylin agonist is acutely administered to said subject.

Claim 26. The method according to any one of Claims 22-25, wherein said amylin agonist is not CGRP, CGRP agonist or CGRP analog.

Claim 27. The method according to any one of Claims 22-25, wherein said amylin agonist is not an AFP-6 or AFP-6 analog.

Claim 28. The method according to any one of Claims 22-25, wherein said amylin agonist is an amylin receptor agonist.

Claim 29. The method according to any one of Claims 22-25, wherein said amylin or amylin agonist is parenterally administered to said subject.

Claim 30. The method according to any one of Claims 22-29, further comprising administering administration of an angiotensin II antagonist, renin inhibitor, calcium channel blocker and/or cardioprotective peptide to said subject.

Claim 31. A method for treating or preventing dysrhythmia in a subject in need thereof, said method comprising:
administering to said subject an amount of amylin or amylin agonist effective to treat or prevent dysrhythmia.

Claim 32. The method according to Claim 31, wherein said amylin molecule is rat amylin.

Claim 33. The method according to Claim 31, wherein said amylin agonist is pramlintide.

Claim 34. The method according to Claim 31, wherein the subject is at risk of developing or has developed a dysrhythmia is selected from the group consisting of premature atrial contractions, premature ventricular contractions, atrial fibrillation, atrial flutter, paroxysmal supraventricular tachycardia, accessory pathway tachycardias, AV nodal reentrant tachycardias, ventricular tachycardia, ventricular fibrillation, long QT syndrome, bradyarrhythmias, sinus node dysfunction and heart block.

Claim 35. The method according to any one of Claims 31-34, wherein said amylin or amylin agonist is acutely administered to said subject.

Claim 36. The method according to any one of Claims 31-34, wherein said amylin or amylin agonist is chronically administered to said subject.
Claim 37. The method according to any one of Claims 31-36, wherein said amylin agonist is not CGRP, CGRP agonist or CGRP analog.

Claim 38. The method according to any one of Claims 31-36, wherein said amylin agonist is not an AFP-6 or AFP-6 analog.

Claim 39. The method according to any one of Claims 31-36, wherein said amylin agonist is an amylin receptor agonist.

Claim 40. The method according to any one of Claims 31-39, wherein said amylin or amylin agonist is parenterally administered to said subject.

Claim 41. The method according to any one of Claims 31-40, further comprising administering administration of an angiotensin II antagonist, renin inhibitor, calcium channel blocker and/or cardioprotective peptide to said subject.

Claim 42. The use of an amylin or amylin agonist in the manufacture of a medicament to treat, prevent or ameliorate a heart condition or cardiovascular disease wherein the medicament comprises an amount of an amylin or amylin agonist effective to provide cardioprotection, myoprotection or both in a subject in need thereof.

Claim 43. The use of claim 42 wherein the amylin or amylin agonist is not a CGRP, CGRP agonist, CGRP analog, AFP-6 or AFP-6 analog.

Claim 44. The use of claim 42 wherein the amylin or amylin agonist is an amylin-salmon calcitonin-amylin chimera.
Type 1 Diabetes

![Graph showing death rates for Type 1 Diabetes with Pramlintide and Placebo groups.]

Type 2 Diabetes

![Graph showing death rates for Type 2 Diabetes with Pramlintide and Placebo groups.]

All Insulin-Treated

![Graph showing death rates for all insulin-treated patients with Pramlintide and Placebo groups.]

Figure 3