



(72) FUSON, Kimberly Sue, US

(72) MAY, Patrick Cornelious, US

(71) ELI LILLY AND COMPANY, US

(51) Int.Cl.⁶ A61K 38/06

(30) 1996/02/05 (60/011,126) US

(54) **PROCEDE DE TRAITEMENT DES TROUBLES LIES A LA
PRESENCE DE PEPTIDE .BETA.-AMYLOIDE AU MOYEN DE
THYROLIBERINE**

(54) **METHOD OF TREATING .BETA.-AMYLOID PEPTIDE
ASSOCIATED CONDITIONS USING TRH**

(57) Cette invention se rapporte à un procédé de traitement d'un trouble physiologique associé à la présence d'un peptide bêta-amyloïde chez un mammifère. Ledit procédé consiste à administrer à un mammifère ayant besoin d'un tel traitement, une quantité efficace de thyrolibérine (TRH), un analogue de thyrolibérine, ou un sel ou un bioprécurseur de thyrolibérine ou dudit analogue.

(57) This invention provides a method for treating a physiological disorder associated with .beta.-amyloid peptide in a mammal which comprises administering to a mammal in need thereof an effective amount of thyrotropin releasing hormone, an analog of thyrotropin releasing hormone, or a salt or prodrug of thyrotropin releasing hormone or said analog.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 5/093, A61K 38/06	A1	(11) International Publication Number: WO 97/28184 (43) International Publication Date: 7 August 1997 (07.08.97)
(21) International Application Number: PCT/US97/01481 (22) International Filing Date: 5 February 1997 (05.02.97) (30) Priority Data: 60/011,126 5 February 1996 (05.02.96) US (71) Applicant (for all designated States except US): ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): FUSON, Kimberly, S. [US/US]; 5834 Petersen Court, Indianapolis, IN 46254-1077 (US). MAY, Patrick, C. [US/US]; 4690 Cheval Place, Carmel, IN 46032 (US). (74) Agents: GAYLO, Paul, J. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, YU, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHOD OF TREATING BETA-AMYLOID PEPTIDE ASSOCIATED CONDITIONS USING TRH		
(57) Abstract This invention provides a method for treating a physiological disorder associated with β-amyloid peptide in a mammal which comprises administering to a mammal in need thereof an effective amount of thyrotropin releasing hormone, an analog of thyrotropin releasing hormone, or a salt or prodrug of thyrotropin releasing hormone or said analog.		

- 1 -

Title

METHOD OF TREATING BETA-AMYLOID PEPTIDE ASSOCIATED CONDITIONS USING TRH

5

Background of the Invention

Alzheimer's disease is a degenerative disorder of the human brain. Clinically, it appears as a progressive dementia. Its histopathology is characterized by degeneration of neurons, gliosis, and the abnormal deposition of proteins in the brain. Proteinaceous deposits (called "amyloid") appear as neurofibrillary tangles, amyloid plaque cores, and amyloid of the congophilic angiopathy. [For reviews, see, Alzheimer's Disease, (B. Reisberg, ed., The Free Press 1983).]

While there is no general agreement as to the chemical nature of neurofibrillary tangles, the major constituent of both the amyloid plaque cores and the amyloid of the congophilic angiopathy has been shown to be a 4500 Dalton protein originally termed β -protein or amyloid A4. Throughout this document this protein is referred to as β -amyloid peptide or protein.

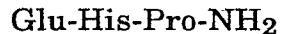
β -amyloid peptide is proteolytically derived from a transmembrane protein, the amyloid precursor protein (APP). Different splice forms of the amyloid precursor protein are encoded by a widely expressed gene. see, e.g., K. Beyreuther and B. Müller-Hill, Annual Reviews in Biochemistry, 58:287-307 (1989). β -Amyloid peptide consists, in its longest forms, of 42 or 43 amino acid residues. J. Kang, et al., Nature (London), 325:733-736 (1987). These peptides, however, vary as to their amino-termini. C. Hilbich, et al., Journal of Molecular Biology, 218:149-163 (1991).

Because senile plaques are invariably surrounded by dystrophic neurites, it was proposed early that β -amyloid peptide is involved in the loss of neuronal cells that occurs in Alzheimer's disease. B. Yankner and co-workers were the first to demonstrate that synthetic β -amyloid peptide could be neurotoxic in vitro and in vivo. B.A. Yankner, et al., Science, 245:417 (1989); See, also, N.W. Kowall, et al., Proceedings of the National Academy of Sciences, U.S.A., 88:7247 (1991).

- 2 -

Other research groups, however, were unable to consistently demonstrate direct toxicity with β -amyloid peptide. See, e.g., Neurobiology of Aging, 13:535 (K. Kosik and P. Coleman, eds. 1992). Even groups receiving β -amyloid peptide from a common source
5 demonstrate conflicting results. D. Price, et al., Neurobiology of Aging, 13:623-625 (1991)(and the references cited therein).

As mentioned supra, cells have alternative mechanisms for processing amyloid precursor protein which can result in the formation of the β -amyloid protein and subsequently, the senile plaques.
10 Thyrotropin releasing hormone (TRH) is a naturally occurring tripeptide having the sequence



15 which is important in the regulation of the synthesis and secretion of thyroid-stimulating hormone (TSH) in the anterior pituitary. Thyrotropin releasing hormone also has a central role in the regulation of the hypothalamic-pituitary-thyroid axis. In addition to its role in the regulation of thyroid function, thyrotropin releasing hormone has long
20 been recognized as a modulatory neuropeptide, with a broad spectrum of central nervous system activity. Abundant extra-hypothalamic TRH receptors have been demonstrated in both rodent and human brain. Although TRH interacts with several classical neurotransmitters, the most robust evidence is for a positive modulatory effect of TRH on the
25 action of acetylcholine in the central nervous system.

Thyrotropin releasing hormone has been reported to have antidepressant properties and to be beneficial in schizophrenia. These studies have, however, proven to be controversial. United States Patents 5,190,923, issued March 2, 1993, and 5,098,888, issued March 24, 1992,
30 the entire contents of which are herein incorporated by reference, teach the use of analogs of TRH to facilitate cholinergic neurotransmission, in particular by promoting synthesis of the neuromediator when the latter is rendered deficient or by enhancing the central effect of a cholinergic agonist. None of these references, either singly or taken in combination,
35 suggest the use of thyrotropin releasing hormone, or an analog thereof,

- 3 -

to inhibit the aggregation of β -amyloid peptide, thereby decreasing the potential for neurotoxicity of this peptide.

Because of the debilitating effects of Alzheimer's disease there continues to exist a need for effective treatments. This invention provides methods for the treatment of Alzheimer's disease and other conditions associated with β -amyloid peptide in mammals. Specifically, this invention provides methods of using thyrotropin releasing hormone, or an analog thereof, for the treatment of a condition associated with β -amyloid-induced neurodegeneration.

Summary of the Invention

This invention provides a method for treating a physiological disorder associated with β -amyloid peptide in a mammal which comprises administering to a mammal in need thereof an effective amount of thyrotropin releasing hormone, an analog of thyrotropin releasing hormone, or a salt or prodrug of thyrotropin releasing hormone or said analog.

Detailed Description and Preferred Embodiments

β -amyloid peptide naturally occurs as a series of peptides which are 39 to 43 amino acids long, with the shorter, more soluble forms being present in cerebrovascular deposits and the longer forms being found primarily in senile plaques. F. Prelli, *et al.*, Journal of Neurochemistry, 51:648-651 (1988). The primary structure of the 43 amino acid long peptide (β 1-43) is depicted in SEQ ID NO:1:

Asp	Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	15
Lys	Leu	Val	Phe	Phe	Ala	Glu	Asp	Val	Gly	Ser	Asn	Lys	Gly	Ala	30
Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala	Thr			43

Even though the full length peptide of SEQ ID NO:1: has sufficient solubility in water for the following experiments, for the purposes of convenience, a more water-soluble form of the peptide is often desired.

- 4 -

For that reason, the following examples were performed using peptides containing just the first 40 amino acids of the β -amyloid peptide (β 1-40). The sequence of this preferred peptide is SEQ ID NO:2:

5	Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln	15
	Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala	30
	Ile Ile Gly Leu Met Val Gly Gly Val Val	40

10 It is understood by those in the art that other fragments of β -amyloid peptide, comprising amino-truncated, carboxy-truncated, or internal deletions, or any combination of these, as well as conservative variants of these peptides, may be employed in this invention so long as that peptide fragment demonstrates the requisite neurotoxicity.

15 While the peptide of SEQ ID NO:1 and SEQ ID NO:2 are referred to as β -amyloid peptide throughout this document, in the body of literature concerning this field, this peptide is alternatively referred to as β -amyloid protein, amyloid β peptide, amyloid β A4, β protein, amyloid A4, β -peptide, and other such names.

20 The term "treating" (or "treat") as used herein includes its generally accepted meaning which encompasses prohibiting, preventing, restraining, and slowing, stopping, or reversing progression, severity, or a resultant symptom. As such, the methods of this invention encompass both therapeutic and prophylactic administration.

25 The term "effective amount" as used herein refers to the amount of compound necessary to treat physiological effects or disorders associated with β -amyloid peptide, or inhibit amyloidogenic production or deposition, or treat Alzheimer's Disease, as the case may be.

30 The term "physiological disorder associated with β -amyloid peptide" includes diseases related to the inappropriate or undesirable deposition of β -amyloid peptide, and as such includes Alzheimer's Disease (including familial Alzheimer's Disease), Down's Syndrome, advanced aging of the brain, hereditary cerebral hemorrhage with amyloidosis of the Dutch-type (HCHWA-D), and the like.

35 The compounds used in the method of the present invention may have one or more asymmetric centers. As a consequence of these

- 5 -

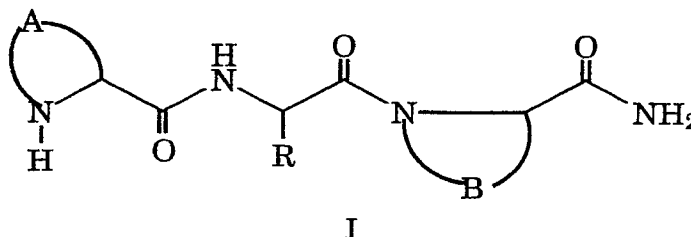
chiral centers, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All asymmetric forms, individual isomers and combinations thereof, are within the scope of the present invention.

This invention also encompasses methods employing the pharmaceutically acceptable salts of the compounds described herein. A compound employed in this invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with any of a number of organic and inorganic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt.

The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds of the above formula which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a pharmaceutically acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts.

This invention also encompasses methods employing the pharmaceutically acceptable solvates of the compounds described herein. Many of these compounds can combine with solvents such as water, methanol, ethanol and acetonitrile to form pharmaceutically acceptable solvates such as the corresponding hydrate, methanolate, ethanolate and acetonitrilate.

United States Patent 5,190,923, issued March 2, 1993, the entire contents of which are herein incorporated by reference, describes a series of compounds of Formula I



- 6 -

wherein:

- 5 A represents, with the nitrogen and carbon atoms to which it is linked, a 2-oxoperhydro-7-azepinyl group, a 2-oxoperhydro-8-azocinyl group, a 2-oxoperhydro-9-azoninyl group, a 2-oxoperhydro-10-azecinyl group, a 2-oxo-2,3,4,7-tetrahydrobenz[e]azepin-7-yl group, a 2-oxo-2,3,6,7-tetrahydrobenz[d]azepin-7-yl group, or a 2-oxo-2,3,6,7-tetrahydrobenz[c]azepin-7-yl group;
- 10 B represents, with the nitrogen and carbon atoms to which it is linked, a polycyclic structure selected from the following structures,
- 15 2-azobicyclo[2.2.1]heptane;
- 2-azabicyclo[2.2.2]octane, optionally substituted at positions 1 and 4 with one or two linear or branched (C₁-C₄) alkyl groups;
- 20 perhydroindole;
- indoline;
- 25 perhydroisoquinoline;
- 1,2,3,4-tetrahydroquinoline;
- 1,2,3,4-tetrahydroisoquinoline;
- 30 cyclopenta[b]pyrrolidine;
- pyrrolidine, optionally substituted with one or two linear or branched (C₁-C₄) alkyl groups;
- 35 piperidine; or

- 7 -

thiazolidine;

R represents:

5

hydrogen;

10

a linear or branched (C₁-C₆) alkyl group optionally substituted with an amino group or a guanidino group;

15

a (4-imidazolyl)methyl group, optionally substituted on one of the nitrogen atoms with a linear or branched (C₁-C₄) alkyl radical;

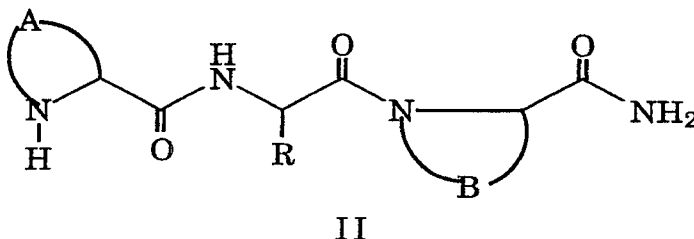
20

a (3-pyrazolyl)methyl group; or

a (2-pyridyl)methyl group, optionally substituted with an amino group;

their enantiomers, diastereoisomers, and epimers, as well as their pharmaceutically acceptable acid addition salts.

25 United States Patent 5,098,888, issued March 24, 1999, the entire contents of which are herein incorporated by reference, describes a series of compounds of Formula II



30 wherein:

- 8 -

A represents, with the nitrogen and carbon atoms to which it is linked, a cycloamide group selected from the list consisting of

- 5 2-oxo-5-pyrrolidinyl,
- 2-oxo-6-piperidyl,
- 2,6-dioxo-1,2,3,6-tetrahydro-4-pyrimidinyl,
- 10 2-oxo-4-thiazolidinyl,
- 2-oxo-4-azetidiny, and
- 15 1-oxo-1,2,3,4-tetrahydro-3-isoquinolinyl;

B represents, with the nitrogen and carbon atoms to which it is linked, a polycyclic structure selected from the following structures,

- 20 2-azobicyclo[2.2.1]heptane; and
- 2-azabicyclo[2.2.2]octane, optionally substituted at positions 1 and 4 with one or two linear or branched
- 25 (C₁-C₄) alkyl groups;

R represents:

- 30 hydrogen;
- a linear or branched (C₁-C₆) alkyl group optionally substituted with an amino group or a guanidino group; or

- 9 -

a (4-imidazolyl)methyl group, optionally substituted on one of the nitrogen atoms with a linear or branched (C₁-C₄) alkyl radical;

- 5 their enantiomers, diastereoisomers, and epimers, as well as their pharmaceutically acceptable acid addition salts.

The neuroprotective ability of thyrotropin releasing hormone is demonstrated by assays showing the ability of thyrotropin releasing hormone to protect neural cells from the neurotoxic effects of beta amyloid peptide. Such assays are described below.

Neurotoxicity Assay Measuring Calcium Levels

15 An aliquot of ED 18 cortical cells are seeded into polyethylenimine-coated tissue culture dishes and cultured for 3-5 days in vitro before treatment with a 25 μ M solution of β -amyloid peptide, either freshly dissolved (predominantly random coil conformation) or aged (7 days, predominantly β -sheet conformation). This neurotoxicity
20 assay is conducted in chemically-defined HEPES-buffered DMEM supplemented with fetal calf serum.

After a two day treatment with the β -amyloid peptide, the elevation of cytosolic calcium (Ca⁺²) concentrations after a glutamate pulse are determined using a fluorescent calcium dye. J. Wahl, et al.,
25 Journal of Neurochemistry, 53:1316 (1989). The elevation of intracellular Ca⁺² levels compromises cell integrity.

Neurotoxicity Assay Measuring XTT

30 An aliquot of ED 18 cortical cells are seeded into polyethylenimine-coated tissue culture dishes and cultured for 3-5 days in vitro. This assay is conducted in chemically-defined HEPES-buffered DMEM supplemented with fetal calf serum.

These cells are cultured for 3 to 5 days in vitro before
35 treatment with a 25 μ M solution of β -amyloid peptide, either freshly dissolved (predominantly random coil conformation) or aged (7 days,

- 10 -

predominantly β -sheet conformation). After two days of treatment, cell viability is assessed by measuring the reduction of the tetrazolium salt XTT [2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide inner salt] as described by N. Roehm, *et al.*, Journal of Immunological Methods, 142:257 (1992).

Neurotoxicity Assay Measuring LDH

Primary hippocampal or cortical cultures are prepared from E18 fetal Sprague-Dawley rat pups. The cells are plated at high density ($1.5 \times 10^5/\text{cm}^2$) in 24-well plates coated with 0.5 mg/ml of polyethylenimine. These cultures are routinely maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 4 mM glutamine, 5 g/liter glucose, 1 mM pyruvate, 20 mM potassium chloride, 50 units/ml penicillin, and 50 $\mu\text{g}/\text{ml}$ streptomycin.

The cells are incubated for 10 days *in vitro* before treatment with a 25 μM solution of β -amyloid peptide, either freshly dissolved or aged. After four days of incubation, cell integrity is determined by measuring the level of the enzyme lactate dehydrogenase (LDH) using a standard colorimetric endpoint assay for pyruvate. P. May, *et al.*, Neurobiology of Aging, 13:605, 606 (1992). Control standards containing normal and elevated levels of serum LDH are run with every assay. The results are expressed in the figures as units of LDH/ml wherein 1 unit is defined as the amount of LDH which reduces 4.8×10^{-4} moles pyruvate/minute at 25°C.

In repeated experiments thyrotropin releasing hormone is neuroprotective against β -amyloid peptide-induced toxicity in a dose-dependent manner with an approximate IC_{50} in the nanomolar range (10 nM-100 nM).

Neurotoxicity Assay Measuring alamarBlue™

The alamarBlue™ assay incorporates a proprietary fluorometric/colorimetric growth indicator (Alamar Biosciences, Inc.)

- 11 -

based on the detection of metabolic activity. Viable cells cause alamarBlue™ to change from an oxidized (non-fluorescent, blue color) to a reduced (fluorescent, red) form. Neuronal viability as assessed by alamarBlue™ is comparable in sensitivity to that obtained by measuring the release of LDH into the culture media or the reduction of the tetrazolium salt XTT. Assays are performed by replacing the culture media with a 10% alamarBlue™ solution in neuronal medium. Reduction of alamarBlue™ is determined using a Millipore Cytofluor 2350 Scanner (excitation, 560 nm; emission, 590 nm) and CytoCalc™ software (v. 01.00.04, Millipore Corporation). Results are expressed as percent of control (untreated) cultures.

The advantage of using this technique over other techniques as described supra is that measuring the alamarBlue™ concentration does not involve lysis of the test cells so a continuing assay, with multiple timepoints, can be performed.

Treatment of mature, high density, rat hippocampal cultures with 50 μ M human amylin (Bachem) results in prominent neurodegeneration, morphologically similar to that observed in cultures treated with 50 μ M β -amyloid peptide. see, European Patent Publication 646,792, published April 5, 1995. These neurodegenerative changes induced by human amylin are accompanied by a concentration-dependent release of LDH into the culture media. Human amylin is more potent than β -amyloid peptide with detectable neurotoxicity observed with 10 μ M amylin. A similar concentration-response curve is observed with β_2 -microglobulin, another amyloidogenic protein. Id.

Human amylin obtained from 3 independent sources induces prominent neurodegeneration in cultured rat hippocampal neurons. In contrast, cultures treated with the non-amyloidogenic rat amylin (Bachem) are indistinguishable from vehicle-treated (H_2O) control wells. Consistent with the assessment by phase-contrast microscopy, LDH values in rat amylin-treated cultures are similar to control values. Rat amylin obtained from a different source (Peninsula Laboratories) is similarly non-toxic to hippocampal neurons (results not shown).

- 12 -

Only full length human amylin (1-37) is neurotoxic. No toxicity is observed with fragments of human amylin including the ten amino acid peptide containing residues 20-29 important for the secondary structure of amylin. A similar lack of neurotoxicity of the peptide consisting of human amylin(20-29) is recently reported by C.J. Pike, et al., Journal of Neurosciences, 13:1676-1687 (1993), but full length human amylin is apparently not included in that study. These results demonstrate that while the region of amino acids 20-29 is important for determining the final conformation attained by amylin [see, e.g., L.R. McLean and A. Balasubramaniam, Biochimica and Biophysica Acta, 112:317-320 (1992)], it is not of sufficient size to elicit neurotoxicity in mature high density hippocampal cultures.

Treatment of mature, high density, human cortical cultures with 12.5-50 μ M human amylin for three days results in complete neurodegeneration. Significant, though not complete, neurodegeneration is also observed in cultures treated with 25-100 μ M human β -amyloid peptide. Human amylin is more potent than β -amyloid peptide with detectable neurotoxicity observed with 3 μ M amylin.

Experimental Design

Primary hippocampal or cortical cultures are exposed to the compound of Formula I in concentrations ranging from 10 nM to 100 μ M in combination with 50 μ M of β -amyloid peptide(1-40). Viability of the hippocampal cultures is assessed after a four day incubation.

In one such experiment using thyrotropin releasing hormone it is observed that low concentrations of thyrotropin releasing hormone provides some protection against β -amyloid-associated neurotoxicity.

PC12/ β -Amyloid Neurotoxicity Assay

An in vitro assay of the effects of β -amyloid peptide on cell viability is developed using the rat pheochromocytoma cell line, PC12. The PC12 cells are exposed to 1-25 μ M β -amyloid peptide in cell culture

- 13 -

media for 12-24 hours. Subsequently, the cells are exposed to the tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), also known as MTT, for 2 hours. Mitochondria in viable cells reduce the yellowish MTT into a blue formazan product which can be extracted from the cells and measured spectrophotometrically. Thus, the conversion of the tetrazolium to the formazan is a measure of the reducing potential of the mitochondria and, hence, a measure of cell viability. Exposure of PC12 cells to β -amyloid peptide (1-40) or a sub-fragment β -amyloid peptide (25-35) led to about a 50% reduction in the amount of formazan production. Thyrotropin releasing hormone prevents the reduction in MTT conversion observed in the presence of β -amyloid peptide.

The aggregation of β -amyloid peptide results in the activation of immune and inflammatory responses in affected areas of the brain. The β -amyloid peptide, upon assuming the conformation necessary for aggregation, also potentiates cytokine secretion, especially interleukin-6 and interleukin-8 release. Thyrotropin releasing hormone, in preventing the formation of β -amyloid aggregates, inhibits this cytokine release and, thereby, ameliorates the inflammatory component of Alzheimer's Disease.

The determination of whether thyrotropin releasing hormone is useful in treating Alzheimer's disease may be made by the utilization of several assays which are known in the art. An additional assay which may be employed involves the use of a transgenic mouse model as described in D. Games, *et al.*, Nature (London), 373:523-527 (1995). The ability of thyrotropin releasing hormone to ameliorate the inflammatory aspects of Alzheimer's disease may be rapidly assessed using this mouse model.

Human clinical trials may also be employed to assess the value of thyrotropin releasing hormone in treating or preventing some of the neurological damage associated with conditions associated with β -amyloid peptide, including Alzheimer's disease. E. Matsubura, *et al.*, Annals of Neurology, 28:561-567 (1990); L. Altstiel, *et al.*, Dementia, 6:17-20 (1995). Human clinical trials may be employed to measure the effects

- 14 -

of therapy on cognition and behavior. The design of clinical trials, use of assessment instruments, and interpretation of these results are well known to clinicians in the field.

While it is possible to administer a compound employed in the methods of this invention directly without any formulation, the compounds are usually administered in the form of pharmaceutical compositions comprising a pharmaceutically acceptable excipient and at least one active ingredient. These compositions can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Many of the compounds employed in the methods of this invention may be effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound. See, e.g., REMINGTON'S PHARMACEUTICAL SCIENCES, (16th ed. 1980).

The instant invention further provides pharmaceutical formulations comprising the claimed compounds. The compounds, preferably in the form of a pharmaceutically acceptable salt, can be formulated for oral or parenteral administration.

For example, the compounds can be admixed with conventional pharmaceutical carriers and excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, wafers and the like. The compositions comprising TRH compounds will contain from about 0.1 to 90% by weight of the active compound, and more generally from about 10 to 30%. The compositions may contain common carriers and excipients such as corn starch or gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride, and alginic acid.

Disintegrators commonly used in the formulations of this invention include croscarmellose, microcrystalline cellulose, corn starch, sodium starch, glycolate and alginic acid.

Tablet binders that can be included are acacia, methyl cellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone (Povidone), hydroxypropyl methylcellulose, sucrose, starch and ethylcellulose.

- 15 -

Lubricants that can be used include magnesium stearate or other metallic stearates, stearic acid, silicone fluid, talc, waxes, oils and colloidal silica.

5 Flavoring agents such as peppermint, oil of wintergreen, cherry flavoring, or the like can also be used.

It may be desirable to add a coloring agent to make the dosage form more attractive in appearance or to help identify the product.

10 For intravenous (IV) use, a water soluble form of compounds of the formula 1 can be dissolved in one of the commonly used intravenous fluids and administered by infusion. Such fluids, for example, physiological saline, Ringer's solution or 5% dextrose solution can be used.

15 For intramuscular preparations, a sterile formulation of a suitable soluble salt form of the compounds of the formula 1, for example the hydrochloride salt, can be dissolved and administered in a pharmaceutical diluent such as pyrogen-free water (distilled), physiological saline or 5% glucose solution. A suitable insoluble form of the compound may be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, e.g. an ester of a
20 long chain fatty acid such as ethyl oleate.

For oral use, a sterile formulation of a suitable salt form of TRH, for example, the hydrochloride salt, formulated in a diluent such as distilled or deionized water, is particularly useful.

25 A preferred formulation of the compounds of the present invention includes the use of poly(alkylcyanoacrylate) particles, commonly known as nanoparticles, as carriers for the TRH protein. The use of nanoparticles is well known in the art as described in J.L. Grangier, *et al*, "Nanoparticles as carriers for growth hormone releasing factor", Journal of Controlled Release, 15:3-13 (1991).
30

In some therapeutic applications, it may be desirable to achieve a prolonged release of the compound. Methods of incorporating the compounds of formula 1 into such delayed release formulations are well known in the art and may be found in general texts on the subject
35 such as PROLONGED RELEASE OF THERAPEUTIC AGENTS.

- 16 -

Alternatively, the unit dosage form of the compound can be solution of the compound, preferably in its salt form, in a suitable diluent in sterile hermetically sealed ampoules. The concentration of the compound in the unit dosage may vary, e.g. from about 1% to about
5 50% depending on the particular form of the compound and its solubility and the dose desired by the physician.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or
10 discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile,
15 or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One
20 such implantable delivery system, used for the transport of biological factors to specific anatomical regions of the body, is described in U.S. Patent 5,011,472, issued April 30, 1991, which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually
25 involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs or prodrugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation
30 across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

The type of formulation employed for the administration of
35 the compounds employed in the methods of the present invention may be dictated by the particular compounds employed, the type of

WO 97/28184

PCT/US97/01481

- 17 -

pharmacokinetic profile desired from the route of administration and the compound(s), and the state of the patient.

- 18 -

We Claim:

- 5 1. A method of treating a physiological disorder associated with β -amyloid peptide which comprises administering to a mammal in need thereof an effective amount of thyrotropin releasing hormone, an analog of thyrotropin releasing hormone, or a salt or prodrug of thyrotropin releasing hormone or said analog.
- 10 2. A pharmaceutical formulation adapted for the treatment of a physiological disorder associated with β -amyloid peptide associated neurotoxicity, which comprises an effective amount of thyrotropin releasing hormone, an analog of thyrotropin releasing
15 hormone, or a salt or prodrug of thyrotropin releasing hormone or said analog.
3. The use of thyrotropin releasing hormone, an analog of thyrotropin releasing hormone, or a salt or prodrug of thyrotropin
20 releasing hormone or said analog, for the manufacture of a medicament for the treatment of a physiological disorder associated with β -amyloid peptide.
4. Thyrotropin releasing hormone, an analog of
25 thyrotropin releasing hormone, or a salt or prodrug of thyrotropin releasing hormone or said analog for treating a physiological disorder associated with β -amyloid peptide.