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(54) **NEUROMEDIN B RECEPTOR ANTAGONISTS**

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• **MOLECULAR PHARMACOLOGY**, Volume 44,
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617.

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EP 1 011 716 B9

DescriptionBACKGROUND OF THE INVENTION

[0001] The mammalian bombesin (Bn)-related peptides, gastrin-releasing peptide (GRP), neuromedin B (NMB), and neuromedin C (NMC) have a wide range of biological effects. These include chemotaxis, contraction of smooth muscle stimulation, and the release of numerous gastrointestinal hormones. GRP and NMB are also active in the central nervous system, affecting thermoregulation, behavioral effects, satiety, maintenance of circadian rhythm, and inhibition of TSH release. Bn-related peptides function as a growth factor in numerous normal cells (e.g., stomal, epithelial, and neuroendocrine cells) as well as neoplastic cells such as human small cell lung cancer cells, non-small cell lung cancer cells, rat hepatocellular tumor cells, prostatic cells and breast adenocarcinoma cells.

[0002] Recent structure and cloning studies demonstrate that Bn-related peptides mediate the actions of two distinct receptor classes. GRP has a high affinity, and NMB has a low affinity, for the GRP-preferring class or subtype (GRP receptor or GRP-R). In contrast, GRP has a low affinity, and NMB has a high affinity, for the other class, the NMB-preferring subtype (NMB receptor or NMB-R). Both receptor classes are present throughout the central nervous system and the gastrointestinal tract.

[0003] Native somatostatin, somatostatin-14 (SS-14), has been shown to inhibit the cross-linking of ^{125}I -GRP to a 120 kD protein in Triton® extracts of 3T3 cells and human small cell lung cancer cells which are known to possess bombesin receptors. Recently, somatostatin octapeptide analogs have also demonstrated binding affinity to NMB-R in Orbuch, et al., Mol. Pharmacol., 44:841 (1993). These analogs, however, also maintain a substantial activity for somatostatin receptors.

[0004] WO94/02163 describes a method of selectivity inhibiting biochemical activity of cells induced by neuromedin B. The method includes the step of contacting cells which contain neuromedin B receptor with a cyclic octapeptide, having lysine or ornithine at position 5.

[0005] EP0389180 describes octapeptides exhibiting GH-releasing-inhibiting activity. The octapeptides have lysine at position 5.

[0006] EP0215171 describes octapeptides active in inhibiting the secretion of GH, insulin and glucagon. The octapeptides have lysine at position 5.

[0007] EP0298732 describes a peptide comprising the sequence - Cys-X-D-Trp-Lys-Val-Cys wherein X represents any amino acid residue.

SUMMARY OF THE INVENTIONAbbreviations**[0008]**

Nal = 3- (2-naphtyl)-alanine or 3- (1-naphthyl) -alanine

Bpa = 3-(4-biphenyl)-alanine

X-Phe = phenylalanine with a *p*-, *o*- or *m*-substituent, such as -OH, CH₃, NO₂, and halogen, on the phenyl ring, e. g., 3-(4-chlorophenyl)-alanine

F₅Phe = 3-(pentafluorophenyl)-alanine

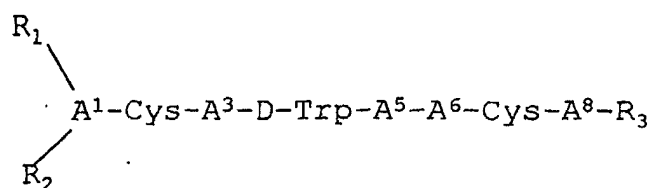
Nle=norleucine

Me-Trp = Trp with a methyl-substituted indolyl nitrogen

Dab = 2,4-diamino butyric acid

Aub = 2-amino butyric acid

[0009] The present invention relates to cyclic octapeptides which have both high affinity and high selectivity for the NMB receptor and are encompassed by the following formula (I):



A¹ is the D-isomer of Nal or Trp, and is preferably D-Nal. A³ is F₅-Phe or ortho-, para-, or meta-substituted X-Phe wherein X is halogen, NO₂, CH₃ or OH. A³ is preferably Phe or para-substituted X-Phe, where X is C1, F, or OH. A⁵ is -NH-CH(Y)-CO- wherein Y is (CH₂)_m-R₄-N(R₅)(R₆) or (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆). In one aspect, A⁵ is -NH-CH(Y)-CO- where Y is (CH₂)_m-R₄-N(R₅)(R₆), and preferably Dab, 7-amino-phenylalanine, and 2,3-diamino propionic acid, provided that A⁵ is not Orn. In another aspect, A⁵ is -NH-CH(Y)-CO- where Y is (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆), and is preferably Arg or 7-guanidiny-phenylalanine. A⁶ is the D- or L- isomer of Thr, Leu, Ile, Nle, Val, Nal, Trp, Me-Trp, Abu, Bpa, Phe, F₅-Phe, or X-Phe wherein X is a halogen, NO₂, CH₃, or OH. A⁶ is preferably the D- or L-isomer of Thr, Leu, Ile, Nle, Trp, Val, and Abu. A⁸ is Nal or Trp, and is preferably Nal. Subscript m is 1, 2, or 3, and preferably 2 or 3; n is 1, 2, 3, 4 or 5, and preferably 2, 3, or 4. Each of R₁ and R₂, independently, is H, E, COE, or COOE. E is a hydrocarbon of between 1 and 25 carbon atoms; substituted or unsubstituted; saturated or unsaturated; straight chain or branched; cyclic, acyclic, or polycyclic. Examples of E include C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, phenyl, naphthyl, C₇₋₁₂ phenylalkyl or alkylphenyl, C₈₋₁₂ phenylalkenyl or alkenylphenyl, C₈₋₁₂ phenylalkynyl or alkynylphenyl, C₁₁₋₂₀ naphthylalkyl or alkynaphthyl, C₁₂₋₂₀ naphthylalkenyl or alkenynaphthyl, or C₁₂₋₂₀ naphthylalkynyl or alkynynaphthyl, provided that when one of R₁ or R₂ is COE or COOE, the other must be H. R₃ is -NH₂ and R₅, R₆, R₈ are each independently H or E. Each of R₅, R₆ and R₈ is preferably H or a C₁₋₁₀ hydrocarbon, such as alkyl, alkenyl, alkylphenyl, phenyl, and phenylalkyl, including C₁₋₅ alkyl. Each of R₅ and R₆ is more preferably H. R₄ is C₆H₄ or absent, and preferably is absent. R₇ is =NR₈, =S, or =O, and preferably =NR₈, and more preferably R₇ is =NH. Preferred octapeptides encompassed by the above formula (I) of the invention include H₂-D-Nal-Cys-Tyr-D-Trp-Arg-Val-Cys-Nal-NH₂ (peptide Arg⁵); and H₂-D-Nal-Cys-Tyr-D-Trp-Dab-Val-Cys-Nal-NH₂ (peptide Dab⁵).

[0010] In formula (I), the N-terminus is at the left and the C-terminus at the right- in accordance with the conventional representation of a polypeptide chain. The symbol A¹, A², or the like in a peptide sequence stands for an amino acid residue, i.e. =N-CH(R)-CO- when it is at the N-terminus or -NH-CH(R)-CO- when it is not at the N-terminus, where R denotes the side chain of that amino acid residue. Thus, R is -CH(CH₃)₂ for Val. Also, when the amino acid residue is optically active, it is the L-form configuration that is intended unless D-form is expressly designated. Note that the two Cys residues (i.e., A² and A⁷) in formula (I) are linked together via a disulfide bond. However, for convenience a line which is used conventionally to denote a disulfide bond between two Cys residues is omitted herein. COE refers to -(C=O)-E and COOE refers to -(C=O)-O-E.

[0011] Administration of a pharmaceutically acceptable salt of an octapeptide covered by formula (I) into a patient whose disorder arises from biochemical activity mediated by NMB is also within the present invention. In other words, the octapeptides can be provided in the form of pharmaceutically acceptable salts such as acid addition salts, or metal complexes such as with zinc or iron. Examples of acid addition salts are (i) those made with organic acids such as acetic, lactic, pantoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic or toluenesulfonic acid; (ii) those made with polymeric acids such as tannic acid or carboxymethyl cellulose; and (iii) those made with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid.

[0012] Other features and advantages of the present invention will be apparent from the following description of the preferred embodiments, and also from the appending claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] We first briefly describe the drawings.

Fig. 1 is a graph showing the suppression of NMB stimulated food intake by an analog of the invention.

Fig. 2 is a graph showing suppression of NMC stimulated food intake by an analog of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0014] Octapeptides of the invention are synthesized on methylbenzhydrylamine resin using standard solid phase procedures and cleaved with hydrogen fluoride/anisole mixtures. The peptides are cyclized in dilute 90% acetic acid solution by titration with I₂ and purified by gel filtration on SEPHADEX™ G-25 (Aldrich, Milwaukee, WI) in 50% acetic acid and gradient elution on C18 silica using acetonitrile/0.1% trifluoroacetic acid buffers. See, e.g., Sasaki, Y. et al. *J. Med. Chem.*, 30:1162 (1987); Stewart, J.M. et al. *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984); and Coy, D.H. et al. *Tetrahedron*, 44:835 (1988). Homogeneity is assessed by thin layer chromatography ("TLC"), analytical HPLC, amino acid analysis and mass spectrometry.

Preferably, homogeneity should be determined to be >96% for each peptide. Example 1 below is a detailed description regarding the synthesis of peptide Dab⁵. Other peptides of the invention can be prepared by making appropriate modifications, within the ability of someone of ordinary skill in the art, of the synthetic methods disclosed herein.

[0015] The NMB analogs of the invention are screened in binding assays to determine their respective affinities for

the NMB, GRP, and somatostatin receptors. See Examples 2, 3, and 4, respectively. Agonists of the NMB receptor have been shown to stimulate the generation of inositol phosphates. Wang, et al, *J. Biochem.*, 286:641-648 (1992). In Example 5 below, an inositol phosphate turnover assay measured the ability of the NMB analogs to antagonize the NMB receptor activation. In Example 6 below, an *in vivo* assay demonstrated the ability of the NMB analogs of the

[0016] The NMB analogs of this invention behave as potent antagonists of the NMB receptor. NMB has been shown to stimulate the growth of cancer cell lines (Moody, et al. *J. Pharmacol.*, 263:1 (1992); Wang, et al., *Biochem. J.*, 286: 641 (1992)). As NMB antagonists the analogs of this invention can be used to treat cancers such as small cell lung tumors and glioblastomas. In addition, NMB has been shown to suppress food intake (Kirkman, et al., Society for the Study of Ingestive Behavior, Toronto, Canada). The analogs of the invention can be used to stimulate food intake to treat eating disorders such as anorexia or those resulting from cancer or AIDS. Furthermore, NMB has also been shown to decrease gastrin release, Kawai, et al., *Endocrinol. Japan*, 37(6):857 (1990). The analogs of the invention, thus, can be used to stimulate gastrin release in patients who are producing insufficient amounts of gastrin.

[0017] The analogs of the invention are also highly selective for the NMB receptor. The analogs of the invention will, therefore, have reduced cross-reactivity with both of these receptors. For example, other agonists of the somatostatin receptors may inhibit growth hormone release or disturb carbohydrate metabolism by the agonists' inhibition of insulin release.

[0018] The dose of the compound of the present invention for treating the above-mentioned diseases varies depending upon the manner of administration, the age and the body weight of the subject and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such amount of the active compound as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount".

[0019] The formulations are presented in unit dosage form and are prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients. In general, the formulations for tablets or powders are prepared by uniformly and intimately blending the active ingredient with finely divided solid carriers, and then, if necessary as in the case of tablets, forming the product into the desired shape and size.

[0020] Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed merely as illustrative, and not limitative of the remainder of the disclosure in any way. All publications cited herein are incorporated by reference.

Example 1

[0021] Synthesis of Boc-D-Nal-S-methylbenzyl-Cys-O-bromobenzyl-oxycarbonyl-Tyr-D-Trp-N-benzyloxycarbonyl-Dab-Val-S-methylbenzyl-Cys-Nal-benzhydrylamine resin was as follows. Benzhydrylamine-polystyrene resin (Advanced Chem Tech, Inc., Louisville, KY) (0.7 g, 0.3 mmole) in the chloride ion form was placed in the reaction vessel of an Advanced Chem Tech™ peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 time for 1 and 25 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform.

[0022] The neutralized resin was stirred with *t*-butoxy-carbonyl("Boc")-Nal and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hr and the resulting amino acid resin was then cycled through steps (a) to (f) in the above wash program. The following amino acids (0.9 mmole) were then coupled successively by the same procedure: Boc-S-methylbenzyl-Cys, Val, Boc-N-benzyloxycarbonyl-Dab, Boc-D-Trp, Boc-O-bromobenzyloxycarbonyl-Tyr, and Boc-S-methyl-benzyl-Cys and Boc-D-Nal. After washing and drying, the completed resin weighed 1.13 g.

[0023] Using the completed resin, H-D-Nal-Cys-Tyr-D-Trp-Lys-Dab-Cys-Nal-NH₂ was prepared. The peptide resin obtained above (1.13 g, 0.5 mmole) was mixed with anisole (5 ml), dithiothreitol (100 mg) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen. Free peptide was precipitated and washed with ether. The crude peptide was then dissolved in 250 ml of 90% acetic acid to which was added a concentrated solution of I₂/MeOH until a permanent brown color was observed. Excess I₂ was removed by addition of ascorbic acid and the solution was reduced to a small volume by evaporation. The crude peptide solution was applied to a column (2.5 x 90 cm) of SEPHADEX™ G-25 and eluted with 50% acetic acid. Fractions containing a major component by UV absorption and TLC were then pooled, reduced to a small volume by evaporation and applied to a column (1.5 x 70 cm) of VYDAC® octadecylsilane silica (10-15 μ) (Vydac, Hesperia, CA) followed by elution with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid in water. Fractions were examined by TLC and analytical high performance liquid chromatography and pooled to give maximum purity.

[0024] Repeated lyophilization of the solution from water gave 97 mg of the product as a white, fluffy powder. The product was found to be homogeneous by HPLC and TLC. Amino acid analysis of an acid hydrolysate and FAB MS

confirmed the composition of the octapeptide.

Example 2

5 NMB Receptor Binding Assay

[0025] The procedure for transfecting the rat NMB receptor into BALB-3T3 fibroblasts is discussed in Wada, et al., *Neuron*, 6:4221-430 (1991) and Benya, et al., *Mol. Pharmacol.*, 42:1058 (1992). Membranes for the NMB receptor binding assay were obtained by homogenizing BALB-3T3 fibroblasts, transfected with the rat NMB receptor, with a POLYTRON™ tissue homogenizer (setting 6, 15 sec) (Brinkman, Westbury, NY) in ice-cold 50 mM Tris-HCl (Buffer A) (Sigma Chemicals, St. Louis, MO) and centrifuging twice at 39,000 x g (10 min), with an intermediate resuspension in fresh Buffer A. The final pellets were resuspended in the 50 mM Tris-HCl, containing 0.1 mg/ml bacitracin (Sigma Chemicals, St. Louis, MO), and 0.1% bovine serum albumin (BSA) (Buffer B) (Sigma Chemicals, St. Louis, MO), and held on ice for the receptor binding assay. Aliquots (0.4 ml) were incubated with 0.05 ml [¹²⁵I-Tyr⁴]bombesin (-2200 Ci/mmmole) (New England Nuclear, Boston, MA) in Buffer B, with and without 0.05 ml of unlabeled NMB analogs. After a 30 min incubation (4°C), the bound [¹²⁵I-Tyr⁴]bombesin was separated from the free by rapid filtration through WHATMAN™ GF/B filters which had been previously soaked in 0.3% polyethyleneimine using a Brandel filtration manifold (Brandel, Gaithersburg, MD). The filters were then washed three times with 5 ml aliquots of ice-cold Buffer A. Specific binding was defined as the total [¹²⁵I]bombesin bound minus that bound in the presence of 1 μM unlabeled NMB. Analogs of the invention had a high binding affinity for the NMB receptor. Examples of the NMB receptor binding assay results for three analogs of the invention were (K_i values in nM) 47.4 ± 10.3 (peptide Arg⁵), and 85.1 ± 2.7 (peptide Dab⁵).

Example 3

25 GRP Receptor Binding Assay

[0026] Membranes for the GRP receptor binding assay were obtained by homogenizing cultured AR42J cells with a Polytron™ tissue homogenizer (setting 6, 15 sec) in ice-cold 50 mM Tris-HCl (Buffer A) and centrifuging twice at 39,000 x g (10 min), with an intermediate resuspension in fresh Buffer A. The final pellets were resuspended in the 50 mM Tris-HCl containing 0.1 mg/ml bacitracin and 0.1% bovine serum albumin (BSA) (Buffer B) and held on ice for the GRP receptor binding assay. Aliquots (0.4 ml) were incubated with 0.05 ml of [¹²⁵I-Tyr⁴]bombesin (-2200 Ci/mmmole) in Buffer B, with and without 0.05 ml of unlabeled NMB analogs. After a 30 min incubation (4°C), the bound [¹²⁵I]-Tyr⁴] bombesin was separated from the free by rapid filtration through WHATMAN™ GF/B filters which had been previously soaked in 0.3% polyethyleneimine using a Brandel™ filtration manifold. The filters were then washed three times with 5 ml aliquots of ice-cold Buffer A. specific binding was defined as the total [¹²⁵I-Tyr⁴]bombesin bound minus that bound in the presence of 1 μM unlabeled GRP. Analogs of the invention had a weak binding affinity for the GRP receptor. Examples of the GRP receptor binding assay results for analogs of the invention were (K_i values in nM) 2921 ± 250 (peptide Arg⁵) and 2632 ± 216 (peptide Dab⁵).

40 Example 4

Somatostatin Receptor Binding Assay

[0027] Membranes for the somatostatin receptor binding assay were obtained by homogenizing cultured AR42J acinar pancreas cells with a Polytron™ tissue homogenizer (setting 6, 15 sec), in ice-cold 50 mM Tris-HCl (Buffer A) and centrifuging twice at 39,000 x g (10 min), with an intermediate resuspension in fresh Buffer A. The final pellets were resuspended in 10 mM Tris-HCl for the receptor binding assay. For determination of the K_i values, the various concentrations of NMB analogs were incubated for 90 min at 25°C with approximately 0.05 nM [¹²⁵I]MK-678 (University of Arizona, School of Medicine, Tucson, AZ) in 50 mM HEPES (pH 7.4) (Sigma Chemicals, St. Louis, MO) containing BSA (fraction V) (10 mg/ml) (Sigma Chemicals, St. Louis, MO), MgCl₂ (5 mM) (Sigma Chemicals, St. Louis, MO), aprotinin (200 KIU/ml) (Sigma Chemicals, St. Louis, MO) bacitracin (0.02 mg/ml), and phenylmethylsulphonyl fluoride (0.02 mg/ml) (Sigma Chemicals, St. Louis, MO). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through GF/C filters (presoaked in 0.3% polyethylenimine) using a BRANDEL™ filtration manifold. Each tube and filter were then washed three times with 5 ml aliquots of ice-cold buffer. Specific binding was defined as the total [¹²⁵I]MK-678 bound minus that bound in the presence of 200 nM MK-678. A known cyclic octapeptide D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Nal-NH₂ (Lys⁵) was disclosed in Orbuch et al., *Mol. Pharmacol.* 44:841 (1993), and had an extremely high affinity for the somatostatin receptor (K_i = 0.84 ± 0.53). In contrast, analogs of the invention had a much lower affinity, in a range of about one hundredth or one thousandth the K_i value of Lys⁵. For example, K_i values

(nM) for analogs of the invention were 407 ± 82 (peptide Dab⁵) and, notably, 1032 ± 113 (peptide Arg⁵).

Example 5

Inositol Phosphate Turnover Assay

[0028] For the measurement of inositol phosphate turnover, BALB-3T3 fibroblasts, transfected with the rat NMB receptor were harvested and resuspended in a phosphate-buffered saline solution containing 25 mM glucose (Sigma Chemicals, St. Louis, MO) and 75 mM sucrose (PBS+GS) and pre-incubated with 25 μ Ci/ml myo-[³H]inositol (New England Nuclear, Boston, MA) for 60 min at 37°C. The cells were washed, resuspended in PBS+GS, and incubated with LiCl (100 mM) (Sigma Chemicals, St. Louis, MO) and the NMB analogs in a final volume of 0.30 ml. The reaction was terminated by the addition of chloroform/methanol (1:2) (Burdick & Johnson, Muskegeon, Michigan; Mallinckrodt, Paris, Kentucky), and the total [³H] inositol phosphates were isolated as described in Snider et al., J. Neurochem., 47: 1214 (1986). Peptide Dab⁵ is a potent agonist of the NMB receptor, with a K_i (μ M) of 78.1 ± 25.9 in the inositol phosphate turnover assay.

[0029] The following example describes a method for the determination of in vivo suppression of food intake using a cyclic octapeptide.

Example 6

[0030] Individually housed male Sprague-Dawley rats (Charles River, n=8) weighing 450-500 g. were maintained in a temperature-controlled room on a 12:12 hr. light: dark cycle. Rats were adapted to a 5 hr. food deprivation schedule followed by 60 min. access to a 0.5 kcal/ml glucose solution. Rats were injected intraperitoneally with either 0.9% saline (1.0 ml/kg) or 100 nmole/kg of peptide Orn⁵. One minute later, rats were injected intraperitoneally with either saline, 32.0 nmole/kg NMB, or 3.2 nmole/kg NMC (GRP18-27), the biologically active portion of GRP. These agonist doses have previously been determined to reliably suppress intake in this experimental paradigm, Ladenheim, et al., Amer. Physiol. Soc. R263-R266 (1991). Five minutes after the second injection, the glucose solution was presented and intake was monitored at 15, 30, 45 and 60 min. Each rat received all four conditions for both NMB and NMC. Administration of either the agonists or antagonist was separated by at least 48 hr. Data were statistically analysed using a 4 (injection) x 4 (time) analysis of the variance followed by planned t-test comparisons for each agonist. Because intake following the baseline condition (Saline+Saline) for both sets of experiments was not significantly different ($p > 0.5$) these were averaged and used to compare with effects of the agonists and peptide Orn⁵.

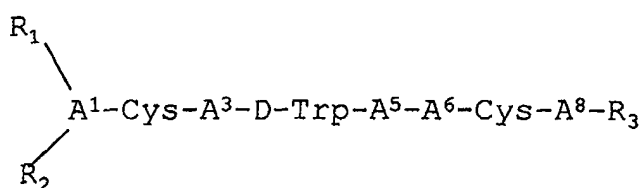
[0031] The results showed that both NMB (Fig. 1) and NMC (Fig.2) significantly suppressed food intake compared to baseline intake at all time points ($p < .01$). Prior administration of 100 nmole/kg of peptide Orn⁵ completely blocked the suppression of glucose intake produced by NMB. Intake for peptide Orn⁵+NMB and peptide Orn⁵+Saline conditions was greater than in the Saline+Saline condition ($p < .01$). In contrast to NMB, prior administration of peptide Orn⁵ had no effect on suppression of intake produced by NMC, in that suppression of intake in the Saline+NMC condition was not significantly different from intake in the peptide Orn⁵+NMC condition ($p > 0.5$).

Other Embodiments

[0032] From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

Claims

1. A cyclic octapeptide of the formula:



wherein:

A¹ is D-Nal or D-Trp;

A³ is Phe, F₅-Phe, or X-Phe wherein X is a halogen, NO₂, CH₃, or OH;

A⁵ is -NH-CH(Y)-CO- wherein Y is (CH₂)_m-R₄-N(R₅)(R₆) or (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆), provided that A⁵ is not Orn;

A⁶ is the D- or L- isomer of an amino acid selected from the group consisting of Thr, Leu, Ile, Nle, Val, Abu, Nal, Trp, Me-Trp, Bpa, F₅-Phe, Phe and X-Phe where X is a halogen, NO₂, CH₃, or OH;

A⁸ is Nal or Trp;

m is 1, 2, or 3;

n is 1, 2, 3, 4 or 5;

each of R₁ and R₂, independently, is H, E, COE, or COOE wherein E is C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, phenyl, naphthyl, C₇₋₁₂ phenylalkyl or alkylphenyl, C₈₋₁₂ phenylalkenyl or alkenylphenyl, C₈₋₁₂ phenylalkynyl or alkynylphenyl, C₁₁₋₂₀ naphthylalkyl or alkyl naphthyl, C₁₂₋₂₀ naphthylalkenyl or alkenyl naphthyl, or C₁₂₋₂₀ naphthylalkynyl or alkynyl naphthyl, provided that when one of R₁ or R₂ is COE or COOE, the other must be H;

R₃ is -NH₂;

R₄ is C₆H₄ or absent;

R₇ is =NR₈, =S, or =O;

each of R₅, R₆, and R₈, independently, is H or E; and the two Cys residues are linked together via a disulfide bond

2. A cyclic octapeptide of claim 1, wherein Y is

a) (CH₂)_m-R₄-N(R₅)(R₆); or

b) (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆), wherein R₇ is absent, and, in the case of b), R₇ is =NR₈.

3. A cyclic octapeptide of claim 2, wherein m is 2 or 3, n is 2, 3 or 4, and each of R₅, R₆ and R₈, where present, independently is H or C₁-C₅ alkyl.

4. A cyclic octapeptide of claim 3, wherein A⁶ is the D- or L- isomer of an amino acid selected from the group consisting of Thr, Leu, Ile, Nle, Trp, Val, and Abu.

5. A cyclic octapeptide of claim 4, wherein A³ is Phe or para-substituted X-Phe where X is Cl, F, or OH.

6. A cyclic octapeptide of claim 5, wherein A¹ is D-Nal and A⁸ is Nal.

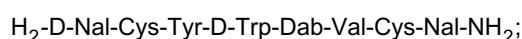
7. A cyclic octapeptide of claim 6, wherein A³ is Tyr and A⁶ is Val.

8. A cyclic octapeptide of claim 3, wherein A⁵, in the case of a), is Dab and, in the case of b), is Arg.

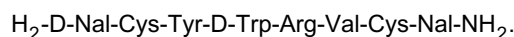
9. A cyclic octapeptide of claim 8, wherein A⁶ is a D- or L- isomer of an amino acid selected from the group consisting of Thr, Leu, Ile, Nle, Trp, Val, and Abu.

10. A cyclic octapeptide of claim 8, wherein A³ is Phe or para-substituted X-Phe where X is Cl, F, or OH; or wherein A¹ is D-Nal and A⁸ is Nal; or wherein A³ is Tyr and A⁶ is Val.

11. A cyclic octapeptide of claim 10, having the formula:



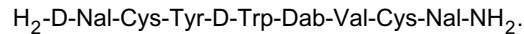
or having the formula:



12. A cyclic octapeptide of claim 9, wherein A¹ is D-Nal and A⁸ is Nal.

13. A cyclic octapeptide of claim 9, wherein A³ is Tyr and A⁶ is Val.

14. A cyclic octapeptide of claim 13, having the formula:



15. A cyclic octapeptide of claim 1, wherein Y is (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆).

16. A cyclic octapeptide of claim 15, wherein R₄ is absent, and R₇ is =NR₈.

17. A cyclic octapeptide of claim 16, wherein n is 2, 3, or 4; and each of R₅, R₆, and R₈, independently, is H or C₁-C₅ alkyl.

18. A cyclic octapeptide of claim 17, wherein A⁶ is a D- or L- isomer of an amino acid selected from the group consisting of Thr, Leu, Ile, Nle, Trp, Val and Abu.

19. A cyclic octapeptide of claim 18, wherein A³ is Phe, or para-substituted X-Phe where X is Cl, F, or OH.

20. A cyclic octapeptide of claim 19, wherein A¹ is D-Nal and A⁸ is Nal.

21. A cyclic octapeptide of claim 20, wherein A³ is Tyr and A⁶ is Val.

22. A cyclic octapeptide of claim 19, wherein A⁵ is Arg.

23. A cyclic octapeptide of claim 22, wherein A⁶ is a D- or L- isomer of an amino acid selected from the group consisting of Leu, Ile, Nle, Trp, Val, and Abu.

24. A cyclic octapeptide of claim 23, wherein A³ is Phe, or para-substituted X-Phe where X is Cl, F, or OH.

25. A cyclic octapeptide of claim 24, wherein A¹ is D-Nal and A⁸ is Nal.

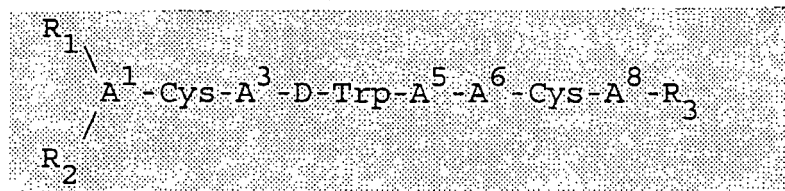
26. A cyclic octapeptide of claim 25, wherein A³ is Tyr and A⁶ is Val.

27. A cyclic octapeptide of claim 26, wherein said octapeptide is of the formula:



Patentansprüche

1. Cyclisches Octapeptid mit der Formel:



in der

A¹ D-Nal oder D-Trp ist;

A³ Phe, F₅-Phe oder X-Phe ist, wobei X Halogen, NO₂, CH₃ oder OH ist;

A⁵ -NH-CH(Y)-CO- ist, wobei Y (CH₂)_m-R₄-N(R₅)(R₆) oder (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆) ist, mit der Maßgabe, dass A⁵ nicht Orn ist;

A⁶ das D- oder L-Isomer einer Aminosäure ausgewählt aus der Gruppe bestehend aus Thr, Leu, Ile, Nle, Val, Abu, Nal, Trp, Me-Trp, Bpa, F₅-Phe, Phe und X-Phe ist, wobei X Halogen, NO₂, CH₃ oder OH ist;
A⁸ Nal oder Trp ist;
m 1, 2 oder 3 ist;
n 1, 2, 3, 4 oder 5 ist;

jedes von R₁ und R₂ unabhängig H, E, COE oder COOE ist, wobei E C₁₋₁₂-Alkyl, C₂₋₁₂-Alkenyl, C₂₋₁₂-Alkynyl, Phenyl, Naphthyl, C₇₋₁₂-Phenylalkyl oder -Alkylphenyl, C₈₋₁₂-Phenylalkenyl oder -Alkenylphenyl, C₈₋₁₂-Phenylalkinyl oder -Alkynylphenyl, C₁₁₋₂₀-Naphthylalkyl oder -Alkyl-naphthyl, C₁₂₋₂₀-Naphthylalkenyl oder -Alkenyl-naphthyl oder C₁₂₋₂₀-Naphthylalkinyl oder -Alkynyl-naphthyl ist, mit der Maßgabe, dass, wenn eines von R₁ oder R₂ COE oder COOE ist, das andere H sein muss;

R₃ -NH₂ ist;
R₄ C₆H₄ ist oder fehlt;
R₇ =NR₈, =S oder =O ist;

jedes von R₅, R₆ und R₈ unabhängig H oder E ist; und die beiden Cys-Reste über eine Disulfidbrücke miteinander verbunden sind.

2. Cyclisches Octapeptid nach Anspruch 1, bei dem Y

- a) (CH₂)_m-R₄-N(R₅) (R₆) oder
b) (CH₂)_n-R₄-NH-C(R₇)-N-(R₅) (R₆) ist, wobei R₄ fehlt und im Fall von b) R₇ =NR₈ ist.

3. Cyclisches Octapeptid nach Anspruch 2, bei dem m 2 oder 3 ist, n 2, 3 oder 4 ist, und jedes von R₅, R₆ und R₈, wo vorhanden, unabhängig H oder C₁- bis C₅-Alkyl ist.

4. Cyclisches Octapeptid nach Anspruch 3, bei dem A⁶ das D- oder L-Isomer einer Aminosäure ausgewählt aus der Gruppe bestehend aus Thr, Leu, Ile, Nle, Trp, Val und Abu ist.

5. Cyclisches Octapeptid nach Anspruch 4, bei dem A³ Phe oder para-substituiertes X-Phe ist, wobei x Cl, F oder OH ist.

6. Cyclisches Octapeptid nach Anspruch 5, bei dem A¹ D-Nal ist und A⁸ Nal ist.

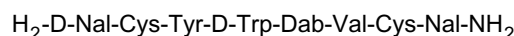
7. Cyclisches Octapeptid nach Anspruch 6, bei dem A³ Tyr ist und A⁶ Val ist.

8. Cyclisches Octapeptid nach Anspruch 3, bei dem A⁵ im Fall von a) Dab ist und im Fall von b) Arg ist.

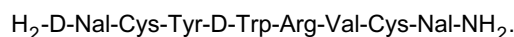
9. Cyclisches Octapeptid nach Anspruch 8, bei dem A⁶ ein D- oder L-Isomer einer Aminosäure ausgewählt aus der Gruppe bestehend aus Thr, Leu, Ile, Nle, Trp, Val und Abu ist.

10. Cyclisches Octapeptid nach Anspruch 8, bei dem A³ Phe oder para-substituiertes X-Phe ist, wobei X Cl, F oder OH ist; oder
bei dem A¹ D-Nal ist und A⁸ Nal ist; oder
bei dem A³ Tyr ist und A⁶ Val ist.

11. Cyclisches Octapeptid nach Anspruch 10 mit der Formel:



oder mit der Formel

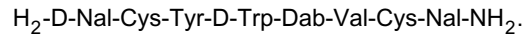


12. Cyclisches Octapeptid nach Anspruch 9, bei dem A¹ D-Nal ist und A⁸ Nal ist.

EP 1 011 716 B9 (W1B1)

13. Cyclisches Octapeptid nach Anspruch 9, bei dem A³ Tyr ist und A⁶ Val ist.

14. Cyclisches Octapeptid nach Anspruch 13 mit der Formel:



15. Cyclisches Octapeptid nach Anspruch 1, bei dem Y (CH₂)_n-R₄-NH-C(R₇)-N(R₅) (R₆) ist.

16. Cyclisches Octapeptid nach Anspruch 15, bei dem R₄ fehlt und R₇ =NR₈ ist.

17. Cyclisches Octapeptid nach Anspruch 16, bei dem n 2, 3 oder 4 ist, und jedes von R₅, R₆ und R₈ unabhängig H oder C₁bis C₅-Alkyl ist.

18. Cyclisches Octapeptid nach Anspruch 17, bei dem A⁶ ein D-oder L-Isomer einer Aminosäure ausgewählt aus der Gruppe bestehend aus Thr, Leu, Ile, Nle, Trp, Val und Abu ist.

19. Cyclisches Octapeptid nach Anspruch 18, bei dem A³ Phe oder para-substituiertes X-Phe ist, wobei X Cl, F oder OH ist.

20. Cyclisches Octapeptid nach Anspruch 19, bei dem A¹ D-Nal ist und A⁸ Nal ist.

21. Cyclisches Octapeptid nach Anspruch 20, bei dem A³ Tyr ist und A⁶ Val ist.

22. Cyclisches Octapeptid nach Anspruch 19, bei dem A⁵ Arg ist.

23. Cyclisches Octapeptid nach Anspruch 22, bei dem A⁶ ein D-oder L-Isomer einer Aminosäure ausgewählt aus der Gruppe bestehend aus Leu, Ile, Nle, Trp, Val und Abu ist.

24. Cyclisches Octapeptid nach Anspruch 23, bei dem A³ Phe oder para-substituiertes X-Phe ist, wobei X Cl, F oder OH ist.

25. Cyclisches Octapeptid nach Anspruch 24, bei dem A¹ D-Nal ist und A⁸ Nal ist.

26. Cyclisches Octapeptid nach Anspruch 25, bei dem A³ Tyr ist und A⁶ Val ist.

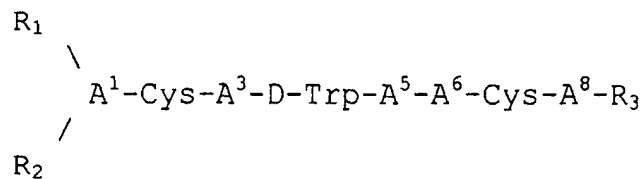
27. Cyclisches Octapeptid nach Anspruch 26, bei dem das Octapeptid die Formel



hat.

Revendications

1. Octapeptide cyclique de la formule :



dans laquelle :

EP 1 011 716 B9 (W1B1)

- A¹ représente D-Nal ou D-Trp ;
- A³ représente Phe, F₅-Phe ou X-Phe, où X représente un halogène, NO₂, CH₃ ou OH ;
- A⁵ représente -NH-CH(Y)-CO-, où Y représente (CH₂)_m-R₄-N(R₅)(R₆) ou (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆), à la condition que A⁵ ne représente pas Orn ;
- A⁶ est l'isomère D ou L d'un acide aminé choisi dans le groupe constitué par Thr, Leu, Ile, Nle, Val, Abu, Nal, Trp, Me-Trp, Bpa, F₅-Phe, Phe et X-Phe, où X représente un halogène, NO₂, CH₃ ou OH ;
- A⁸ représente Nal ou Trp ;
- m vaut 1, 2 ou 3 ;
- n vaut 1, 2, 3, 4 ou 5 ;
- R₁ et R₂ représentent chacun indépendamment H, E, COE ou CODE, où E représente alkyle en C₁₋₁₂, alcényle en C₂₋₁₂, alcynyle en C₂₋₁₂, phényle, naphtyle, phénylalkyle ou alkylphényle en C₇₋₁₂, phénylalcényle ou alcénylphényle en C₈₋₁₂, phénylalcynyle ou alcynylphényle en C₈₋₁₂, naphtylalkyle ou alkylnaphtyle en C₁₁₋₂₀, naphtylalcényle ou alcénylnaphtyle en C₁₂₋₂₀, ou naphtylalcynyle ou alcynylnaphtyle en C₁₂₋₂₀, à la condition que, lorsque l'un parmi R₁ ou R₂ représente COE ou CODE, l'autre doit être H ;
- R₃ représente -NH₂ ;
- R₄ représente C₆H₄ ou est absent ;
- R₇ représente =NR₈, =S ou =O ;
- R₅, R₆ et R₈ représentent chacun indépendamment H ou E ; et
- les deux résidus Cys sont liés ensemble par l'intermédiaire d'une liaison disulfure.

2. Octapeptide cyclique selon la revendication 1, dans lequel Y représente :

(a) (CH₂)_m-R₄-N(R₅)(R₆) ; ou

(b) (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆), où R₄ est absent et, dans le cas de (b), R₇ représente =NR₈.

3. Octapeptide cyclique selon la revendication 2, dans lequel m vaut 2 ou 3, n vaut 2, 3 ou 4, et R₅, R₆ et R₈, s'ils sont présents, représentent chacun indépendamment H ou alkyle en C₁-C₅.

4. Octapeptide cyclique selon la revendication 3, dans lequel A⁶ est l'isomère D ou L d'un acide aminé choisi dans le groupe constitué par Thr, Leu, Ile, Nle, Trp, Val et Abu.

5. Octapeptide cyclique selon la revendication 4, dans lequel A³ représente Phe ou X-Phe substitué en para, où X représente Cl, F ou OH.

6. Octapeptide cyclique selon la revendication 5, dans lequel A¹ représente D-Nal et A⁸ représente Nal.

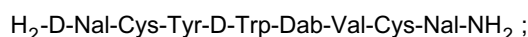
7. Octapeptide cyclique selon la revendication 6, dans lequel A³ représente Tyr et A⁶ représente Val.

8. Octapeptide cyclique selon la revendication 3, dans lequel A⁵, dans le cas de (a), représente Dab et, dans le cas de (b), représente Arg.

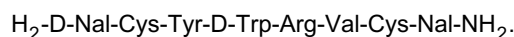
9. Octapeptide cyclique selon la revendication 8, dans lequel A⁶ est un isomère D ou L d'un acide aminé choisi dans le groupe constitué par Thr, Leu, Ile, Nle, Trp, Val et Abu.

10. Octapeptide cyclique selon la revendication 8, dans lequel A³ représente Phe ou X-Phe substitué en para, où X représente Cl, F ou OH ; ou dans lequel A¹ représente D-Nal et A⁸ représente Nal ; ou dans lequel A³ représente Tur et A⁶ représente Val.

11. Octapeptide cyclique selon la revendication 10, ayant la formule :



ou ayant la formule :

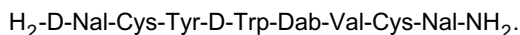


EP 1 011 716 B9 (W1B1)

12. Octapeptide cyclique selon la revendication 9, dans lequel A¹ représente D-Nal et A⁸ représente Nal.

13. Octapeptide cyclique selon la revendication 9, dans lequel A³ représente Tyr et A⁶ représente Val.

5 14. Octapeptide cyclique selon la revendication 13, ayant la formule :



10 15. Octapeptide cyclique selon la revendication 1, dans lequel Y représente (CH₂)_n-R₄-NH-C(R₇)-N(R₅)(R₆).

16. Octapeptide cyclique selon la revendication 15, dans lequel R₄ est absent, et R₇ représente =NR₈.

15 17. Octapeptide cyclique selon la revendication 16, dans lequel n vaut 2, 3 ou 4 ; et R₅, R₆ et R₈ représentent chacun indépendamment H ou alkyle en C₁-C₅.

18. Octapeptide cyclique selon la revendication 17, dans lequel A⁶ représente un isomère D ou L d'un acide aminé choisi dans le groupe constitué par Thr, Leu, Ile, Nle, Trp, Val et Abu.

20 19. Octapeptide cyclique selon la revendication 18, dans lequel A³ représente Phe ou X-Phe substitué en para, où X représente Cl, F ou OH.

20. Octapeptide cyclique selon la revendication 19, dans lequel A¹ représente D-Nal et A⁸ représente Nal.

25 21. Octapeptide cyclique selon la revendication 20, dans lequel A³ représente Tyr et A⁶ représente Val.

22. Octapeptide cyclique selon la revendication 19, dans lequel A⁵ représente Arg.

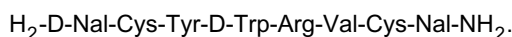
30 23. Octapeptide cyclique selon la revendication 22, dans lequel A⁶ représente un isomère D ou L d'un acide aminé choisi dans le groupe constitué par Leu, Ile, Nle, Trp, Val et Abu.

24. Octapeptide cyclique selon la revendication 23, dans lequel A³ représente Phe ou X-Phe substitué en para, où X représente Cl, F ou OH.

35 25. Octapeptide cyclique selon la revendication 24, dans lequel A¹ représente D-Nal et A⁸ représente Nal.

26. Octapeptide cyclique selon la revendication 25, dans lequel A³ représente Tyr et A⁶ représente Val.

40 27. Octapeptide cyclique selon la revendication 26, dans lequel ledit octapeptide est représenté par la formule :



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