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Patent
Attorney Docket No. 213/080

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Enclosed Documents:

1. Fee Transmittal (1 page) (with 1 additional copy)
2. Patent Term Extension Application under 35 U.S.C. 156 (16 pages) (with 4 additional copies)

Including:

- Exhibit A – Power of Attorney (3 pages)
- Exhibit B – U.S. Patent No. 5,686,411 (23 pages)
- Exhibit C – Certificate of Correction Issued on U.S. Patent No. 5,686,411 (2 pages)
- Exhibit D – Maintenance Fee Statement receipts received for U.S. Patent No. 5,686,411 for years four and eight (3 pages)

3. Return Postcard

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Effective on 12/08/2004.
 Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 1103).

FEE TRANSMITTAL

For FY 2005

Complete if Known

Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$) 1,120.00

Application Number	08/447,849
Filing Date	May 23, 1995
First Named Inventor	Gaeta
Examiner Name	N/A
Art Unit	N/A
Attorney Docket No.	213/080

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METHOD OF PAYMENT (check all that apply)

Check Credit Card Money Order None Other (please identify): _____

Deposit Account Deposit Account Number: 01-0535 Deposit Account Name: Amylin Pharmaceuticals Inc

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

Charge fee(s) indicated below Charge fee(s) indicated below, except for the filing fee

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	
Utility	300	150	500	250	200	100	_____
Design	200	100	100	50	130	65	_____
Plant	200	100	300	150	160	80	_____
Reissue	300	150	500	250	600	300	_____
Provisional	200	100	0	0	0	0	_____

2. EXCESS CLAIM FEES

Fee Description	Fee (\$)	Small Entity Fee (\$)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims **Extra Claims** **Fee (\$)** **Fee Paid (\$)**

_____ - 20 or HP = _____ x _____ = _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims **Extra Claims** **Fee (\$)** **Fee Paid (\$)**

_____ - 3 or HP = _____ x _____ = _____

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets **Extra Sheets** **Number of each additional 50 or fraction thereof** **Fee (\$)** **Fee Paid (\$)**

_____ - 100 = _____ / 50 = _____ (round up to a whole number) x _____ = _____

4. OTHER FEE(S)

Non-English Specification, \$130 fee (no small entity discount) **Fees Paid (\$)**

Other (e.g., late filing surcharge): Patent Term Extension Application Fee under 37 CFR 1.20(i)(1) 1,120.00

SUBMITTED BY

Signature		Registration No. (Attorney/Agent)	40,022	Telephone (858) 642-7084
Name (Print/Type)	Molly A. Holman, Ph.D., J.D.	Date	5/12/05	

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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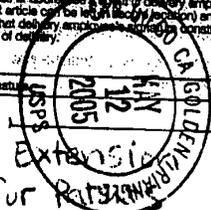
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Patents
Attorney Reference: 213/080

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant in re: Gaeta et al.)
)
Patent No.: 5,686,411)
)
Serial No: 08/447,849)
)
Issued: November 11, 1997)
)
Filed: May 23, 1995)
)
For: AMYLIN AGONIST PEPTIDES AND)
USES THEREFOR)

PATENT TERM EXTENSION APPLICATION
UNDER 35 U.S.C. 156

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Mail Stop Patent Extension
Commissioner for Patents
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Dear Sir:

Amylin Pharmaceuticals, Inc. respectfully requests a patent term extension for U.S. Letters Patent No. 5,686,411. Submitted herewith is an Application for Patent Term Extension under 35 U.S.C. 156 based on the regulatory review period for SYMLIN[®] (pramlintide acetate), in compliance with 37 CFR 1.710 - 1.740.

This application is being submitted by a registered practitioner on behalf of Amylin

CERTIFICATE OF EXPRESS MAILING
(37 C.F.R. §1.10)

I hereby certify that this paper (along with anything referred to as being attached or enclosed) is being deposited with the United States Postal Service as "Express Mail". Label No. ED 127779653 US on the date shown below with sufficient postage in an envelope addressed to Mail Stop Patent Extension, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

12 May 2005
Date of Deposit

Diane Trus
Name of Person Mailing Paper
Diane Trus
Signature of Person Mailing Paper

05/13/2005 SMIHSS1 00000073 010535 08447849

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Pharmaceuticals, Inc., which is the assignee of the entire right, title and interest in U.S. Patent No. 5,686,411 by virtue of the assignment recorded on January 6, 1992 at the U.S. Patent and Trademark Office at Reel 5934, Frame 0449, and a name change recorded on July 16, 1997 at Reel 8635, Frame 0563. A Power of Attorney from Amylin Pharmaceuticals, Inc. granting power to Molly A. Holman is attached as Exhibit A.

Pursuant to the provisions of 37 C.F.R. § 1.730, applicant hereby applies for an extension of the term of U.S. Patent No. 5,686,411 under 35 U.S.C. § 156 of 1586 days, based on the materials set forth herein and in the accompanying papers.

The required information for this application is submitted in accordance with 35 USC 156(d) and 37 CFR 1.710 et seq., and follows the numerical format set forth in 37 CFR 1.740(a). A response to each requirement follows each numerical requirement. The required information starts on page 3 of this application.

- (1) A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics.

The approved product is pramlintide, the active ingredient of SYMLIN[®] (pramlintide acetate) injection. SYMLIN[®] (pramlintide acetate) injection is an antihyperglycemic drug for use in patients with diabetes treated with insulin. Pramlintide is a synthetic analog of human amylin, a naturally occurring neuroendocrine hormone synthesized by pancreatic beta cells that contributes to glucose control during the postprandial period. Pramlintide is provided as an acetate salt of the synthetic 37-amino acid polypeptide, which differs in amino acid sequence from human amylin by replacement with proline at positions 25 (alanine), 28 (serine), and 29 (serine) (*i.e.*, ^{25,28,29}Pro-h-amylin).

The structural formula of pramlintide acetate is as shown:


Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Pro-Ile-Leu-Pro-Pro-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH₂ acetate (salt) with a disulfide bridge between the two Cys residues.

Pramlintide acetate is a white powder that has a molecular formula of C₁₇₁H₂₆₇N₅₁O₅₃S₂• x C₂H₄O₂ (3 ≤ x ≤ 8); the molecular weight is 3949.4. The CAS registry number for pramlintide acetate is 196078-30-5.

We note that the term "product," for purposes of patent term extension for a drug product, is defined as "the active ingredient of a new drug, antibiotic drug, or human biological product...including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient." 35 U.S.C. § 156(f)(2). Nothing in this application should be construed as limiting the term "product" for purposes of the requested patent term extension to the specific form of pramlintide approved in SYMLIN[®].

- (2) A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred.

The regulatory review for SYMLIN[®] (pramlintide acetate) occurred under Section 505 of the Federal Food, Drug, and Cosmetic Act, 21 USC § 355.

- (3) An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred.

SYMLIN[®] (pramlintide acetate) injection received permission for commercial marketing and use under Section 505 of the Federal Food, Drug, and Cosmetic Act on March 16, 2005.

- (4) In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.

The active ingredient in SYMLIN[®] (pramlintide acetate) is pramlintide (^{25,28,29}Pro-h-amylin). That active ingredient has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

- (5) A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted.

This application for patent term extension is being submitted within the sixty-day period permitted for submission pursuant to § 1.720(f). The product was approved on March 16, 2005. The last date within the sixty-day period is May 15, 2005. Since May 15, 2005 is a Sunday, the application may be timely filed under 35 USC 21(b) on May 16, 2005, the next succeeding business day. As evident from the Certificate of Mailing by "Express Mail" pursuant to 37 C.F.R. 1.10, this application is timely filed.

- (6) A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration.

A complete identification of the patent for which an extension is being sought is as follows:

Inventors: Laura S. L. Gaeta, Howard Jones, and Elisabeth Albrecht

Patent Number: 5,686,411

Date of Issue: November 11, 1997

Date of Expiration: November 11, 2014

- (7) A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings.

A copy of U.S. Patent No. 5,686,411 for which an extension is being sought is attached as Exhibit B.

- (8) A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent.

U.S. Patent No. 5,686,411 has a Certificate of Correction, a copy of which is attached as Exhibit C. Copies of the Maintenance Fee Statement receipts received for U.S. Patent No. 5,686,411 for years four and eight are attached as Exhibit D.

- (9) A statement that the patent claims the approved product, or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on:

(i) The approved product, if the listed claims include any claim to the approved product;

(ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product; and

(iii) The method of manufacturing the approved product, if the listed claims include any claim to the method of manufacturing the approved product.

Patent No. 5,686,411 claims the approved product. Claims 1-12, 19, 24-29, and 38-43 claim the approved product. The approved product is pramlintide, the active ingredient of SYMLIN[®] (pramlintide acetate).¹

Claim 19 reads on pramlintide as follows.

19. ^{25,28,29}Pro-h-amylin.

Pramlintide is ^{25,28,29}Pro-h-amylin.

Patent No. 5,686,411 claims a method of using the approved product. Claims 30-37 claim a method of using the approved product. The approved product is pramlintide, the active ingredient of SYMLIN[®] (pramlintide acetate).

Claim 35 reads on a method of using the approved product as follows.

35. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of ^{25,28,29}Pro-h-amylin.

Pramlintide is ^{25,28,29}Pro-h-amylin.

SYMLIN[®] (pramlintide acetate) is given at mealtimes and is indicated for:

- Type 1 diabetes, as an adjunct treatment in patients who use mealtime insulin therapy and who have failed to achieve desired glucose control despite optimal insulin therapy.
- Type 2 diabetes, as an adjunct treatment in patients who use mealtime insulin therapy and who have failed to achieve desired glucose control despite optimal insulin therapy, with or without a concurrent sulfonylurea agent and/or metformin.

¹ We note that the term "product," for purposes of patent term extension for a drug product, is defined as "the active ingredient of a new drug, antibiotic drug, or human biological product...including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient." 35 U.S.C. § 156(f)(2). Nothing in this application should be construed as limiting the term product for purposes of the requested patent term extension to the specific form of pramlintide approved in SYMLIN[®].

- (10) A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. 156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:
- (i) For a patent claiming a human drug, antibiotic, or human biological product:
 - (A) The effective date of the investigational new drug (IND) application and the IND number;
 - (B) The date on which a new drug application (NDA) or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and
 - (C) The date on which the NDA was approved or the Product License Issued.

U.S. Patent No. 5,686,411 claims a human drug product. The relevant dates and information pursuant to 35 U.S.C. 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

- (A) The IND for pramlintide acetate injection was accepted by the FDA on June 24, 1992. The effective date of the IND application was July 29, 1992. The IND number is 38,897.
 - (B) The NDA was initially submitted to the FDA on December 7, 2000. The NDA number is 21-332.
 - (C) The NDA was approved by the FDA on March 16, 2005.
- (11) A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities.

During the applicable regulatory review period, Amylin was diligently involved in obtaining FDA approval for pramlintide. As discussed in (10) above, the IND for pramlintide was accepted by the FDA on June 24, 1992, the IND Exemption became effective on July 29, 1992, the NDA was submitted on December 7, 2000, and the NDA was approved on March 16, 2005. Amylin was in close consultation with the FDA during the clinical studies conducted under the IND. Similarly, subsequent to the submission of the NDA, Amylin had numerous contacts and meetings with the FDA with respect to the approval. A brief description of the significant activities undertaken by Amylin during the applicable regulatory review period with respect to pramlintide acetate and the significant dates applicable to such activities appears in the table below.

Date	Regulatory Review Activities
6/22/1992	Amylin submitted IND Application for AC-137 to FDA/CDER; Protocol AP92-01 submitted with IND Application
6/30/1992	Agency acknowledged receipt of IND on 7/6/92; IND Number Assigned: 39,897
7/29/1992	Amylin submitted Protocol Amendment to Study No. AP92-01 in response to FDA request; revision to Investigator's Brochure. Per discussions with FDA, Amylin given permission to proceed with study upon submission of revised protocol
8/3/1992	Amylin submitted Protocol AP92-02
10/1/1992	Amylin submitted clinical information on first 3 doses of AP92-02
11/12/1992	Amylin submitted Protocol AP92-03
11/30/1992	Meeting with FDA to discuss clinical program, clinical data to date.
1/15/1993	Amylin submitted clinical data from AP92-01
2/17/1993	Amylin submitted Protocol AP93-01
6/1/1993	Amylin submitted Protocol AP93-03
6/4/1993	Amylin submitted Protocol AP93-02
8/17/1993	Amylin submitted Protocol AP93-04
10/22/1993	Informal meeting between Amylin and FDA
11/9/1993	Amylin submitted Protocol AP93-08
1/14/1994	Amylin submitted final clinical report for AP92-02
1/26/1994	Amylin submitted Protocols AP93-07, AP93-09, 137-101
2/14/1994	Amylin submitted final clinical report for AP92-01
8/24/1994	Amylin submitted Protocol 137-102
9/14/1994	Amylin submitted Protocols 137-103, 137-104, 137-105, 137-106, and AP93-09
12/6/1994	Amylin submitted final report for clinical study AP92-03
12/13/1994	Meeting at FDA between Amylin and FDA for CMC discussion
4/25/1995	Amylin submitted the final report for clinical study AP93-04
5/1/1995	End-of-Phase II meeting between Amylin and FDA
5/30/1995	Amylin submitted Protocols 137-109, 137-110, and 137-111
6/30/1995	Amylin submitted the final report for study 137-101
7/31/1995	Amylin submitted Protocols 137-107, 137-108
10/11/1995	Amylin submitted Protocols 137-112, 137-113, 137-114, 137-115

11/13/1995	Amylin submitted final report for clinical study AP93-01.
12/15/1995	Amylin submitted Protocol 137-116
2/13/1996	Amylin submitted Protocol 137-118
2/21/1996	Amylin submitted final report for clinical study AP93-02
2/27/1996	Amylin submitted final report for clinical study 137-103.
5/10/1996	Amylin submitted the final report for AP93-08
5/20/1996	Amylin submitted the final report for 137-106.
5/13/1996	Amylin submitted the final report for 137-109.
6/24/1996	Amylin submitted Protocols 137-111E, 137-112E, 137-119, 137-120
10/10/1996	Amylin submitted Protocols 137-117, 137-118, 137-120, 137-121, 137-122, 137-123, 137-125, 137-126
10/17/1996	Clinical meeting between Amylin and FDA
1/15/1997	Teleconference regarding IND Amendment between Amylin and FDA
3/13/1997	Amylin submitted Protocol 137-123
5/22/1997	Pre-clinical CMC meeting between Amylin and FDA
6/2/1997	Amylin submitted Protocols 137-124, 137-127
6/13/1997	Amylin submitted the final report for AP93-03
6/18/1997	Teleconference between FDA and Amylin re clinical pharmacokinetic and drug interaction program, etc.
9/12/1997	Amylin submitted Protocols 137-130, 137-117E, 137-123E
10/28/1997	Clinical Pre-NDA meeting between Amylin and FDA
2/2/1998	Amylin submitted Protocols 137-136, 137-137
4/9/1998	Clinical meeting between Amylin and FDA
4/24/1998	Amylin submitted Protocol 137-133
6/15/1998	Submission of final clinical study report for protocol 137-130
6/16/1998	Amylin submitted Protocol 137-138
6/30/1998	Amylin submitted Protocol 137-140
7/29/1998	Amylin submitted Protocol 137-134
11/6/1998	Teleconference between Amylin and FDA to review pivotal trials
12/8/1998	Clinical meeting between Amylin and FDA
2/5/1999	Submission of statistical analysis plan for 137-121.
9/23/1999	Meeting between Amylin and FDA
9/30/1999	Amylin submitted Protocol 137-141
11/9/1999	Amylin submitted Protocol 137-142
2/9/2000	Pre-NDA Meeting re CMC between Amylin and FDA
6/9/2000	Amylin submitted Protocols 137-143, 137-144
9/7/2000	Pre-NDA Meeting between Amylin and FDA
12/7/2000	New Drug Application (NDA 21-332) for SYMLIN (pramlintide acetate) Injection submitted by Amylin Pharmaceuticals
4/5/2001	Amylin submitted 120-Day Safety Update for SYMLIN, including data from now completed studies 137-143, 137-144 and 137-145.
7/18/2001	Amylin submitted Protocol 137-147
7/26/2001	Endocrine and Metabolism Drug Advisory Committee Meeting and Amylin

	presentation
11/21/2001	End-of-Review Conference held between FDA and Amylin
12/17/2001	Amylin submitted Protocol 137-152
12/19/2001	Amylin submitted Protocol 137-151
2/27/2002	Amylin submitted Protocol 137-149
3/26/2002	Amylin submitted Protocol 137-150
4/9/2002	Teleconference held between FDA and Amylin to discuss protocols 137-150 and 137-151.
4/29/2002	Amylin submitted Protocol 137-153
8/5/2002	Amylin submitted Protocol 137-154
10/25/2002	Amylin submitted Protocol 137-150E
3/26/2003	Amylin submitted Protocol 137-155
6/16/2003	Amylin submitted NDA Resubmission to FDA
8/13/2003	137-150 Final Clinical Study Report sent to FDA
11/20/2003	Amylin submitted Protocol 137-157
1/14/2004	Telephone Conference between Amylin and FDA re clarification of issues in 17 December 2003 Action Letter
1/15/2004	Amylin submitted Protocol 137-158
3/26/2004	Amylin submitted Protocol 137-160
7/21/2004	Meeting between Amylin and FDA re overall development program for SYMLIN
9/17/2004	Complete Response of Amylin Pharmaceuticals, Inc. to the second Approvable Letter dated December 17, 2003, for SYMLIN® (pramlintide acetate) Injection
1/31/2005	Amylin submitted a summary of the final study report of Protocol 137-158
3/16/2005	Approval letter sent to Amylin by FDA

- (12) A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as the length of the extension claimed, including how the length of the extension was determined.

It is the opinion of the Applicant that U.S. Patent No. 5,686,411 is eligible for patent term extension under 35 USC 156.

The length of extension claimed is 1586 days, for an extension to March 16, 2019. The period of this extension was calculated under 35 USC 156(c) and 37 CFR 1.775(d), using 35 U.S.C. 156(g) and 37 CFR 1.775(c) to calculate regulatory review period, as described below.

Calculation of Regulatory Review Period: Under 37 CFR 1.775(c), the length of the regulatory review period for a human drug is the sum of:

- (1) The number of days in the period beginning on the date an exemption under subsection (i) of section 505 or subsection (d) of section 507 of the Federal Food, Drug, and Cosmetic Act became effective for the approved product and ending on the date an application was initially submitted for such product under these sections or under section 351 or the Public Health Service Act; and**
- (2) The number of days in the period beginning on the date the application was initially submitted for the approved product under section 351 or the Public Health Service Act, subsection (b) of section 505 or section 507 of the Federal Food, Drug, and Cosmetic Act, and ending on the date such application was approved under such section.**

The period under 37 CFR 1.775(c)(1) is 3052 days. An Investigational New Drug exemption under subsection 505 of the Federal Food, Drug, and Cosmetic Act became effective for pramlintide acetate on July 29, 1992. A New Drug Application was initially submitted for pramlintide acetate on December 7, 2000. The period under 37 CFR 1.775(c)(2) is 1560 days. The New Drug Application for pramlintide acetate was initially submitted on December 7, 2000. This application was approved on March 16, 2005.

The regulatory review period is the sum of the number of days above listed sections (c)(1) and (c)(2), which is 4,612 days.

Calculation of Patent Term Extension: Under 37 CFR 1.775(d) (bolded text), the term of US 5,686,411 as extended for a human drug product has been calculated to be until March 16, 2019, fourteen years from approval of the product, as shown below:

- (1) Subtracting from the number of days ...[in] the Regulatory Review Period:**
 - (i) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section which were on and before the date on which the patent issued;**
 - (ii) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section during which it is determined under 35 U.S.C. 156(d)(2) (B) by the Secretary of Health and Human Services that applicant did**

not act with due diligence;

- (iii) One-half the number of days remaining in the period defined by paragraph (c)(1) of this section after that period is reduced in accordance with paragraphs (d)(1)(i) and (ii) of this section; half days will be ignored for this section;**

(i) U.S. Patent No. 5,686,411 issued on November 11, 1997. The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section which were on and before the date that the patent issued is 1,930 days.

(ii) The Secretary of Health and Human Services has not determined that Applicant did not act with due diligence, as defined in U.S.C. 156(d)(3), and in fact Applicant did act with the degree of attention, continuous directed effort, and timeliness reasonably expected from and exercised by a person during the regulatory review period. Therefore, no reduction in the number of days of the regulatory review period is appropriate.

(iii) The number of days remaining in the period defined by paragraph (c)(1) of this section after that period is reduced in accordance with paragraphs (d)(1)(i) and (ii) is 1122 days. One-half of this number is 561 days.

Thus, the number of days to be subtracted under 37 CFR 1.775(d)(1)(i) – (iii) is 2491 days. By subtracting this number of days (2491) from the number of days determined to be the Regulatory Review Period in accordance with paragraphs (c)(1) and (c)(2) of this section (4612), 2121 days remain.

- (2) By adding the number of days determined in paragraph (d)(1) of this section to the original term of the patent as shortened by any terminal disclaimer;**

The number of days determined in paragraph (d)(1) of this section is 2121 days. There is no terminal disclaimer for this patent and therefore no reduction in patent term extension under this subpart. With a term extension of 2121 days, the extension period for US 5,686,411 would expire September 1, 2020.

- (3) By adding 14 years to the date of the approval of the application under subsection 505 of the Federal Food, Drug, and Cosmetic Act;**

When 14 years is added to the date of the approval of the application under subsection 505 of the Federal Food, Drug, and Cosmetic Act, the date is March 16, 2019.

- (4) By comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) of this section with each other and selecting the earlier date;**

The earlier date calculated under paragraphs (d)(2) and (d)(3) of section 1.775 is March 16, 2019, under subsection (d)(3).

(5) If the original patent issued after September 24, 1984,

- (i) By adding 5 years to the original expiration date of the patent or any earlier date set by terminal disclaimer; and**
- (ii) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section with each other and selecting the earlier date;**

U.S. Patent No. 5,686,411 issued after September 24, 1984. When 5 years is added to the original expiration date of the patent, November 11, 2014, the date is November 11, 2019. By comparing this date to the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section, the earlier date is March 16, 2019.

U.S. Patent No. 5,686,411 issued after September 24, 1984. Therefore, section (d)(6) does not apply.

In summary, Applicant concludes that it is entitled to a period of extension for this patent to March 16, 2019.

- (13) A statement that applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

Applicant acknowledges a duty toward the Commissioner of Patent and Trademarks and the Secretary of Health and Human Services to disclose any information which is material to the determination of entitlement to the patent term extension sought.

- (14) The prescribed fee for receiving and acting upon the application for extension.

The Commissioner is hereby authorized to charge the \$1,120.00 fee under 37 CFR 1.20(j)(1) for receiving and acting upon the application for patent term extension to Applicant's Deposit Account No. 01-0535. Additionally, the Commissioner is authorized to charge any other fees related to this filing or credit any overpayment to Applicant's Deposit Account. A Fee Transmittal Form (PTO/SB/17) is enclosed for this purpose.

- (15) The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed.

Please direct all inquiries and correspondence relating to this application to:

Molly A. Holman, Ph.D., J.D.
Executive Director, Intellectual Property
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This Application is submitted together with two duplicate copies as required under 37 C.F.R. § 1.740(b) and two additional duplicate copies of the application pursuant to M.P.E.P. § 2753, for a total of four copies and one original.

A Return Receipt Postcard is also attached so that the Office can notify Applicant that this complete application has been received.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Molly A. Holman". The signature is fluid and cursive, with the first name "Molly" being the most prominent.

Molly A. Holman, Ph.D., J.D.
Registration No. 40,022

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EXHIBIT A

Power of Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant in re: Gaeta et al.)
)
Patent No.: 5,686,411)
)
Serial No: 08/447,849)
)
Issued: November 11, 1997)
)
Filed: May 23, 1995)
)
For: AMYLIN AGONIST PEPTIDES AND)
USES THEREFOR)

**Revocation of Prior Power of Attorney,
Appointment of New Attorneys of Record and
Change of Correspondence Address**

Mail Stop Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Amylin Pharmaceuticals, Inc. is the assignee of the entire right, title and interest in the above-identified patent by virtue of the assignment recorded on January 6, 1992 at the U.S. Patent and Trademark Office at Reel 5934, Frame 0449, and a name change recorded on July 16, 1997 at Reel 8635, Frame 0563. The undersigned, having express authority to represent Amylin Pharmaceuticals, Inc. as assignee, hereby revokes all powers of attorney heretofore given in the above-captioned application and appoints the attorneys listed below with full power of substitution, association, and revocation, to prosecute said application and to transact all business in the U.S. Patent and Trademark Office connected therewith.

Molly A. Holman, Reg. No. 40,022
James E. Butler, Reg. No. 40,931

Timothy Torchia, Reg. No. 36,700
Joanna L. Moore, Reg. No. 44,950

of Amylin Pharmaceuticals, Inc.

and

Practitioners at Arnold & Porter, Customer No. 44638

Please address future correspondence to:

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5 May 2005
Date



Lloyd A. Rowland
Vice President Legal and General Counsel
AMYLIN PHARMACEUTICALS, INC.

EXHIBIT B

U.S. Patent No. 5,686,411



US005686411A

United States Patent [19]

[11] Patent Number: 5,686,411

Gaeta et al.

[45] Date of Patent: Nov. 11, 1997

[54] AMYLIN AGONIST PEPTIDES AND USES THEREFOR

[75] Inventors: Laura S. L. Gaeta, Foster City; Howard Jones, Poway; Elisabeth Albrecht, San Diego, all of Calif.

[73] Assignee: Amylin Pharmaceuticals, Inc., San Diego, Calif.

[21] Appl. No.: 447,849

[22] Filed: May 23, 1995

Related U.S. Application Data

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

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

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

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

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Attorney, Agent, or Firm—Lyon & Lyon, L.L.P.

[57] ABSTRACT

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

45 Claims, 3 Drawing Sheets

FIGURE 1

¹Lys-Cys-Asn-Thr-⁵Ala-Thr-Cys-Ala-Thr-¹⁰Gln-Arg-Leu-Ala-
Asn-¹⁵Phe-Leu-Val-His-Ser-²⁰Ser-Asn-Asn-Phe-Gly-²⁵Ala-
Ile-Leu-Ser-Ser-³⁰Thr-Asn-Val-Gly-Ser-³⁵Asn-Thr-Tyr-NH₂

FIGURE 2

Amylin

human	KCNTATCATQRLANFLVHSSNFGAILSSTNVGSNTY-NH ₂
cat	-----IR---L---P-----
dog	-----RT---L---P-----
rat	-----R---L-PV-PP-----
mouse	-----R---L-PV-PP-----
hamster	-----N---L-PV--P-----
guinea pig	-----T---R--H-L--A-LP-D-----

FIGURE 3

¹A₁-X-Asn-Thr-⁵Ala-Thr-Y-Ala-Thr-¹⁰Gln-Arg-Leu-
B₁-Asn-¹⁵Phe-Leu-C₁-D₁-E₁-²⁰F₁-G₁-Asn-H₁-Gly-²⁵I₁-J₁-
Leu-K₁-L₁-³⁰Thr-M₁-Val-Gly-Ser-³⁵Asn-Thr-Tyr-Z

AMYLIN AGONIST PEPTIDES AND USES THEREFOR

This is a continuation of application Ser. No. 07794,266 filed on Nov. 19, 1991, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 077667,040 filed Mar. 8, 1991 (abandoned), which is hereby incorporated by reference.

BACKGROUND

1. Field of the Invention

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

2. Description of Related Art and Introduction to the Invention

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

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

Amyloid is the name given to extracellular deposits of β sheet protein filaments. Deposits of amyloid material have been reported to be found in pancreas of patients with Type 2 diabetes mellitus. Other studies have indicated that the degree of amyloid depositions increases with the degree of hyperglycemia in humans and the severity of Type 2 diabe-

tes. Chemical analysis of pancreatic amyloid led to the surprising and unexpected discovery of the peptide hormone, amylin. Clark, A., et al., *Lancet* ii: 231-234 (1987). This peptide was discovered to be comprised of 37 amino acids, none of which are acidic residues, to have a disulfide linkage between the cysteine residues at positions 2 and 7, and to be C-terminally amidated. Amylin is the major protein constituent of the amyloid which is reported to be found in the pancreatic Islets of Langerhans in patients with type 2 diabetes mellitus.

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

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

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

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

SUMMARY OF THE INVENTION

The present invention is directed to novel analogues of the peptide hormone amylin. These compounds mimic the effects of amylin, and are referred to as amylin agonists or as agonist analogues of amylin.

The invention is also directed to pharmaceutical compositions comprising the agonist analogues of the present invention, and to methods of treatment and prevention of hypoglycemic conditions and other conditions in which enhanced amylin action is of benefit, including insulin-requiring states such as diabetes mellitus, comprising administering an agonist analogue of amylin to an animal (alone or in conjunction with an insulin or a glucagon).

Definitions

As used herein, the following terms have the following meanings unless expressly stated to the contrary:

The term "alkyl" refers to both straight- and branched-chain alkyl groups. The term "lower alkyl" refers to both straight- and branched-chain alkyl groups having a total of from 1 to 6 carbon atoms and includes primary, secondary and tertiary alkyl groups. Typical lower alkyls include, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, n-pentyl, n-hexyl, and the like.

The term "aryl" refers to carbocyclic aromatic groups of 6 to 14 carbon atoms such as phenyl and naphthyl, as well as heterocyclic aromatic groups containing 1 to 3 heteroatoms (nitrogen, oxygen, sulfur, etc.) such as pyridyl, triazolopyrazine, pyrimidine and the like.

The term "aralkyl" refers to an "aryl" group of 6 to 10 carbon atoms directly attached to an "alkyl" group of 1 to 4 carbon atoms and includes for example benzyl, p-chlorobenzyl, p-methylbenzyl, and 2-phenylethyl.

The term "cycloalkyl" refers to cyclic alkyl groups of 5 to 8 carbon atoms.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the amino acid sequence of human amylin.

FIG. 2 depicts a comparison of amino acid sequences of amylin isolated from several mammals.

FIG. 3 depicts the amino acid sequence of novel amylin agonist peptides.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, novel agonist analogues of amylin are provided. These analogues are useful as agonists of amylin, including as hyperglycemics, and may be represented by FIG. 3.

In one aspect, the present invention is directed to agonist analogues of FIG. 3, wherein A₁ is hydrogen Lys, Ser, Ala, des-α-amino Lys, or acetylated Lys; B₁ is Ala, Ser or Thr; C₁ is Val, Leu or Ile; D₁ is His or Arg; E₁ is Ser or Thr; F₁ is Ser, Thr, Gln or Asn; G₁ is Asn, Gln or His; H₁ is Phe, Leu or Tyr; I₁ is Ala or Pro; J₁ is Ile, Val, Ala or Leu; K₁ is Ser, Pro, Leu, Ile or Thr; L₁ is Ser, Pro or Thr; M₁ is Asn, Asp or Gln; X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage; and Z is hydroxy, amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; provided that (a) when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is His, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Phe, I₁ is Ala, J₁ is Ile, K₁ is Ser, L₁ is Ser, and M₁ is Asn; (b) when A₁ is Lys, B₁ is Ala, C₁ is Ile, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Ala, J₁ is Ile, K₁ is Ser, L₁ is Pro, and M₁ is Asn; (c)

when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Thr, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Ala, J₁ is Ile, K₁ is Ser, L₁ is Pro, and M₁ is Asn; (d) when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Pro, J₁ is Val, K₁ is Pro, L₁ is Pro, and M₁ is Asn; (e) when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is His, E₁ is Ser, F₁ is Asn, G₁ is Asn, H₁ is Leu, I₁ is Pro, J₁ is Val, K₁ is Ser, L₁ is Pro and M₁ is Asn; or (f) when A₁ is Lys, B₁ is Thr, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is His, H₁ is Leu, I₁ is Ala, J₁ is Ala, K₁ is Leu, L₁ is Pro and M₁ is Asp; then one or more of any of A₁ to M₁ is not an L-amino acid and Z is not amino.

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. Preferred alkyl chains include lower alkyl groups having from about 1 to about 6 carbon atoms.

An additional aspect of the present invention is directed to agonist analogues of FIG. 3 which are not bridged, and wherein X and Y are independently selected from Ala, Ser, Cys, Val, Leu and Ile or alkyl, aryl, or aralkyl esters and ethers of Ser or Cys.

Biologically active derivatives of the above FIG. 3 agonist analogues 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 agonist analogues modified by glycosylation of Asn, Ser and/or Thr residues.

Biologically active agonist analogues of amylin are included within the scope of this invention which contain less peptide character. 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—), β-enaminonitriles (—C(=CH—CN)—NH—), thioamides (—CS—NH—), thiomethylenes (—S—CH₂— or —CH₂—S—), methylenes (—CH₂—C₂—) and retro-amides (—NH—CO—).

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 preferred.

The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

The compounds of the invention include various stereoisomers. In the preferred compounds of this invention, the chiral centers on the peptide backbone are all S.

Compounds of the present invention may be prepared by using certain conventional coupling reactions known in the

peptide art. The analogues of this invention are prepared by successively adding the desired amino acid to a growing peptide chain. Typically, an α -N-carbamoyl protected amino acid and an amino acid attached to the growing peptide chain on a resin support are reacted at room temperature in an inert solvent such as N-methylpyrrolidone, dimethylformamide or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide 1-hydroxybenzotriazole in the presence of a base such as diisopropylethylamine. The α -N-carbamoyl protecting group is removed from the resultant peptide with a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired N-protected amino acid. Suitable N-protecting groups are known in the art, with t-butyloxycarbonyl herein preferred.

Certain preferred methods for synthesis are described in the commonly-assigned copending and commonly assigned patent application Ser. No. 667,040 ("Synthetic Preparation of Amylin and Amylin Analogs", filed Mar. 8, 1991). These methods provide for solid phase synthesis of a peptide which comprises amylin or an amylin analog which has enhanced biological activity and is substantially free of deletion and other contaminating peptides wherein said peptide is synthesized using successive synthesis cycles, whereby in each such synthesis cycle, a designated amino acid is added to a growing peptide chain attached to an insoluble resin support by formation of a peptide linkage between an α -amino group of the growing peptide chain and on α -carboxyl of the designated amino acid; and wherein each synthesis cycle comprises: (a) treating the growing peptide chain under α -amino deprotecting conditions to remove an α -amino group; (b) activating the α -carboxyl group of the α -amino protected designated amino acid; (c) contacting the growing peptide chain and the designated amino acid under coupling conditions to form a peptide linkage between the free α -amino for the peptide chain and the activated α -carboxyl of the designated amino acid; and (d) repeating steps (b) and (c) if the coupling efficiency of step (c) is less than about 97%. It is preferred to repeat steps (b) and (c) if the coupling efficiency is less than about 99%. In another preferred aspect, steps (b) and (c) are repeated in each synthesis cycle. Optionally, the coupling efficiency is measured after each coupling step.

Suitable coupling conditions include use of a solvent system which maximizes swelling of the solid support, minimizes secondary structure elements of the peptide chain during synthesis cycles, and minimizes intrapeptide and interpeptide hydrogen bonding. Preferably the synthesis cycle includes a capping step after the coupling step(s) wherein unreacted α -amino groups of the peptide chain are rendered unreactive. The synthesis cycle is successively repeated using appropriate protected α -amino acids to give amylin or an amylin analog of specified sequence. After completions of the successive synthesis cycles, said amylin or amylin analog is cleaved from the solid support. It is preferred that the cysteine residues of the peptide chain are selectively deprotected and an intramolecular disulfide bond is formed before cleaving the peptide bond from the solid support.

Suitable α -amino protective groups include t-butyloxycarbonyl and 9-fluorenylmethoxycarbonyl. In one preferred aspect, when t-butyloxycarbonyl is used as the α -amino protecting group, the α -carboxyl groups are activated using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to form 1-hydroxybenzotriazole esters. A particularly preferred solvent system comprise N-methylpyrrolidone.

The preparation of certain agonist analogues of amylin within the invention is described in Examples 1 to 17 herein. In addition, other agonist analogues which may be prepared according to the above procedures are set forth in Table II herein. The compounds of the invention may also be prepared using recombinant DNA techniques, using methods now known in the art. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d Ed., Cold Spring Harbor (1989).

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

The agonist analogues of amylin of this invention are useful in view of their pharmacological properties. In particular, compounds of this invention possess activity as amylin agonist agents, as will be evidenced by activity in the receptor binding assay and the soleus muscle assay described in Examples 18 and 19, respectively. Amylin agonist activity of compounds may also be assessed by the ability to induce hyperlactemia and/or hyperglycemia in mammals. In addition to the description of compounds pursuant to FIG. 3, certain preferred compounds are set forth in Table I. The preferred compounds des-¹Lys-h-amylin, ²⁸Pro-h-amylin, ^{25,28,29}Pro-h-amylin, ¹⁸Arg^{25,28}Pro-h-amylin, and des-¹Lys¹⁸Arg^{25,28}Pro-h-amylin, all show amylin activity in vivo in treated test animals, provoking marked hyperlactemia followed by hyperglycemia. In addition to having activities characteristic of amylin, certain of the preferred compounds of the invention have also been found to possess more desirable solubility and stability characteristics when compared to human amylin. These preferred compounds include ²⁵Pro²⁶Val^{28,29}Pro-h-amylin, ^{25,28,29}Pro-h-amylin, and ¹⁸Arg^{25,28}Pro-h-amylin.

Compounds described herein which are especially preferred include ¹⁸Arg^{25,28}Pro-h-amylin, des-¹Lys¹⁸Arg^{25,28}Pro-h-amylin, ¹⁸Arg^{25,28,29}Pro-h-amylin, des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin, ^{25,28,29}Pro-h-amylin, des-¹Lys^{25,28,29}Pro-h-amylin, and ²⁵Pro²⁶Val^{28,29}Pro-h-amylin. Still further amylin agonist peptide compounds are listed in Table II. They include:

- ²³Leu²⁵Pro²⁶Val^{28,29}Pro-h-amylin;
- ²³Leu²⁵Pro²⁶Val²⁸Pro-h-amylin;
- des-¹Lys²³Leu²⁵Pro²⁶Val²⁸Pro-h-amylin;
- ¹⁸Arg²³Leu²⁵Pro²⁶Val²⁸Pro-h-amylin;
- ¹⁸Arg²³Leu^{25,28,29}Pro-h-amylin;
- ¹⁸Arg²³Leu^{25,28}Pro-h-amylin;
- ¹⁷Ile²³Leu^{25,28,29}Pro-h-amylin;
- ¹⁷Ile^{25,28,29}Pro-h-amylin;
- des-¹Lys¹⁷Ile²³Leu^{25,28,29}Pro-h-amylin;
- ¹⁷Ile¹⁸Arg²³Leu-h-amylin;
- ¹⁷Ile¹⁸Arg²³Leu²⁶Val²⁹Pro-h-amylin;
- ¹⁷Ile¹⁸Arg²³Leu²⁵Pro²⁶Val^{28,29}Pro-h-amylin;

¹³Thr²¹His²³Leu²⁶Ala²⁸Leu²⁹Pro³¹Asp-h-amylin;
¹³Thr²¹His²³Leu²⁶Ala²⁸Pro³¹Asp-h-amylin;
 des-¹Lys¹³Thr²¹His²³Leu²⁶Ala²⁸Pro³¹Asp-h-amylin;
¹³Thr¹⁸Arg²¹His²³Leu²⁶Ala²⁸Pro³¹Asp-h-amylin;
¹³Thr¹⁸Arg²¹His²³Leu^{28,29}Pro³¹Asp-h-amylin; and
¹³Thr¹⁸Arg²¹His²³Leu²⁵Pro²⁶Ala^{28,29}Pro³¹Asp-h-amylin.

The compounds of this invention can be combined with pharmaceutical carriers to prepare pharmaceutical forms suitable for parenteral administration. Experimental responses of the compounds support the clinical application of such pharmaceutical compositions in the treatment of diabetes mellitus and other insulin-requiring states, as well as in the prevention and treatment of episodes of hypoglycemia. The compounds of this invention can also be combined with insulin for the treatment of diabetes mellitus and other insulin-requiring states. By "insulin" is meant a polypeptide or its equivalent useful in regulation of blood glucose levels. A general description of such insulins is provided in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press (1990). Such insulins can be fast acting, intermediate acting, or long acting. Various derivatives of insulin exist and are useful in this invention. See, e.g., U.S. Pat. Nos. 5,049,547, 5,028, 587, and 5,016,643. Insulin peptides are also useful (see, e.g., U.S. Pat. No. 5,008,241), as are analogues (see, e.g., U.S. Pat. Nos. 4,992,417 and 4,992,418). Such compositions can be administered by any standard route, including nasal administration (see, e.g., U.S. Pat. Nos. 4,988,512 and 4,985,242, and 2 *BioWorld Today*, No. 125 (1991)). The compounds of this invention are also useful in combination with a glucagon for the prevention and treatment of hypoglycemia. See Young et al., U.S. application Ser. No. 07/640,478, filed Jan. 10, 1991, entitled "Hyperglycemic Compositions," which is incorporated herein by reference.

Compositions or products of the invention may conveniently be provided in the form of solutions suitable for parenteral (including intravenous, intramuscular and subcutaneous) or nasal or oral administration. In many cases, it will be convenient to provide an agonist analogue of amylin and an insulin or glucagon in a single composition or solution for administration together. In other cases, it may be more advantageous to administer an insulin or a glucagon separately from said agonist analogue. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., *Remington's Pharmaceutical Sciences* by E. W. Martin. See also Wang, Y. J. and Hanson, M. A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," *Journal of Parenteral Science and Technology*, Technical Report No. 10, Supp. 42:2S (1988). Suitable formulations including insulin or glucagon are known in the art.

The agonist preparations of the invention may be stabilized at neutral pH. Since the products of the invention are amphoteric they may be utilized as free bases, as acid addition salts or as metal salts. The salts must, of course, be pharmaceutically acceptable, and these will include metal salts, particularly alkali and alkaline earth metal salts, e.g., potassium or sodium salts. A wide variety of pharmaceutically acceptable acid addition salts are available, as described above. These include those prepared from both organic and inorganic acids, preferably mineral acids. Typical acids which may be mentioned by way of example include citric, succinic, lactic, hydrochloric and hydrobro-

mic acids. Such products are readily prepared by procedures well known to those skilled in the art.

The products of the invention will normally be provided as parenteral compositions for injection or infusion. They can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, or olive oil. Alternatively, they can be suspended in an aqueous isotonic buffer solution at a pH of about 5.6 to 7.4. Useful buffers include sodium citrate-citric acid and sodium phosphate-phosphoric acid. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection.

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

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

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

For use by the physician, the compositions will be provided in dosage unit form containing an amount of an agonist compound with or without insulin or glucagon which will be effective in one or multiple doses to control or reestablish blood sugar at the selected level. Therapeutically effective amounts of an agonist analogue of amylin as described herein for the treatment of hypoglycemia are those that increase blood sugar levels, preferably to above 80 mg/dl. Therapeutically effective amounts of such agonist analogues for the treatment of diabetes mellitus and other insulin-requiring states are those sufficient to provide for reduced incidence of insulin overdose or undesired hypoglycemia. 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 blood sugar level to be obtained, and other factors. Typical dosage units for treatment of diabetes mellitus will contain from about 0.1 to 5 mg of an amylin agonist compound and, if desired, about 0.5 to about 10 mg of an insulin. Typical dosage units for the treatment of hypoglycemia will contain about 0.5 to 1.0 mg of an amylin agonist compound and, if desired, the art recognized quantity, or less, of a glucagon.

As set forth above, compositions useful in the invention are formulated by standard procedure. These compositions are also administered by standard procedure. Suitable doses are readily determined by those in the art, examples of which are provided above.

To assist in understanding the present invention, the following examples are included which describe the results of a series of experiments. The following examples relating

to this invention should not, of course, be construed as specifically limiting the invention. Such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the present invention as hereinafter claimed.

EXAMPLES

Example 1

Preparation of ²⁸Pro-human-Amylin

Solid phase synthesis of this analogue of human ("h-") amylin using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment of Ac_m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid hydrofluoric acid ("HF") in the presence of dimethylsulfide and anisole. The ²⁸Pro-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+1)⁺=3914.

Example 2

Preparation of ²⁵Pro²⁶Val^{28,29}Pro-h-Amylin

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ²⁵Pro²⁶Val^{28,29}Pro-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+1)⁺=3936.

Example 3

Preparation of ^{2,7}Cyclo-[²Asp,⁷Lys]-h-Amylin

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. ²Asp and ⁷Lys were introduced with Boc-²Asp(Fmoc)-OH and Boc-⁷Lys(Fmoc)-OH. Following selective side-chain deprotection with piperidine the side-chain to side-chain (²Asp-⁷Lys) cyclization was carried out using benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent). Cyclization was as described in Di Maio, J., et al., *J. Med. Chem.* 33:661-667 (1990); Felix, A. M., et al., *Int J. Pept. Prot. Res.* 32:441 (1988). The ^{2,7}cyclo-[²Asp,⁷Lys] amylin-MBHA-resin obtained after cyclization was cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{2,7}cyclo-[²Asp,⁷Lys]-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the struc-

ture confirmed by amino acid analysis and sequence analysis. FAB mass spec: (M+1)⁺=3925.

Example 4

Preparation of des-¹Lys-h-Amylin

Solid phase synthesis of des-¹Lys-h-amylin (also represented as ²⁻³⁷h-amylin) using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment of Ac_m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,775.

Example 5

Preparation of ¹Ala-h-Amylin

Solid phase synthesis of ¹Ala-h-amylin using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment of Ac_m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ¹Ala-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,847.

Example 6

Preparation of ¹Ser-h-Amylin

Solid phase synthesis of ¹Ser-h-amylin using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment of Ac_m-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ¹Ser-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,863.

Example 7

Preparation of ²⁹Pro-h-Amylin

Solid phase synthesis of this analogue of human amylin using methylbenzhydrylamine anchor-bond resin and N^α-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment of Ac_m-

protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ²⁵Pro-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3916.

Example 8

Preparation of ^{25,28}Pro-h-Amylin

Solid phase synthesis of ^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^ε-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide]amylin-MBHA-resin was obtained by treatment of AcM-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,939.

Example 9

Preparation of des-¹Lys^{25,28}Pro-h-Amylin

Solid phase synthesis of des-¹Lys^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^ε-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide]amylin-MBHA-resin was obtained by treatment of AcM-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,811.

Example 10

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

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

Example 11

Preparation of des-¹Lys¹⁸Arg^{25,28}Pro-h-Amylin

Solid phase synthesis of des-¹Lys¹⁸Arg^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and

N^ε-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide]amylin-MBHA-resin was obtained by treatment of AcM-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys¹⁸Arg^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,832.

Example 12

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

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

Example 13

Preparation of des-¹Lys¹⁸Arg^{25,28,29}Pro-h-Amylin

Solid phase synthesis of des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^ε-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide]amylin-MBHA-resin was obtained by treatment of AcM-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+H)⁺=3,843.

Example 14

Preparation of ^{25,28,29}Pro-h-Amylin

Solid phase synthesis of ^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^ε-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The 2,7-[disulfide]amylin-MBHA-resin was obtained by treatment of AcM-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and

capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^+=3,949$.

Example 15

Preparation of des-¹Lys^{25,28,29}Pro-h-Amylin

Solid phase synthesis of des-¹Lys^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N²-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide] amylin-MBHA-resin was obtained by treatment of Acn-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^+=3,823$.

Example 16

Preparation of des-¹Lys²⁵Pro²⁶Val^{28,29}-Pro-h-Amylin

Solid phase synthesis of this h-amylin analogue using methylbenzhydrylamine anchor-bond resin and N²-Boc/benzyl-side chain protection is carried out by standard peptide synthesis methods, and the ^{2,7}-[disulfide]amylin-MBHA-resin obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization is achieved the resin and side chain protecting groups are cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-¹Lys²⁵Pro²⁶Val^{28,29}Pro-h-amylin is then purified by preparative HPLC.

Example 17

Preparation of [(D)-¹⁴Arg]-Amylin

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N²-Boc/benzyl-side chain protection is carried out by standard peptide synthesis methods. (D)-¹⁴Arg is introduced with Boc-(D)-¹⁴Arg(Mtr)-OH. The ^{2,7}-[disulfide]amylin-MBHA-resin, obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid, is cyclized and the resin and side chain protecting groups are cleaved with liquid HF in the presence of dimethylsulfide and anisole. The [(D)-¹⁴Arg]-amylin is then purified by preparative HPLC.

Example 18

Receptor Binding Assay

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

Male Sprague-Dawley rats (200-250) grams were sacrificed by decapitation. Brains were removed to cold phosphate-buffered saline (PBS). From the ventral surface,

cuts were made rostral to the hypothalamus, bounded laterally by the olfactory tracts and extending at a 45° angle medially from these tracts. This basal forebrain tissue, containing the nucleus accumbens and surrounding regions, was weighed and homogenized in ice-cold 20 mM HEPES buffer (20 mM HEPES acid, pH adjusted to 7.4 with NaOH at 23° C.). Membranes were washed three times in fresh buffer by centrifugation for 15 minutes at 48,000×g. The final membrane pellet was resuspended in 20 mM HEPES buffer containing 0.2 mM phenylmethylsulfonyl fluoride (PMSF).

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

In this assay, purified human amylin binds to its receptor at a measured IC₅₀ of about 50 pM. Results for test compounds of the invention are set forth in Table I, showing that each of the compounds has significant receptor binding activity.

Example 19

Soleus Muscle Assay

Evaluation of the amylin agonist activity of compounds of the invention was carried out using the soleus muscle assay as follows. Male Harlan Sprague-Dawley rats of approximately 200 g mass were used in order to maintain mass of the split soleus muscle less than 40 mg. The animals were fasted for 4 hours prior to sacrifice by decapitation. The skin was stripped from the lower limb which was then pinned out on corkboard. The *tendo achilles* was cut just above *os calcis* and *m. gastrocnemius* reflected out from the posterior aspect of the tibia. *M. soleus*, a small 15-20 mm long, 0.5 mm thick flat muscle on the bone surface of *m. gastrocnemius* was then stripped clear and the perimysium cleaned off using fine scissors and forceps. *M. soleus* was then split into equal parts using a blade passed antero-posteriorly through the belly of the muscle to obtain a total of 4 muscle strips from each animal. After dissecting the muscle from the animal, it was kept for a short period in physiological saline. It was not necessary that the muscle be held under tension as this had no demonstrable effects on radioglucose incorporation into glycogen.

Muscles were added to 50 mL Erlenmeyer flasks containing 10 mL of a pregassed Krebs-Ringer bicarbonate buffer containing (each liter) NaCl 118.5 mmol (6.93 g), KCl 5.94 mmol (443 mg), CaCl₂ 2.54 mmol (282 mg), MgSO₄ 1.19 mmol (143 mg), KH₂PO₄ 1.19 mmol (162 mg), NaHCO₃ 25 mmol (2.1 g), 5.5 mmol glucose (1 g) and recombinant human insulin (Humulin-R, Eli Lilly, Indiana) and the test compound, as detailed below. pH at 37° C. was verified as

being between 7.1 and 7.4. Muscles were assigned to different flasks so that the 4 muscle pieces from each animal were evenly distributed among the different assay conditions. The incubation media were gassed by gently blowing carbogen (95% O₂, 5% CO₂) over the surface while being continuously agitated at 37° C. in an oscillating water bath. After a half-hour "preincubation" period, 0.5 μCi of U-¹⁴C-glucose was added to each flask which was incubated for a further 60 minutes. Each muscle piece was then rapidly removed, blotted and frozen in liquid N₂, weighed and stored for subsequent determination of ¹⁴C-glycogen.

¹⁴C-glycogen determination was performed in a 7 mL scintillation vial. Each frozen muscle specimen was placed in a vial and digested in 1 mL 60% potassium hydroxide at 70° C. for 45 minutes under continuous agitation. Dissolved glycogen was precipitated out onto the vial by the addition of 3 mL absolute ethanol and overnight cooling at -20° C. The supernatant was gently aspirated, the glycogen washed again with ethanol, aspirated and the precipitate dried under vacuum. All ethanol is evaporated to avoid quenching during scintillation counting. The remaining glycogen was redissolved in 1 mL water and 4 mL scintillation fluid and counted for ¹⁴C.

The rate of glucose incorporation into glycogen (expressed in μmol/g/hr) was obtained from the specific activity of ¹⁴C-glucose in the 5.5 mM glucose of the incubation medium, and the total ¹⁴C counts remaining in the glycogen extracted from each muscle. Dose/response curves were fitted to a 4-parameter logistic model using a least-squares iterative routine (ALLFIT, v2.7, NIH, Maryland) to derive EC₅₀'s. Since EC₅₀ is log-normally distributed, it is expressed ± standard error of the logarithm. Pairwise comparisons were performed using t-test based routines of SYSTAT (Wilkinson, "SYSTAT: the system for statistics," SYSTAT Inc., Evanston Ill. (1989)).

some commercial preparations which are less than 90% pure have higher EC₅₀'s due to the presence of contaminants that result in a lower measured activity. Results for test compounds are set forth in Table I, showing that each of the compounds has amylin activity.

TABLE I

	Receptor Binding	
	Assay EC ₅₀ (pM)	Soleus Muscle Assay EC ₅₀ (nM)
1) ²⁸ Pro-h-Amylin	15.0	2.64
2) ²⁸ Pro ²⁶ Val ^{28,29} Pro-h-Amylin	18.0	4.68
3) ^{2,7} Cyclo-[² Asp, ⁷ Lys]-h-Amylin	310.0	6.62
4) ²⁻²⁷ h-Amylin	236.0	1.63
5) ¹ Ala-h-Amylin	148.0	12.78
6) ¹ Ser-h-Amylin	33.0	8.70
7) ²⁸ Pro-h-Amylin	64.0	3.78
8) ^{25,28} Pro-h-Amylin	26.0	13.20
9) des- ¹ Lys ^{25,28} Pro-h-Amylin	85.0	7.70
10) ¹⁸ Arg ^{25,28} Pro-h-Amylin	32.0	2.83
11) des- ¹ Lys ¹⁸ Arg ^{25,28} Pro-h-Amylin	82.0	3.77
12) ¹⁸ Arg ^{25,28,29} Pro-h-Amylin	21.0	1.25
13) des- ¹ Lys ¹⁸ Arg ^{25,28,29} Pro-h-Amylin	21.0	1.86
14) ^{25,28,29} Pro-h-Amylin	10.0	3.71
15) des- ¹ Lys ^{25,28,29} Pro-h-Amylin	14.0	4.15

TABLE II

	A ₁	B ₁	C ₁	D ₁	E ₁	F ₁	G ₁	H ₁	I ₁	J ₁	K ₁	L ₁	M ₁	Z
16) Lys	Ala	Val	His	Ser	Ser	Asn	Leu	Pro	Val	Pro	Pro	Asn	-NH ₂	
17) Lys	Ala	Val	His	Ser	Ser	Asn	Leu	Pro	Val	Pro	Ser	Asn	-NH ₂	
18) Hydrogen	Ala	Val	His	Ser	Ser	Asn	Leu	Pro	Val	Pro	Ser	Asn	-NH ₂	
19) Lys	Ala	Val	Arg	Ser	Ser	Asn	Leu	Pro	Val	Pro	Ser	Asn	-NH ₂	
20) Lys	Ala	Val	Arg	Ser	Ser	Asn	Leu	Pro	Ile	Pro	Pro	Asn	-NH ₂	
21) Lys	Ala	Val	Arg	Ser	Ser	Asn	Leu	Pro	Ile	Pro	Ser	Asn	-NH ₂	
22) Lys	Ala	Ile	His	Ser	Ser	Asn	Leu	Pro	Ile	Pro	Pro	Asn	-NH ₂	
23) Lys	Ala	Ile	His	Ser	Ser	Asn	Phe	Pro	Ile	Pro	Pro	Asn	-NH ₂	
24) Hydrogen	Ala	Ile	His	Ser	Ser	Asn	Leu	Pro	Ile	Pro	Pro	Asn	-NH ₂	
25) Lys	Ala	Ile	Arg	Ser	Ser	Asn	Leu	Ala	Ile	Ser	Ser	Asn	-NH ₂	
26) Lys	Ala	Ile	Arg	Ser	Ser	Asn	Leu	Ala	Val	Ser	Pro	Asn	-NH ₂	
27) Lys	Ala	Ile	Arg	Ser	Ser	Asn	Leu	Pro	Val	Pro	Pro	Asn	-NH ₂	
28) Lys	Thr	Val	His	Ser	Ser	His	Leu	Ala	Ala	Leu	Pro	Asp	-NH ₂	
29) Lys	Thr	Val	His	Ser	Ser	His	Leu	Ala	Ala	Ser	Pro	Asp	-NH ₂	
30) Hydrogen	Thr	Val	His	Ser	Ser	His	Leu	Ala	Ala	Pro	Ser	Asp	-NH ₂	
31) Lys	Thr	Val	Arg	Ser	Ser	His	Leu	Ala	Ala	Ser	Pro	Asp	-NH ₂	
32) Lys	Thr	Val	Arg	Ser	Ser	His	Leu	Ala	Ile	Pro	Pro	Asp	-NH ₂	
33) Lys	Thr	Val	Arg	Ser	Ser	His	Leu	Pro	Ala	Pro	Pro	Asp	-NH ₂	

Dose response curves were generated with muscles added to media containing 7.1 nM (1000 μU/mL) insulin and each test compound added at final (nominal) concentrations of 0, 1, 3, 10, 30, 100, 300 and 1000 nM. Each assay also contained internal positive controls consisting of a single batch of archived rat amylin, lyophilized and stored at -70° C.

Human amylin is a known hyperglycemic peptide, and EC₅₀ measurements of amylin preparations in the soleus muscle assay range typically from about 1-10 nM, although

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 33

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
1          5          10          15
Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Pro Ser Thr
          20          25          30
Asn Val Gly Ser Asn Thr Tyr
          35

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acids
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
1          5          10          15
Leu Val His Ser Ser Asn Asn Phe Gly Pro Val Leu Pro Pro Thr
          20          25          30
Asn Val Gly Ser Asn Thr Tyr
          35

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Lys Asp Asn Thr Ala Thr Lys Ala Thr Gln Arg Leu Ala Asn Phe
1          5          10          15
Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr
          20          25          30
Asn Val Gly Ser Asn Thr Tyr
          35

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 1 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn
 20 25 30
 Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 1 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
 20 25 30
 Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
 1 5 10 15
 Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr
 20 25 30
 Asn Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
 1 5 10 15
 Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Pro Thr
 20 25 30
 Asn Val Gly Ser Asn Thr Tyr
 35

-continued

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
 1 5 10 15
 Leu Val His Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Ser Thr
 20 25 30
 Asn Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 36 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 1 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Ser Thr Asn
 20 25 30
 Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
 1 5 10 15
 Leu Val Arg Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Ser Thr
 20 25 30
 Asn Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 36 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

-continued

Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Arg Asn Phe Leu
 1 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Ser Thr Asn
 20 25 30
 Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
 1 5 10 15
 Leu Val Arg Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Pro Thr
 20 25 30
 Asn Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 1 5 10 15
 Val Arg Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Pro Thr Asn
 20 25 30
 Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 1 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Pro Thr Asn Val
 20 25 30
 Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids

-continued

- (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 1 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Pro Ile Leu Pro Pro Thr Asn
 20 25 30
 Val Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Lys Ala Val His Ser Ser Asn Leu Pro Val Pro Pro Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Lys Ala Val His Ser Ser Asn Leu Pro Val Pro Ser Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Val His Ser Ser Asn Leu Pro Val Pro Ser Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Lys Ala Val Arg Ser Ser Asn Leu Pro Val Pro Ser Asn
 1 5 10

-continued

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Lys Ala Val Arg Ser Ser Asn Leu Pro Ile Pro Pro Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Lys Ala Val Arg Ser Ser Asn Leu Pro Ile Pro Ser Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Lys Ala Ile His Ser Ser Asn Leu Pro Ile Pro Pro Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Ala Ile His Ser Ser Asn Phe Pro Ile Pro Pro Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ala Ile His Ser Ser Asn Leu Pro Ile Pro Pro Asn
 1 5 10

-continued

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Thr Val His Ser Ser His Leu Ala Ala Pro Ser Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Lys Thr Val Arg Ser Ser His Leu Ala Ala Ser Pro Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Lys Thr Val Arg Ser Ser His Leu Ala Ile Pro Pro Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Lys Thr Val Arg Ser Ser His Leu Pro Ala Pro Pro Asp
 1 5 10

55

We claim:

1. An agonist analogue of amylin having the amino acid sequence:

¹A₁-X-Asn-Thr-²Ala-Thr-Y-Ala-Thr-¹⁰Gln-Arg-
 60 Leu-B₁-Asn-¹⁵Phe-Leu-C₁-D₁-E₁-²⁰P₁-G₁-
 Asn-H₁-Gly-²⁵Pro-I₁-Leu-Pro-I₂-³⁰Thr-K₁-
 Val-Gly-Ser-³⁵Asn-Thr-Tyr-Z

wherein

A₁ is Lys, Ala, Ser or hydrogen;
 B₁ is Ala, Ser or Thr;
 C₁ is Val, Leu or Ile;

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

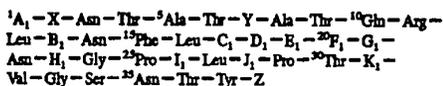
65 X and Y are independently selected amino acid residues having side chains which are chemically bonded to each other to form an intramolecular linkage, wherein said

intramolecular linkage is a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; and provided that when A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is Arg, E₁ is Ser, F₁ is Ser, G₁ is Asn, H₁ is Leu, I₁ is Val, J₁ is Pro, and K₁ is Asn; then one or more of A₁ to K₁ is a D-amino acid and Z is selected from the group consisting of alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy.

2. An agonist analogue of amylin according to claim 1 wherein X and Y are Cys residues linked by a disulfide bond.

3. An agonist analogue of amylin according to claim 2 wherein Z is amino.

4. An agonist analogue of amylin having the amino acid sequence:



wherein

A₁ is Lys, Ala, Ser or hydrogen;

B₁ is Ala, Ser or Thr;

C₁ is Val, Leu or Ile;

D₁ is His or Arg;

E₁ is Ser or Thr;

F₁ is Ser, Thr, Gln or Asn;

G₁ is Asn, Gln or His;

H₁ is Phe, Leu or Tyr;

I₁ is Ile, Val, Ala or Leu;

J₁ is Ser, Pro, Leu, Ile or Thr;

K₁ is Asn, 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, wherein said intramolecular linkage is a disulfide bond, a lactam or a thioether linkage; and Z is amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; and provided that when

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

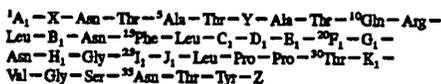
(b) A₁ is Lys, B₁ is Ala, C₁ is Val, D₁ is His, E₁ is Ser, F₁ is Asn, G₁ is Asn, H₁ is Leu, I₁ is Val, J₁ is Ser and K₁ is Asn;

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

5. An agonist analogue of amylin according to claim 4 wherein X and Y are Cys residues linked by a disulfide bond.

6. An agonist analogue of amylin according to claim 5 wherein Z is amino.

7. An agonist analogue of amylin having the amino acid sequence:



wherein

A₁ is Lys, Ala, Ser or hydrogen;

B₁ is Ala, Ser or Thr;

C₁ is Val, Leu or Ile;

D₁ is His or Arg;

E₁ is Ser or Thr;

F₁ is Ser, Thr, Gln or Asn;

G₁ is Asn, Gln or His;

H₁ is Phe, Leu or Tyr;

I₁ is Ala or Pro;

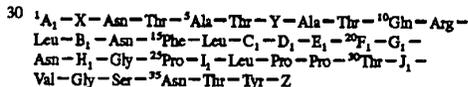
J₁ is Ile, Val, Ala or Leu;

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

8. An agonist analogue of amylin according to claim 7 wherein X and Y are Cys residues linked by a disulfide bond.

9. An agonist analogue of amylin according to claim 8 wherein Z is amino.

10. An agonist analogue of amylin having the amino acid sequence:



wherein

A₁ is Lys, Ala, Ser or hydrogen;

B₁ is Ala, Ser or Thr;

C₁ is Val, Leu or Ile;

D₁ is His or Arg;

E₁ is Ser or Thr;

F₁ is Ser, Thr, Gln or Asn;

G₁ is Asn, Gln or His;

H₁ is Phe, Leu or Tyr;

I₁ is Ile, Val, Ala or Leu;

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

11. An agonist analogue of amylin according to claim 10 wherein X and Y are Cys residues linked by a disulfide bond.

12. An agonist analogue of amylin according to claim 11 wherein Z is amino.

13. An agonist analogue of amylin according to any of claims 1-12 wherein D₁ is Arg.

14. An agonist analogue of amylin according to any of claims 1-6 or 10-12 wherein I₁ is Val.

15. An agonist analogue of amylin according to any of claims 7-9 where J₁ is Val.
16. An agonist analogue of amylin according to any of claims 1-12 wherein A₁ is hydrogen.
17. ¹⁸Arg^{25,28,29}Pro-h-amylin.
18. des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin.
19. ^{25,28,29}Pro-h-amylin.
20. des-¹Lys^{25,28,29}Pro-h-amylin.
21. ¹⁸Arg^{25,28,29}Pro-h-amylin.
22. des-¹Lys¹⁸Arg^{25,28,29}Pro-h-amylin.
23. ²⁵Pro²⁸Val^{28,29}Pro-h-amylin.
24. An agonist analogue of amylin according to any of claims 1-16 which is an acetate salt.
25. An agonist analogue of amylin according to any of claims 1-16 which is a trifluoroacetate salt.
26. An agonist analogue of amylin according to any of claims 1-16 which is a hydrochloride salt.
27. A compound according to any of claims 17-23 which is an acetate salt.
28. A compound according to any of claims 17-23 which is a trifluoroacetate salt.
29. A compound according to any of claims 17-23 which is a hydrochloride salt.
30. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of an agonist analogue of amylin according to claim 3.
31. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of an agonist analogue of amylin according to claim 6.
32. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of an agonist analogue of amylin according to claim 9.
33. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of an agonist analogue of amylin according to claim 12.
34. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of

- a therapeutically effective amount of an agonist analogue of amylin according to any of claims 17-23.
35. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of ^{25,28,29}Pro-h-amylin.
36. The method of any of claims 30-35 further comprising the administration to said mammal of a therapeutically effective amount of an insulin.
37. A method for the treatment of diabetes mellitus in a mammal comprising the administration to said mammal of a therapeutically effective amount of ^{25,28,29}Pro-h-amylin and a therapeutically effective amount of an insulin.
38. A composition comprising a therapeutically effective amount of an agonist analogue of amylin according to claim 3 in a pharmaceutically acceptable carrier.
39. A composition comprising a therapeutically effective amount of an agonist analogue of amylin according to claim 6 in a pharmaceutically acceptable carrier.
40. A composition comprising a therapeutically effective amount of an agonist analogue of amylin according to claim 9 in a pharmaceutically acceptable carrier.
41. A composition comprising a therapeutically effective amount of an agonist analogue of amylin according to claim 12 in a pharmaceutically acceptable carrier.
42. A composition comprising a therapeutically effective amount of an agonist analogue of amylin according to any of claims 17-23 in a pharmaceutically acceptable carrier.
43. A composition comprising a therapeutically effective amount of ^{25,28,29}Pro-h-amylin in a pharmaceutically acceptable carrier.
44. A composition comprising a therapeutically effective amount of an agonist analogue of amylin according to any of claims 3, 6, 9, 11 and 17-23 and an insulin admixed in a pharmaceutically acceptable carrier.
45. A composition comprising a therapeutically effective amount of ^{25,28,29}Pro-h-amylin and an insulin admixed in a pharmaceutically acceptable carrier.

* * * * *

EXHIBIT C

**Certificate of Correction
Issued on U.S. Patent No. 5,686,411**

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,686,411
DATED : November 11, 1997
INVENTOR(S) : Gaeta et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 34,

Line 20, replace "aralkylamino" with -- alkylamino --; and

Line 56, replace "K₁" with -- J₁ --.

Signed and Sealed this

Seventh Day of October, 2003

A handwritten signature in black ink, appearing to read "James E. Rogan", written over a horizontal line.

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

EXHIBIT D

**Maintenance Fee Statement receipts received for U.S.
Patent No. 5,686,411 for years four and eight**



Customer Num: 22249

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SUITE 4700
LOS ANGELES CA 90071

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "STAT", below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "STAT" below. **TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).**

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. **THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.**

PATENT NUMBER	FEE CODE	FEE AMT	SUR CHARGE	APPLICATION NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT	ATTY DKT NUM
5,686,411	183	\$850.00	\$0.00	08/447,849	11/11/97	05/23/95	04	NO	PAID	213/080

**DIRECT YOUR RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
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PATENT NUMBER	FEE AMT	SUR CHARGE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	STAT	ATTY DKT NUMBER
5,686,411	\$2,300.00	\$0.00	08/447,849	11/11/97	05/23/95	08	NO	PAID	213/080

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