METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS

Inventors: Lance E. Steward, Irvine, CA (US); George Sachs, Encino, CA (US); Kel Roger Aoki, Coto de Caza, CA (US)

Correspondence Address:
Dean G. Stathakis, Ph.D.
ALLERGAN, INC.
T2-7H
2525 Dupont Drive
Irvine, CA 92612 (US)

Assignee: Allergan, Inc.

Appl. No.: 11/014,795

Filed: Dec. 15, 2004

Related U.S. Application Data
Division of application No. 09/548,409, filed on Apr. 13, 2000, now Pat. No. 6,843,998, which is a continuation-in-part of application No. 09/288,326, filed on Apr. 8, 1999, now Pat. No. 6,776,990.

Publication Classification
Int. Cl7 ........................................... A61K 39/395
U.S. Cl ............................................. 424/155.1; 424/178.1

ABSTRACT
Methods and compositions for the treatment of acute pancreatitis in a mammal. Particular compositions comprise a binding element, a translocation element, and a therapeutic element able to prevent accumulation of digestive enzymes within the pancreas.
METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS

[0001] This application is a continuation-in-part of application Ser. No. 09/288,326, filed Apr. 8, 1999.

FIELD OF THE INVENTION

[0002] The present invention includes methods and compositions for the treatment of acute pancreatitis. In a preferred embodiment the invention concerns the use of agents to reduce or prevent the secretion of pancreatic digestive enzymes within the pancreas. Such agents are targeted to pancreatic cells, and serve to prevent the exocytotic fusion of vesicles containing these enzymes with the plasma membrane. The invention is also concerned with methods of treating a mammal suffering from pancreatitis through the administration of such agents.

BACKGROUND OF THE INVENTION

[0003] Pancreatitis is a serious medical condition involving an inflammation of the pancreas. In acute or chronic pancreatitis the inflammation manifests itself in the release and activation of pancreatic enzymes within the organ itself, leading to autodigestion. In many cases of acute pancreatitis, the condition can lead to death.

[0004] In normal mammals, the pancreas, a large gland similar in structure to the salivary gland, is responsible for the production and secretion of digestive enzymes, which digest ingested food, and bicarbonate for the neutralization of the acidic chyme produced in the stomach. The pancreas contains acinar cells, responsible for enzyme production, and ductal cells, which secrete large amounts of sodium bicarbonate solution. The combined secretion product is termed “pancreatic juice”; this liquid flows through the pancreatic duct past the sphincter of Oddi into the duodenum. The secretion of pancreatic juice is stimulated by the presence of chyme in the upper portions of the small intestine, and the precise composition of pancreatic juice appears to be influenced by the types of compounds (carbohydrate, lipid, protein, and/or nucleic acid) in the chyme.

[0005] The constituents of pancreatic juice includes proteases (trypsin, chymotrypsin, carboxypeptidase), nucleases (RNase and DNase), pancreatic amylase, and lipases (pancreatic lipase, cholesterol esterase and phospholipase). Many of these enzymes, including the proteases, are initially synthesized by the acinar cells in an inactive form aszymogens: thus trypsin is synthesized as trypsinogen, chymotrypsin as chymotrypsinogen, and carboxypeptidase as procarboxypeptidase. These enzymes are activated according to a cascade, wherein, in the first step, trypsin is activated through proteolytic cleavage by the enzyme enteropeptidase. Trypsinogen can also be autoactivated by trypsin; thus one activation has begun, the activation process can proceed rapidly. Trypsin, in turn, activates both chymotrypsinogen and procarboxypeptidase to form their active protease counterparts.

[0006] The enzymes are normally activated only when they enter the intestinal mucosa in order to prevent autodigestion of the pancreas. In order to prevent premature activation, the acinar cells also co-secrete a trypsin inhibitor that normally prevents activation of the proteolytic enzymes within the secretory cells and in the ducts of the pancreas. Inhibition of trypsin activity also prevents activation of the other proteases.

[0007] Pancreatitis can occur when an excess amount of trypsin saturates the supply of trypsin inhibitor. This, in turn, can be caused by underproduction of trypsin inhibitor, or the overabundance of trypsin within the cells or ducts of the pancreas. In the latter case, pancreatic trauma or blockage of a duct can lead to localized overabundance of trypsin; under acute conditions large amounts of pancreatic zymogen secretion can pool in the damaged areas of the pancreas. If even a small amount of free trypsin is available activation of all the zymogenic proteases rapidly occurs, and can lead to digestion of the pancreas (acute pancreatitis) and in particularly severe cases to the patient’s death.

[0008] Pancreatic secretion is normally regulated by both hormonal and nervous mechanisms. When the gastric phase of stomach secretion occurs, parasympathetic nerve impulses are relayed to the pancreas, which initially results in acetylcholine release, followed by secretion of enzymes into the pancreatic acini for temporary storage.

[0009] When acid chyme thereafter enters the small intestine, the mucosal cells of the upper intestine release a hormone called secretin. In humans, secretin is a 27 amino acid (3400 Dalton) polypeptide initially produced as the inactive form prosecretin, which is then activated by proteolytic cleavage. Secretin is then absorbed into the blood. Secretin causes the pancreas to secrete large quantities of a fluid containing bicarbonate ion. Secretin does not stimulate the acinar cells, which produce the digestive enzymes. The bicarbonate fluid serves to neutralize the chyme and to provide a slightly alkaline optimal environment for the enzymes.

[0010] Another peptide hormone, cholecystokinin (CCK) is released by the mucosal cells in response to the presence of food in the upper intestine. As described in further detail below, human CCK is synthesized as a proteoprotein of 115 amino acids. Active CCK forms are quickly taken into the blood through the digestive tract, and normally stimulate the secretion of enzymes by the acinar cells. However, stimulation of the CCK receptor by the CCK analogs cerulein and CCK-octapeptide (CCK-8) appears to lead to a worsening of morbidity and mortality in mammals in whom pancreatitis is induced. See Tani et al., Pancreas 5:284-290 (1990).

[0011] As indicated above, the digestive enzymes are synthesized aszymogens; protoenzyme synthesis occurs in the rough endoplasmic reticulum of the acinar cells. Thezymogens are then packaged within vesicles having a single lipid bilayer membrane. Thezymogens are packed within the vesicles so densely that they appear as quasi-crystalline structures when observed under light microscopy and thezymogen granules are electron-dense when observed under the electron microscope. The vesicles are localized within the cytoplasm of the acinar cells. Secretion of zymogens by the acinar cells occurs through vesicle docking and subsequent fusion with the plasma membrane, resulting in the liberation of the contents into the extracellular milieu.

[0012] Nerve cells appear to secrete neurotransmitters and other intercellular signaling factors through a mechanism of membrane fusion that is shared with other cell types, see e.g., Rizo & Sudhof, Nature Struct. Biol. 5:839-842 (October 1998), hereby incorporated by reference herein, including the pancreatic acinar cells.

[0013] Although the Applicants do not wish to be bound by theory, it is believed that a vesicle first contacts the
intracellular surface of the cellular membrane in a reaction called docking. Following the docking step the membrane fuses with and becomes part of the plasma membrane through a series of steps that currently remain relatively uncharacterized, but which clearly involve certain vesicle and membrane-associated proteins, as has been illustrated using neural models.

[0014] In neurons, neurotransmitters are packaged within synaptic vesicles, formed within the cytoplasm, then transported to the inner plasma membrane where the vesicles dock and fuse with the plasma membrane. Recent studies of nerve cells employing clostridial neurotoxins as probes of membrane fusion have revealed that fusion of synaptic vesicles with the cell membrane in nerve cells depends upon the protein core of specific proteins that are associated with either the vesicle or the target membrane. See id. These proteins have been termed SNAREs. As discussed in further detail below, a protein alternatively termed synaptobrevin or VAMP (vesicle-associated membrane protein) is a vesicle-associated SNARE (v-SNARE). There are at least two isoforms of synaptobrevin; these two isoforms are differentially expressed in the mammalian central nervous system, and are selectively associated with synaptic vesicles in neurons and secretory organelles in neuroendocrine cells. The target membrane-associated SNAREs (t-SNAREs) include syntaxin and SNAP-25. Following docking, the VAMP protein forms a core complex with syntaxin and SNAP-25; the formation of the core complex appears to be an essential step to membrane fusion. See Rizo & Sudhof, id. and Neumann et al., Trends in Cell Biol. 4:179-183 (May 1994), hereby incorporated by reference herein.

[0015] Recently evidence has increasingly indicated that the SNARE system first identified in neural cells is a general model for membrane fusion in eukaryotic cells. A yeast exocytic core complex similar to that of the synaptic vesicles of mammalian neural cells has been characterized, and found to contain three proteins: Sso1 (syntaxin 1 homolog), Snc1 (synaptobrevin homolog), and sec9 (SNAP-25 homolog). Rizo & Sudhof, id. These proteins share a high degree of amino acid sequence homology with their mammalian synaptosomal counterparts.

[0016] All mammalian non-neuronal cells appear to contain cellubrevin, a synaptobrevin analog—this protein is involved in the intracellular transport of vesicles, and is cleaved by TeFx, BoNT/E, BoNT/F, and BoNT/G. Homologs of syntaxin have been identified in yeast (e.g., sso1p and sso2p) and mammalian non-neuronal cells (syn2p, syn3p, syn4p and syn5p). Finally, as indicated above, a yeast SNAP-25 homolog, sec9 has been identified; this protein appears to be essential for vesicle fusion with the plasma membrane.

[0017] Intoxication of neural cells by clostridial neurotoxins exploits specific characteristics of the SNARE proteins. These neurotoxins, most commonly found in Clostridium botulinum and Clostridium tetani, are highly potent and specific poisons of neural cells. These Gram positive bacteria secrete two related but distinct toxins, each comprising two disulfide-linked amino acid chains: a light chain (L) of about 50 KDa and a heavy chain (H) of about 100 KDa, which are wholly responsible for the symptoms of botulism and tetanus, respectively.

[0018] The tetanus and botulinum toxins are among the most lethal substances known to man; both toxins function by inhibiting neurotransmitter release in affected neurons. The tetanus neurotoxin (TeNT) acts mainly in the central nervous system, while botulinum neurotoxin (BoNT) acts at the neuromuscular junction; both toxins inhibit acetylcholine release from the nerve terminal of the affected neuron into the synapse, resulting in paralysis or reduced target organ function.

[0019] The tetanus neurotoxin (TeNT) is known to exist in one immunologically distinct type; the botulinum neurotoxins (BoNT) are known to occur in seven different immunologically distinct serotypes, termed BoNT/A through BoNT/G. While all of these latter types are produced by isolates of C. botulinum, two other species, C. baratii and C. Butyricum also produce toxins similar to /T and /E, respectively. See, e.g., Coiffe and others, The Site and Mechanism of Action of Botulinum Neurotoxin in Therapy with Botulinum Toxin 3-13 (Jankovic J. & Hallett M. eds. 1994), the disclosure of which is incorporated herein by reference.

[0020] Regardless of type, the molecular mechanism of intoxication appears to be similar. In the first step of the process, the toxin binds to the presynaptic membrane of the target neuron through a specific interaction between the heavy chain and a neuronal cell surface receptor; the receptor is thought to be different for each type of botulinum toxin and for TeNT. The carboxy terminal (C-terminal) half of the heavy chain is required for targeting of the toxin to the cell surface. The cell surface receptors, while not yet conclusively identified, appear to be distinct for each neurotoxin serotype.

[0021] In the second step, the toxin crosses the plasma membrane of the poisoned cell. The toxin is first engulfed by the cell through receptor-mediated endocytosis, and an endosome containing the toxin is formed. The toxin (or light chain thereof) then escapes the endosome into the cytoplasm of the cell. This last step is thought to be mediated by the amino terminal (N-terminal) half of the heavy chain, which triggers a conformational change of the toxin in response to a pH of about 5.5 or lower. Endosomes are known to possess a proton pump that decreases intra-endosomal pH. The conformational shift exposes hydrophobic residues in the toxin, which permits the toxin to embed itself in the endosomal membrane. The toxin then translocates through the endosomal membrane into the cytosol.

[0022] Either during or after translocation the disulfide bond joining the heavy and light chain is reduced, and the light chain is released into the cytoplasm. The entire toxic activity of botulinum and tetanus toxins is contained in the light chain of the holotoxin; the light chain is a zinc (Zn++) endopeptidase which selectively cleaves the SNARE proteins essential for recognition and docking of neurotransmitter-containing vesicles with the cytoplasmic surface of the plasma membrane, and fusion of the vesicles with the plasma membrane. The light chain of TsNT, BoNT/B, BoNT/D, BoNT/F, and BoNT/G cleaves VAMP, an integral protein. During proteolysis, most of the VAMP present at the cytosolic surface of the synaptic vesicle is inactivated as a result of any one of these cleavage events. Each toxin cleaves a different specific peptide bond.

[0023] BoNT/A and /E selectively cleave the plasma membrane-associated SNARE protein SNAP-25; this protein is bound to and present on the cytoplasmic surface of the plasma membrane. BoNT/C1 cleaves syntaxin, which exists
as an integral protein having most of its mass exposed to the cytosol. Syntaxin interacts with the calcium channels at presynaptic terminal active zones. See Tonello et al., *Tetanus and Botulism Neurotoxins in Intracellular Protein Catabolism* 251-260 (Suzuki K & Bond J. eds. 1996), the disclosure of which is incorporated by reference as part of this specification. Bo/NTC1 also appears to cleave SNAP-25.

[0024] Both TeNT and BONT are specifically taken up by cells present at the neuromuscular junction. BONT remains within peripheral neurons and, as indicated above, blocks release of the neurotransmitter acetylcholine from these cells.

[0025] By contrast, TeNT through its receptor, enters vesicles that move in a retrograde manner along the axon to the soma, and is discharged into the intrasynaptic space between motor neurons and the inhibitory neurons of the spinal cord. At this point, TeNT binds receptors of the inhibitory neurons, is again internalized, and the light chain enters the cytosol to block the release of the inhibitory neurotransmitters 4-aminobutyric acid (GABA) and glycine from these cells. Id.

[0026] International Patent Publication No. WO 96/33273 relates to derivatives of botulinum toxin designed to prevent neurotransmitter release from sensory afferent neurons to treat chronic pain. Such derivatives are targeted to nociceptive neurons using a targeting moiety that binds to a binding site of the surface of the neuron.

[0027] International Patent Publication No. 98/07864 discusses the production of recombinant toxin fragments that have domains that enable the polypeptide to translocate into a target cell or which increase the solubility of the polypeptide, or both.

**SUMMARY OF THE INVENTION**

[0028] The present invention concerns methods and compositions useful for the treatment of acute pancreatitis. This condition is largely due to the defective secretion of zymogen granules by acinar cells, and by the premature co-mingling of the secreted zymogens with lysosomal hydrolases capable of activating trypsin, thereby triggering the protease activation cascade and resulting in the destruction of pancreatic tissue.

[0029] In one embodiment of this aspect, the invention is a therapeutic agent comprising a chimeric protein containing an amino acid sequence-specific endopeptidase activity, which will specifically cleave at least one synaptic vesicle-associated protein selected from the group consisting of SNAP-25, syntaxin or VAMP, in combination with the translocation activity of the N-terminus of a clostridial neurotoxin heavy chain, wherein the chimeric protein further comprises a recognition domain which will bind a human cholecystokinin (CCK) receptor. Upon binding of the recognition domain of the protein to the CCK receptor, the protein is specifically transported into cells containing CCK receptors (pancreatic acinar cells) through receptor-mediated endocytosis. In a preferred embodiment, the CCK receptor is the CCK A receptor.

[0030] Once inside the acinar cell, the chimeric protein functions in a manner similar to that of a clostridial neurotoxin within its target neuron. The toxin moiety is translocated from the endosome into the cytoplasm, where it acts to cleave a SNARE protein identical or homologous to SNAP-25, syntaxin or VAMP. The cleavage of this protein prevents formation of a core complex between the SNARE proteins and thus prevents or reduces the extent of fusion of the vesicle with the target membrane. This, in turn, results in inhibition of zymogen release from the acinar cells and of zymogen activation by lysosomal hydrolases. The autodigestion of pancreatic tissue in acute pancreatitis is therefore reduced or eliminated.

[0031] Another embodiment of the present invention concerns a method of treating a patient suffering from acute pancreatitis by administering an effective amount of such a chimeric protein.

[0032] Another embodiment of the invention concerns a therapeutic composition that contains the translocation activity of a clostridial neurotoxin heavy chain in combination with a recognition domain able to bind a specific cell type and a therapeutic activity derived from the endopeptidase activity of a clostridial neurotoxin light chain. A non-exclusive list of certain such therapeutic elements includes: hormones and hormone agonists and antagonists, nucleic acids capable of being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins, and the like.

[0033] In a preferred embodiment, the specific cell type is a pancreatic cell, most preferably a pancreatic acinar cell.

[0034] Another embodiment is drawn to methods for the treatment of acute pancreatitis comprising contacting an acinar cell with an effective amount of a composition comprising a chimeric protein containing an amino acid sequence-specific endopeptidase activity which will specifically cleave at least one synaptic vesicle-associated protein selected from the group consisting of SNAP-25, syntaxin or VAMP, in combination with the translocation activity of the N-terminus of a clostridial neurotoxin heavy chain, wherein the chimeric protein further comprises a recognition domain able to bind to a cell surface protein characteristic of an human pancreatic acinar cell. Preferably the cell surface protein is a CCK receptor protein; most preferably the protein is the human CCK A protein. CCK receptors (CCK-A receptor and CCK-B receptor) are found mainly in on the surface of pancreatic acinar cells, although they are also found in some brain cells and, to a lesser extent on the surface of gastrointestinal cells.

[0035] Any suitable route of administration may be used in this aspect of the invention. Applicants currently prefer to administer the therapeutic agent in an intravenous infusion solution; however methods such as ingestion (particularly when associated with an activity other than NAPs), see Sharma et al., *J. Nat. Toxins* 7:239-253(1998), incorporated by reference herein), direct delivery to the pancreas, injection and the like may also be used. The agent is substantially specifically targeted to pancreatic cells; when the agent contains a CCK receptor-binding domain, the blood-brain barrier prevents the agent from interacting with brain cells.

[0036] In yet another embodiment the invention provides a composition comprising a drug or other therapeutic agent having an activity other than that of a clostridial neurotoxin light chain for intracellular delivery, said agent joined to the translocation domain of a clostridial neurotoxin heavy chain and a binding element able to recognize a cell surface receptor of a target cell. In a preferred embodiment, the target cell is not a neuron. Also, in this embodiment it is
preferred that the drug or other therapeutic agent has an enzymatic, catalytic, or other self-perpetuating mode of activity, so that the effective dose of drug is greater than the number of drug molecules delivered within the target cell. A non-exclusive list of certain such drugs would include: hormones and hormone-agonists and antagonists, nucleic acids capable being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins (such as diphtheria toxin or ricin), and the like.

[0037] In this embodiment the drug may be cleavably linked to the remainder of the composition in such a way as to allow for the release of the drug from the composition within the target cell.

[0038] The presently claimed compositions may be provided to the patient by intravenous administration, may be administered during surgery, or may be provided parenterally.

[0039] WO 95/32738, which shares ownership with the present application, describes transport proteins for the therapeutic treatment of neural cells. This application is incorporated by reference herein as part of this specification.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

[0040] In a basic and presently preferred form, the invention comprises a therapeutic polypeptide comprising three features: a binding element, a translocation element, and a therapeutic element.

[0041] The binding element is able to bind to a specific target cell provided that the target cell is not a motor neuron or a sensory afferent neuron. Preferably, the binding element comprises an amino acid chain; also an independently, it is preferably located at or near the C-terminus of a polypeptide chain. By “binding element” is meant a chemical moiety able to preferentially bind to a cell surface marker characteristic of the target cell under physiological conditions. The cell surface marker may comprise a polypeptide, a polysaccharide, a lipid, a glycoprotein, a lipoprotein, or may have structural characteristics of more than one of these. By “preferentially interact” is meant that the dissociation constant ($K_d$) of the binding element for the cell surface marker is at least one order of magnitude less than that of the binding element for any other cell surface marker. Preferably, the dissociation constant is at least 2 orders of magnitude less, even more preferably the dissociation constant is at least 3 orders of magnitude less than that of the binding element for any other cell surface marker to which the therapeutic polypeptide is exposed. Preferably, the organism to be treated is a human.

[0042] In one embodiment the cell surface receptor comprises the histamine receptor, and the binding element comprises an variable region of an antibody which will specifically bind the histamine receptor.

[0043] In an especially preferred embodiment, the cell surface marker is a cholecystokinin (CCK) receptor. Cholecystokinin is a bioactive peptide that functions as both a hormone and a neurotransmitter in a wide variety of physiological settings. Thus, CCK is involved in the regulation of gall bladder contraction, satiety, gastric emptying, and gut motility; additionally it is involved in the regulation of pancreatic exocrine secretion.

[0044] There are two types of CCK receptors, CCK-A and CCK-B; the amino acid sequences of these receptors have been determined from cloned cDNA. Despite the fact that both receptors are G protein-coupled receptors and share approximately 50% homology, there are distinct differences between their physiological activity. The CCK-A receptor is expressed in smooth muscle cells of the gall bladder, smooth muscle and neurons within the gastrointestinal tract, and has a much greater affinity ($>10^7$ times higher) for CCK than the related peptide hormone gastrin. The CCK-B receptor, found in the stomach and throughout the CNS, has roughly equal ability to bind CCK and gastrin.

[0045] The varied activities of CCK can be partly attributed to the fact that CCK is synthesized as procholecystokinin, a proprotein of 115 amino acids, and is then post-translationally cleaved into a number of active fragments all sharing the same C-terminus. The amino acid sequence of human procholecystokinin is shown below; amino acid residues not present in the biologically active cleavage products are in lower case. All amino acid sequences herein are shown from N-terminus to C-terminus, unless expressly indicated otherwise:

**[0046] Human procholecystokinin, having the amino acid sequence SEQ ID NO:1:**

```
mgcvcclvlmvlalaqltgvpvpdpgesglpcreaspqrgr
```

**VSQRT DGSREHLGA LLARYIQAR KAPSGRMSIV KNQLNLDPH RISDROGYNW MDF grraseeytype**

[0047] Biologically active cleavage products of the full length CCK chain include:

**[0048] CCK-58, having the amino acid sequence SEQ ID NO:2:**

```
VSQRT DGSREHLGA LLARYIQAR KAPSGRMSIV KNQLNLDPH RISDROGYNW MDF;
```

[0049] CCK-39, having the amino acid sequence SEQ ID NO: 3:

```
YIQQAR KAPSGRMSIV KNQLNLDPH RISDROGYNW MDF;
```

**[0050] CCK-33, having the amino acid sequence SEQ ID NO: 4:**

```
KAPSGRMSIV KNQLNLDPH RISDROGYNW MDF;
```

[0051] CCK-12, having the amino acid sequence SEQ ID NO: 5:

```
ISDROGYNW MDF;
```

**[0052] and CCK-8, having the amino acid sequence SEQ ID NO: 6:**

```
RDROGYNW MDF.
```
[0053] In each case, the biologically active polypeptides contain post-translational modifications; in the case of CCK species binding the CCK-A receptor, amidation of the C-terminal phenylalanine, and sulfation of the tyrosine residue located seven residues from the C-terminus of the biologically active species are required for high affinity binding to the receptor. In the case of CCK-B, only the C-terminal amidation is necessary; sulfation of the tyrosine appears to make little difference in CCK-B binding. These modifications appear to be necessary for full biological activity, although both the unmodified C-terminal pentapeptide and tetrapeptide of CCK retains some biological activity. Kennedy et al., J. Biol. Chem. 272: 2920-2926 (1997), hereby incorporated by reference herein.

[0054] In a preferred embodiment, the biologically active therapeutic polypeptide of the present invention comprises a CCK binding element containing the post-translational modifications described above. This polypeptide can be produced by synthetic chemistry or, preferably, can be produced by a combination of recombinant and synthetic means using the “expressed protein ligation” (EPL) method. See Cotton & Muir, Chemistry & Biology 6:R247 (1999), hereby incorporated by reference herein. In this method the therapeutic polypeptide is expressed without the C-terminal binding element as a fusion protein with an “intein” polypeptide sequence positioned at the C-terminus thereof. The intein comprises a conserved cysteine, serine, or threonine residue at its amino terminus; the carboxyl terminus of the intein contains a functional binding sequence such as chitin binding domain (CBD), poly His (6 or more consecutive histidine residues), or another amino acid sequence capable of affinity binding. The coding sequence of this recombinantly expressed polypeptide is constructed using standard recombinant DNA methods.

[0055] Additionally, standard solid phase peptide synthesis methods are employed to construct a synthetic peptide comprising a C-terminal amidated phenylalanine and the desired CCK amino acid sequence. Such methods are described in e.g., Bodansky, M. and Bodansky, A. The Practice of Peptide Synthesis (2d ed. Trost B. M., ed. Springer Laboratory 1994), hereby incorporated by reference herein. The synthetic peptide also contains an sulfated tyrosine at the position 7 residues from the carboxyl terminus. This can be done either by incorporation of commercially available Fmoc-1yr(OSO$_3$)$_3$—OH into the peptide chain at the 7th amino acid position prior to cleavage of the synthetic peptide from the solid support hereby incorporated by reference herein, or by standard peptide synthesis using tyrosine at position 7, followed by a sulfation reaction of the peptide resulting in tyrosine sulfate at the 7 position. See e.g., Koeller, K. M., J. Am. Chem. Soc. 122:742-743 (2000). The synthetic peptide is constructed with a cysteine (or serine or threonine) residue at the amino terminus.

[0056] It will be understood that one can use either hydroxyl-containing amino acids or cysteine as the amino terminal residue of the intein and the synthetic peptide, and either thiolephol, phenol or another nucleophile capable of creating a reactive ester or thioester linkage in accordance with the expressed protein ligation methods described herein. However, thiol-containing amino acid residues and thiophenol or another sulfur-containing nucleophile are preferred.

[0057] Thus, according to one embodiment of the expressed protein ligation method, the fusion protein is immobilized following expression by incubation under selective binding conditions with a surface to which the binding partner of the carboxyl terminal has been joined (e.g., where the binding moiety is CBP, the surface may be a resin to which chitin is conjugated). The immobilized fusion protein is then permitted to react in a transhistoesterification reaction with a S- or O-containing reagent (such as thiolephol or phenol) and the synthetic modified peptide described above. In this step, the intein which is joined to the carboxyl terminus of the therapeutic polypeptide is cleaved at the thioester (or ester) linkage, thus liberating the protein from the surface to which it was bound. The intein may be transiently replaced with the thiolephol group, and the resulting thioester is then itself attacked by the cysteine (or serine or threonine) residue of the synthetic peptide; this reaction is then spontaneously followed by a shift of the carboxyl bond from S (or O) to the N terminal nitrogen of the synthetic peptide, to form a peptide bond. The resultant therapeutic polypeptide thus comprises a therapeutic domain, a translocation domain, and a binding domain comprising a CCK sequence modified to contain the naturally occurring post-translational modifications.

[0058] As intended herein, the term “extein” refers to a portion of a chimeric polypeptide that borders one or more intein, and is subsequently ligated to either another extein or a synthetic polypeptide in the EPL reaction referred to herein.

[0059] As intended herein, the term “intein” refers to a portion of a chimeric polypeptide containing an N-terminal cysteine, serine, or threonine which is excised from said polypeptide during the EPL reaction referred to herein.

[0060] Of course, the Applicants contemplate that this method of producing a CCK-containing therapeutic polypeptide is exemplary only, and that variations and modification of the above-described method will be well within the ability and knowledge of those of ordinary skill in the art in light of the present patent application.

[0061] While it will be understood that the applicants do not wish to be bound by theory, the following findings may assist an understanding the nature of the interaction between CCK and the CCK receptors, and thus between the CCK receptor binding element of an embodiment of the present invention and its CCK receptor target.

[0062] In pancreatic acinar cells the CCK A receptor undergoes internalization to intracellular sites within minutes after agonist exposure. Pohl et al., J. Biol. Chem. 272: 18179-18184 (1997), hereby incorporated by reference herein. The CCK B receptor has also shown the same ligand-dependant internalization response in transfected NIH 3T3 cells. In the CCK B receptor, but not the CCK A receptor, the endocytic feature of the receptor has been shown to be profoundly decreased by the deletion of the C terminal 44 amino acids of the receptor chain, corresponding in both receptors to an cytoplasmic portion of the receptor chain.

[0063] Recent studies of the interaction between the CCK A receptor and CCK have shown that the primary receptor sequence region containing amino acid residues 38 through 42 is involved in the binding of CCK. Residues Trp$^{39}$ and Gln$^{40}$ appear to be essential for the binding of a synthetic
CCK C-terminal nonapeptide (in which the methionine residues located at residue 3 and 6 from the C-terminus are substituted by norleucine and threonine respectively) to the receptor. Kennedy et al., supra. These residues do not appear to be essential for the binding of CCK analogs JM180 (corresponding to the synthetic C-terminal heptapeptide of CCK in which the phenylalanine residue is substituted by a phenylethyl ester and the threonine is substituted with norleucine), and JM179 (in which the phenylalanine residue and the L-tryptophan residues of the synthetic CCK nonapeptide are substituted by a phenylethyl ester and D-tryptophan, respectively and the threonine is substituted with norleucine). Id.

These and similar studies have shed light on the structure of the CCK-A receptor active site. Based on receptor binding experiments, a current structural model indicates that CCK residues Trp32, and Met13, (located at positions 4 and 3, respectively, from the C-terminus of mature CCK-8) reside in a hydrophobic pocket formed by receptor residues Leu440, Pro325, Ile353, and Ile350. CCK residue Asp32 (located at amino acid position 2 measured from the C terminus of CCK-8) seems to be involved in an ionic interaction with receptor residue Lys125. CCK Tyr-sulfate27 (the CCK-8 residue 7 amino acids from C terminus) appears involved in an ionic interaction with receptor residue Lys106, and a stacking interaction with receptor residue Phe210. Ji, et al., 272 J. Biol. Chem. 24393-24401 (1997).

Such structural models provide detailed guidance to the person of ordinary skill in the art as to the construction of a variety of binding elements able to retain the binding characteristics of biologically active CCK peptides for the CCK-A receptor, for example, as, for example, by site directed mutagenesis of a clostridial neurotoxin heavy chain. Similarly, models deduced using similar methodologies have been proposed for the CCK B receptor, see e.g., Jaggerschmidt, A. et al., Mol. Pharmacol. 48:783-789 (1995), and can be used as a basis for the construction of binding elements that retain binding characteristics similar to the CCK B receptor.

It will be appreciated that the CCK-B receptor is known to exist on the surface of neurons associated with the central nervous system. In one alternative embodiment of the present invention the therapeutic polypeptide may be directed (for example, by transthecal application) to these neurons rather than to the pancreas; in such a case, the binding element may comprise a CCK containing the C terminal amidation only. Such a binding element may be constructed using the expressed protein ligation (EPL) methods described above. Indeed, EPL methods may be used to introduce and desired or required modifications to the therapeutic element, the translocation element, and/or the binding element of the claimed therapeutic polypeptide.

Additionally, the binding element may comprise a variable region of an antibody which will bind the CCK-A or CCK-B receptor.

Nucleic acids encoding polypeptides containing such a binding element may be constructed using molecular biology methods well known in the art; see e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press 2d ed. 1989), and expressed within a suitable host cell. The disclosure of this latter reference is incorporated by reference herein in its entirety.

The translocation element comprises a portion of a clostridial neurotoxin heavy chain having a translocation activity. By “translocation” is meant the ability to facilitate the transport of a polypeptide through a vesicular membrane, thereby exposing some or all of the polypeptide to the cytoplasm.

In the various botulinum neurotoxins translocation is thought to involve an allosteric conformational change of the heavy chain caused by a decrease in pH within the endosome.

This conformational change appears to involve and be mediated by the N terminal half of the heavy chain and to result in the formation of pores in the vesicular membrane; this change permits the movement of the proteolytic light chain from within the endosomal vesicle into the cytoplasm. See e.g., Lacy, et al., Nature Struct. Biol. 5:898-902 (October 1998).

The amino acid sequence of the translocation-mediating portion of the botulinum neurotoxin heavy chain is known to those of skill in the art; additionally, the amino acid residues within this portion that are known to be essential for conferring the translocation activity are also known.

It would therefore be well within the ability of one of ordinary skill in the art, for example, to employ the naturally occurring N-terminal peptide half of the heavy chain of any of the various Clostridium tetani or Clostridium botulinum neurotoxin subtypes as a translocation element, or to design an analogous translocation element by aligning the primary sequences of the N-terminal halves of the various heavy chains and selecting a consensus primary translocation sequence based on conserved amino acid, polarity, steric and hydrophobicity characteristics between the sequences. The therapeutic element of the present invention may comprise, without limitation: active or inactive (i.e., modified) hormone receptors (such as androgen, estrogen, retinoid, peroxysome proliferator and edysyne receptors etc.), and hormone-agonists and antagonists, nucleic acids capable of being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins (including apoptosis-inducing agents), and the like.

In a preferred embodiment, the therapeutic element is a polypeptide comprising a clostridial neurotoxin light chain or a portion thereof retaining the SNARE-protein sequence-specific endopeptidase activity of a clostridial neurotoxin light chain. The amino acid sequences of the light chain of botulinum neurotoxin (BONT) subtypes A-G have been determined, as has the amino acid sequence of the light chain of the tetanus neurotoxin (TeNT). Each chain contains the Zn2+-binding motif His-Glu-x-x-His (N terminal direction at the left) characteristic of Zn2+-dependent endopeptidases (HELNH in TeNT, BoNT/A/B and BoNT/E; HELNH in BoNT/C; and HELTH in BoNT/D).

Recent studies of the BoNT/A light chain have revealed certain features important for the activity and specificity of the toxin towards its target substrate, SNAP-25. Thus, studies by Zhou et al. Biochemistry 34:15175-15181 (1995) have indicated that when the light chain amino
acid residue His227 is substituted with tyrosine, the resulting polypeptide is unable to cleave SNAP-25; Kurazono et al., *J. Biol. Chem.* 14721-14729 (1992) performed studies in the presynaptic cholinergic neurons of the buccal ganglia of *Aplysia californica* using recombinant BoNT/A light chain that indicated that the removal of 10 N-terminal or 32 C-terminal residues did not abolish toxicity, but that removal of 10 N-terminal or 57 C-terminal residues abolished toxicity in this system. Most recently, the crystal structure of the entire BoNT/A holotoxin has been solved; the active site is indicated as involving the participation of His222, Glu223, His322, Glu261 and Tyr365. Lacy et al, supra. (These residues correspond to His222, Glu223, His262, Glu261 and Tyr365 of the BoNT/A chain of Kurazono et al., supra.) Interestingly, an alignment of BoNT/A through E and TeNT light chains reveals that each such chain invariably has these residues in positions analogous to BoNT/A. Kurazono et al., supra.

**[0076]** The catalytic domain of BoNT/A is very specific for the C-terminus of SNAP-25 and appears to require a minimum of 16 SNAP-25 amino acids for cleavage to occur. The catalytic site resembles a pocket; when the light chain is linked to the heavy chain via the disulfide bond between Cys270 and Cys289, the translocation domain of the heavy chain appears to block access to the catalytic pocket until the light chain gains entry to the cytosol. When the disulfide bond is reduced, the two polypeptide chains dissociate, and the catalytic pocket is then “opened” and the light chain is fully active.

**[0077]** As described above, VAMP and syntaxin are cleaved by BoNT/B, D, F, and G and TeNT, and BoNT/C1, respectively, while SNAP-25 is cleaved by BoNT/A and E.

**[0078]** The substrate specificities of the various clostridial neurotoxin light chains other than BoNT/A are known. Therefore, the person of ordinary skill in the art could easily determine the toxin residues essential in these subtypes for cleavage and substrate recognition (for example, by site-directed mutagenesis or deletion of various regions of the toxin molecule followed by testing of proteolytic activity and substrate specificity), and could therefore easily design variants of the native neurotoxin light chain that retain the same or similar activity.

**[0079]** Additionally, construction of the therapeutic agents set forth in this specification would be easily constructed by the person of skill in the art. It is well known that the clostridial neurotoxins have three functional domains analogous to the three elements of the present invention. For example, and without limitation, the BoNT/A neurotoxin light chain is present in amino acid residues 1-448 of the BoNT/A holotoxin (i.e., before nicking of the prototoxin to form the disulfide-linked dichain holotoxin); this amino acid sequence is provided below as SEQ ID NO: 7. Active site residues are underlined:

```
MPFVVRQPNKGPVKGIDAYLIKIPNAGQMQPVFMAFHKIHKW
```

**[0080]** The heavy chain N-terminal (Hn) translocation domain is contained in amino acid residues 449-871 of the BoNT/A amino acid sequence, shown below as SEQ ID NO: 8; a gated ion channel-forming domain probably essential for the translocation activity of this peptide is underlined (see Oblatt-Montal et al., *Protein Sci.* 4:1490-1497(1995), hereby incorporated by reference herein.)
The heavy chain C-terminal neural cell binding domain is contained in amino acid residues 872-1296 (SEQ ID NO: 9) of the BoNT/A prototoxin.

The amino acid sequence of the BoNT/A prototoxin is encoded by nucleotides 358 to 4245 of the neurotoxin cDNA sequence, set forth herein below as SEQ ID NO: 10.
-continued
gtaaatagc caataatatg cacgtatatc ttaagaaata caaatattgac agcc acctttct
aatggtcata atacagaaat taataatag aattttacta actaaacaaa ttattatgga
ttggttgaat tttataagtt gctatgtgta agagggtaaa taacctctaa aactaataca
ttagataaaag gatacaataa ggatttaaat gattttagta tcaaaagttaa taatggggac
ttgttttttta gttcttcgaga agaatatttt actaatgtcc tcaaaaggg agaaggatct
acaatacata ctaatataag acagcagaga gaaatattta gtttagattt aataaaacaa
atatatatt ccctttacatt ttgtaaatga cctgaaataa tttcaataga aatactttca
agtcgaccata cagcggcaatt aaatcattct ccaaatttac ccattttcga attttgacat
agtaccatgtag ctagaataa cattttttcc catttttaag catttaaagat ttttttgcgc
gttatatctttcita caaatgtga aagaaagttc ataagacctc gggccccatt
agtttattga gatggtgtaga caaatagtga taatttttaa cgtagaatga tagcagattc
agttcattgcc gcgatatgta ctaatttttc cattttatttt taattttttta
aatattagta atatatatta taaatgtatg ttgtagctgt ctttatatt ttcagattc
gttatctgt tagaattttt accagagatt gcaataactg ttattgtac tttgacctt
gtatataa ttggatattga gttctaaacc gttcaaacaa tagataatgc ttatgtaaa
agtagctgaa aatgggtattc ggtctataaa tatagatga caaatgggtt agccaaaatt
aataacacaga ttagattaag aagaaaaaaa atgaaagagt cttggaagaac ccaagcagaa
goaaacaagg ctataataaa ctataatgt aatacaatata ctagggaga gaaaaataa
atatattta atatatattg aaaaaaactg aacatatattc ggttataacct aatatgctag
atatatata atatatatttt ggatatattt ttggttttctt attatatgaa tttcattgct
cttctagttg tttaacggttt agaaagattt gatgtattgc ttaagatgct attatatag
atatatatag atatatattg aggttttaatt ggtcagagtt atagattaaaa aagaaaaattt
aataaatcc ttagtagtaga tatcattttt cattttcctt catttttaca aatacaaga
atatataca cattatatga attaatagag aacatatatt aatatataa atatattata aataatatg
agtagtataa gtaaatcttt atataaacttt cttaaatggtag catcacaatt aataatgtgt
agtaagattaa attttgttcc aataagaaaa atcaatattt ctttatattt attaaattttt
agtagttgtt ttggtataag aatctcttag tatttttaaa cttattttg ttaagatggt
atatataata taaattgttt ggatatattt cctgggtgga aagtattacta taatatagtgt
gaatatcatt ggaattttaa ggaatctcag gaaatataaa aagagagtgt ttttaatatt
agttcgaaata ttagatattc ccattattta catttttagt tggattttgg aatattgttt
atatataata atataataaa aatgtgaatg taatatgtaa aaaaaaattt
aatatatata atatatatttt ggatatattt ttttttcttt tttgtagaga attatttttt
aatatatata atatatatttt ggatatattt ttttttcttt tttgtagaga attatttttt
aatatatata atatatatttt ggatatattt ttttttcttt tttgtagaga attatttttt
At the conclusion of the invention, which is defined solely by the claims that conclude this specification.

**Example 1**

An agent for the treatment of acute pancreatitis is constructed as follows.

A culture of *Clostridium botulinum* is permitted to grown to confluence. The cells are then lysed and total RNA is extracted according to conventional methods and in the presence of an RNAase inhibitor. The RNA preparation is then passed over a oligo(dT) cellulose column, the polyadenylated messenger RNA is permitted to bind, and the column is washed with 5-10 column volumes of 20 mM Tris pH 7.6, 0.5 M NaCl, 1 mM EDTA (ethylene diamine tetraacetic acid), 0.1% (w/v) SDS (sodium dodecyl sulfate). Polyadenylated RNA is then eluted with 2-3 column volumes of STE (10 mM Tris (pH 7.6), 1 mM EDTA, 0.05% (w/v) SDS). The pooled RNA is then precipitated in 2 volumes of ice cold ethanol, pelleted in a centrifuge at 10,000xg for 15 minutes, then redissolved in a small volume of STE.

The BoNT/A MRNA is used as a template for DNA synthesis using Moloney murine leukemia virus reverse transcriptase (MMLV-RT), then the L chain and then H N chain of the neurotoxin is amplified from the cDNA by the polymerase chain reaction (PCR) using appropriate oligonucleotide primers whose sequences are designed based on the BoNT/A neurotoxin cDNA sequence of SEQ ID NO: 9. These procedures are performed using the standard techniques of molecular biology as detailed in, for example, Sambrook et al., already incorporated by reference herein. The primer defining the beginning of the coding region (5' side of the L chain fragment) is given a Stul site. The PCR primer defining the 3' end of the HN-encoding domain has the following features (from 3' to 5'): a 5' region sufficiently complementary to the 3' end of the HN-encoding domain to it.
anneal thereto under amplification conditions, a nucleotide sequence encoding the human immunoglobulin hinge region \(\gamma_1\) (SEQ ID NO:11), a nucleotide sequence encoding the human CCK-8 octapeptide (SEQ ID NO:6), and a unique restriction endonuclease cleavage site.

[0089] The PCR product (termed BoNT/A\(^{\text{LHN-\gamma-CC}}\)) is purified by agarose gel electrophoresis, and cloned into a pBluescript II SK vector. The resulting plasmid is used to transform competent E. coli cells, and a preparation of the resulting plasmid is made. The BoNT/A\(^{\text{LHN-\gamma-CC}}\) fragment is excised from the pBluescript vector and cloned into a mammalian expression vector immediately downstream of a strong promoter. The resulting vector is used to transfect a culture of the appropriate host cell, which is then grown to confluence. Expression of the BoNT/A\(^{\text{LHN-\gamma-CC}}\) polypeptide is induced, and the cells are lysed. The polypeptide is purified by gel exclusion chromatography, the fractions containing the recombinant therapeutic agent are pooled, then the BoNT/A\(^{\text{LHN-\gamma-CC}}\) polypeptide is further purified using an anti-lg affinity column wherein the antibody is directed to the \(\gamma_1\) hinge region of a human immunoglobulin.

EXAMPLE 2

[0090] Method of Treating a Patient Suffering from Acute Pancreatitis

[0091] A therapeutically effective amount of the BoNT/A\(^{\text{LHN-\gamma-CC}}\) agent constructed and purified as set forth in Example 1 is formulated in an acceptable infusion solution. Properties of pharmacologically acceptable infusion solutions, including proper electrolyte balance, are well known in the art. This solution is provided intravenously to a patient suffering from acute pancreatitis on a single day over a period of one to two hours. Additionally, the patient is fed intravenously on a diet low in complex carbohydrates, complex fats and proteins.

[0092] At the beginning of treatment, the patient’s pancreas shows signs of autodigestion, as measured by blood amylase levels. After the treatment regimen, autodigestion has ceased, and the patient’s pancreas has stabilized.

EXAMPLE 3

[0093] Alternative Treatment Method

[0094] In this example, a patient suffering from acute pancreatitis is treated as in Example 2, with the therapeutic agent given continuously over a period of two weeks. After the treatment regimen, autodigestion has ceased, and the patient’s pancreas has stabilized.

EXAMPLE 4

[0095] Alternative Treatment Method

[0096] In this example, a patient suffering from acute pancreatitis is given a single pharmacologically effective amount of the therapeutic agent of Example 1 by parenteral administration. Two days after the treatment regimen, autodigestion has ceased and the patient’s pancreas has stabilized.

[0097] It will be understood that the present invention is not to be limited by the embodiments and examples described herein, and that the invention is defined solely by the claims that conclude this specification.
[Pinkacted text from the image]
Arg Asp Tyr Met Gly Trp Met Asp Phe

<210> SEQ ID NO 7
<211> LENGTH: 448
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 7

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1  5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20  25 30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35  40 45
Asp Thr Phe Thr Asn Pro Glu Glu Asp Leu Asn Pro Pro Glu
50  55 60
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65  70 75 80
Asp Asn Glu Lys Asp Asn Tyr Lys Gly Val Thr Lys Leu Phe Glu
85  90 95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110
Arg Gly Ile Pro Phe Trp Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140
Arg Ser Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
165 170 175
Arg Asp Gly Tyr Gly Ser Thr Glu Tyr Arg Phe Ser Pro Asp Phe
180 185 190
Thr Phe Gly Phe Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255
Glu Val Ser Phe Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
260 265 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Aan
275 280 285 290
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
295 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335 335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn 355 360 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr 370 375 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn 385 390 395 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu 405 410 415

Lys Asn Phe Thr Gly Leu Phe Gly Phe Tyr Lys Leu Leu Cys Val Arg 420 425 430

Gly Ile Ile Thr Ser Lys Thr Leu Leu Asp Lys Gly Tyr Asn Lys 435 440 445

<210> SEQ ID NO 8
<211> LENGTH: 423
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 8

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Aep Leu Phe Phe 1 5 10 15

Ser Pro Ser Glu Asp Asn Phe Thr Asn Aep Leu Lys Gly Glu Glu 20 25 30

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Lys Asn Ile Ser Leu 35 40 45

Asp Leu Ile Gln Gln Tyr Leu Thr Phe Asn Phe Aep Lys Aep Pro 50 55 60

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Aep Ile Ile Gly Gln Leu 65 70 75 80

Glu Leu Met Pro Aep Ile Glu Arg Phe Pro Aep Gly Lys Tyr Glu 85 90 95

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu 100 105 110

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu 115 120 125

Leu Aep Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Aep Tyr Val Lys 130 135 140

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu 145 150 155 160

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr 165 170 175

Asp Lys Ile Ala Asp Ile Thr Ile Pro Tyr Ile Gly Pro Ala 180 185 190

Leu Aep Ile Gly Aep Met Leu Tyr Lys Asp Aep Phe Val Gly Ala Leu 195 200 205

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala 210 215 220

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys 225 230 235 240

Val Leu Thr Val Glu Thr Ile Aep Ala Ala Leu Ser Lys Arg Asn Glu 245 250 255

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Aep Thr Leu Ala Lys
Val Asn Thr Gln Ile Asp 265 270
Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Asn Tyr Gln Tyr Asn 260 275
Gln Tyr Thr Gln Glu Gln Asn Ile Asn Phe Asn Ile Asp Asp 305 310 315
Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile 325 330 335
Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met 340 345 350
Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys 355 360 365
Asp Ala Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly 370 375 380
Gln Val Asp Arg Leu Lys Asp Val Asn Thr Leu Ser Thr Asp 385 390 395 400
Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Gln Arg Leu Leu Ser 405 410 415
Thr Phe Thr Gln Tyr Ile Lys 420

<210> SEQ ID NO 9
<211> LENGTH: 382
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 9
Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn 1 5 10 15
Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp 20 25 30
Ile Arg Ile Pro Tyr Phe Asn Ser Ile Ser Leu Asn Asn Gly Tyr 35 40 45
Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu 50 55 60
Asn Tyr Gly Glu Ile Ile Thr Leu Gln Asp Thr Gln Glu Ile Lys 65 70 75 80
Gln Arg Val Val Phe Lys Tyr Ser Glu Met Ile Asn Ile Ser Asp Tyr 85 90 95
Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Arg Leu Asn Asn 100 105 110
Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Asp Ile Ser 115 120 125
Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp 130 135 140
Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu 145 150 155 160
Phe Asp Lys Glu Leu Asn Glu Glu Ile Lys Asp Leu Tyr Asp Asn 165 170 175
Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln 180 185 190
-continued

```
Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr 195 200 205
Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly 210 215 220
Pro Arg Gly Ser Val Met Thr Thr Aan Ile Tyr Leu Aen Ser Ser Leu 225 230 235 240
Tyr Arg Gly Thr Lys Phe Ile Ile Lys Tyr Ala Ser Gly Asn Lys 245 250 255
Asp Aan Ile Val Arg Aan Aen Asp Arg Val Tyr Ile Aen Val Val Val 260 265 270
Lys Aen Lys Glu Tyr Arg Leu Ala Thr Aan Ala Ser Gln Ala Gly Val 275 280 285
Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Aen Gly Aen Leu Ser 290 295 300
Gln Val Val Met Lys Ser Aen Asp Aen Gly Aen Asp Aen Gly Ile Gly Phe Ile 305 310 315 320
Cys Lys Met Aen Leu Gln Aen Asp Aen Gly Aen Asp Aen Gly Phe Ile 325 330 335
Gly Phe His Gln Phe Aen Asn Ile Ala Leu Val Ala Ser Aen Trp 340 345 350
Tyr Aen Arg Glu Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp 355 360 365
Glu Phe Ile Pro Val Aen Asp Gly Trp Gly Glu Arg Pro Leu 370 375 380
```

<210> SEQ ID NO 10
<211> LENGTH: 4835
<212> TYPE: DNA
<213> ORGANISM: Clostridium botulinum

```
aagctttaca atttaaat ta ttaaagctaa tttaaattaa aacaaatgaa ccaaaacttt 60
ataggttaat aattcttgta ttaagataata tggaaagata tatagataata tctgaaagata 120
atatagtgaoc aactatagat aacaaatgaa aagaaagagaa gagtagaatat agtagaatat 180
atatgttctc caattgttta acctctatctt ataacagttaa atatatagatg ttagcgatag 240
aagttggaac ccaaatagatg ataggttaata taatatagatg ttagcgatag ttagcgatag 300
ggtcatatttta atatatttaata atatatataa tattatatag ttaaatagatggttaaatagatg 360
ccattttgtaa atatataatgaa atataatagatg ttagcgatag ttagcgatag 420
ataaattttt ccattttgtaa ccaaaattg aa ccattttgtaa ttagcgatag ttagcgatag 480
ataggttaat ttcggagaag gatataatgaa aaaaaagggaa atataatgaa ttagcgatag 540
ccataagagaa aacatataa aaaaatagatg ttagcgatag ttagcgatag 600
aatattaaat atacatatgaa aaaaaatagatg ttagcgatag ttagcgatag 660
gcattttgtaa gataggtttc aacatataa aaaaatagatg ttagcgatag ttagcgatag 720
aacatataa aacatataa aaaaatagatg ttagcgatag ttagcgatag 780
ggtaggttata gttcggagaac aacatataa gtaaagagaa ttagcgatag ttagcgatag 840
catattttttc gtaaaagctttttaa gttttagaattttaa ttagcgatag ttagcgatag 900
tttatattat taggtttagaattttaa gttttagaattttaa gttttagaattttaa 960
```
<table>
<thead>
<tr>
<th>Started at</th>
<th>Ended at</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>3300</td>
<td>3360</td>
<td>tattagatga ggaatatat acaggtacgga agtactactac taaaattggtt</td>
</tr>
<tr>
<td>3420</td>
<td>3480</td>
<td>agtgaatg ttatatata cagttatata cccaggtgga ttttgggtat taaaactaat</td>
</tr>
<tr>
<td>3540</td>
<td>3600</td>
<td>taaatatttag ggaatatat acaggtacgga agtactactac taaaattggtt</td>
</tr>
<tr>
<td>3660</td>
<td>3720</td>
<td>ggtggtattta tcacatatgct taataatac ctaaattagca attatatagc tcaaatataaa</td>
</tr>
<tr>
<td>3780</td>
<td>3840</td>
<td>tagttccagacg ttaatattag caaggtacgga agtactactac taaaattggtt</td>
</tr>
<tr>
<td>3900</td>
<td>3960</td>
<td>atataatag caggtactactac ctaaattagca attatatagc tcaaatataaa</td>
</tr>
<tr>
<td>4020</td>
<td>4080</td>
<td>gtagtttagc ttaaatattag caaggtacgga agtactactac taaaattggtt</td>
</tr>
<tr>
<td>4140</td>
<td>4200</td>
<td>aataatagc gaaataat caggtactactac ctaaattagca attatatagc tcaaatataaa</td>
</tr>
<tr>
<td>4260</td>
<td>4320</td>
<td>tagttccagacg ttaatattag caaggtacgga agtactactac taaaattggtt</td>
</tr>
<tr>
<td>4380</td>
<td>4440</td>
<td>atataatag caggtactactac ctaaattagca attatatagc tcaaatataaa</td>
</tr>
<tr>
<td>4500</td>
<td>4560</td>
<td>gtagtttagc ttaaatattag caaggtacgga agtactactac taaaattggtt</td>
</tr>
<tr>
<td>4620</td>
<td>4680</td>
<td>aataatagc gaaataat caggtactactac ctaaattagca attatatagc tcaaatataaa</td>
</tr>
<tr>
<td>4740</td>
<td>4800</td>
<td>cagttactag agataagttc aatctctttg ttctctact tcacataatagc gaaataat</td>
</tr>
<tr>
<td>4835</td>
<td></td>
<td>tataaatgctata gtaaagttc aatctctttg ttctctact tcacataatagc gaaataat</td>
</tr>
</tbody>
</table>
1-13. (canceled)

14. A method for making a polypeptide comprising:
   a) expressing within a host cell a recombinant chimeric polypeptide comprising an amino terminal side extein and a carboxyl terminal side intein
      wherein said extein comprises able to facilitate the transfer of a polypeptide across a vesicular membrane and a therapeutic element able, when present in the cytoplasm of a pancreatic cell, to inhibit enzymatic secretion by said pancreatic cell, and
      wherein said intein comprises a binding element capable of affinity binding under selective conditions with a binding partner and an amino terminal end first amino acid selected from the group consisting of cysteine, serine or threonine.
   b) contacting said chimeric polypeptide with a synthetic peptide and a nucleophilic reagent
      wherein said synthetic peptide comprises a CCK binding element able to selectively bind a pancreatic cell surface marker under physiological conditions, a carboxyl terminal end amidated phenylalanine modification and an amino terminal end second amino acid selected from the group consisting of cysteine, serine or threonine,
      wherein said nucleophilic reagent is able to cause cleavage of said intein from the said extein, and
      wherein subsequent formation of a peptide bond occurs between carboxyl terminal end of said extein and amino terminal end of said synthetic peptide.

15. The method of claim 14 wherein said first and second amino acids are cysteine.

16. The method of claim 15 wherein said nucleophilic reagent is selected from the group consisting of phenol or thiphenol.

17. The method of claim 14 wherein said synthetic polypeptide further comprises a sulfated tyrosine at the position 7 amino acids from a natural C terminus of said sequence, and said therapeutic polypeptide preferentially binds a CCK-A receptor.

18. The method of claim 17 wherein said first and second amino acids are cysteine.

19. The method of claim 18 wherein said nucleophilic reagent is selected from the group consisting of phenol or thiphenol.

20. The method of claim 14 wherein said first and second amino acids are serine.

21. The method of claim 14 wherein said first and second amino acids are threonine.

22. The method of claim 17 wherein said first and second amino acids are serine.

23. The method of claim 17 wherein said first and second amino acids are threonine.

24. A method for making a polypeptide comprising:
   a) expressing within a host cell a recombinant chimeric polypeptide comprising an amino terminal side extein and a carboxyl terminal side intein
      wherein said extein comprises a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane and a therapeutic element able, when present in the cytoplasm of a pancreatic cell, to inhibit enzymatic secretion by said pancreatic cell, and
      wherein said intein comprises a binding element capable of affinity binding under selective conditions with a binding partner and an amino terminal end first amino acid selected from the group consisting of cysteine, serine or threonine.
   b) contacting said chimeric protein with a synthetic peptide and a nucleophilic reagent
      wherein said synthetic peptide comprises a binding element able to selectively bind a pancreatic cell surface marker under physiological conditions, a carboxyl terminal end amidated phenylalanine modification and an amino terminal end second amino acid selected from the group consisting of cysteine, serine or threonine,
      wherein said nucleophilic reagent is able to cause cleavage of said intein from the said extein, and
      wherein subsequent formation of a peptide bond occurs between carboxyl terminal end of said extein and amino terminal end of said synthetic peptide.

25. The method of claim 24 wherein said therapeutic element will cleave a SNARE protein.

26. The method of claim 25 wherein said SNARE protein is selected from the group consisting of syntxin, SNAP-25 and VAMP.

27. The method of claim 24 wherein said binding element of said synthetic peptide comprises a CCK sequence.

28. The method of claim 27 wherein said CCK sequence comprises a human CCK A amino acid sequence.

29. The method of claim 28 wherein said CCK A amino acid sequence comprises SEQ ID NO: 6.

30. The method of claim 28 wherein said CCK A amino acid sequence comprises SEQ ID NO: 5.

31. The method of claim 28 wherein said CCK A amino acid sequence comprises SEQ ID NO: 4.

32. The method of claim 28 wherein said CCK A amino acid sequence comprises SEQ ID NO: 2.

33. The method of claim 28 wherein said CCK A amino acid sequence comprises SEQ ID NO: 2.

34. The method of claim 24 wherein said first and second amino acids are cysteine.
35. The method of claim 24 wherein said nucleophilic reagent is selected from the group consisting of phenol or thiophenol.

36. The method of claim 24 wherein said first and second amino acids are serine.

37. The method of claim 24 wherein said first and second amino acids are threonine.

38. The method of claim 20 wherein said binding element of said synthetic peptide further comprises a sulfated tyrosine at the position 7 residues from the carboxyl terminal end.

39. The method of claim 38 wherein said binding element of said synthetic peptide comprises a CCK sequence.

40. The method of claim 39 wherein said CCK sequence comprises a human CCK A amino acid sequence.

41. The method of claim 39 wherein said CCK A amino acid sequence comprises SEQ ID NO: 6.

42. The method of claim 39 wherein said CCK A amino acid sequence comprises SEQ ID NO: 5.

43. The method of claim 39 wherein said CCK A amino acid sequence comprises SEQ ID NO: 4.

44. The method of claim 39 wherein said CCK A amino acid sequence comprises SEQ ID NO: 3.

45. The method of claim 39 wherein said CCK A amino acid sequence comprises SEQ ID NO: 2.