Example 2-1: Synthesis of oxyntomodulin derivatives

In order to measure in vitro activities of oxyntomodulin derivatives, oxyntomodulin derivatives having the following amino acid sequences were synthesized (Table 1).

【Table 1】

Oxyntomodulin and oxyntomodulin derivatives

SEQ ID NO.	**Amino acid sequence**	**Note**
SEQ ID NO. 1	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA	
SEQ ID NO. 2	CA-SQGTFTSDYSKYLDEEAVRLFIEWLMNTKRNRNNIA	
SEQ ID NO. 3	CA-SQGTFTSDYSKYLDERRAQDFVAWLKNTGPSSGAPPPS	
SEQ ID NO. 4	CA-GQGTFTSDYSRYLEEEAVRLFIEWLKNGGPSSGAPPPS	
SEQ ID NO. 5	CA-GQGTFTSDYSRQMEEEAVRLFIEWLKNGGPSSGAPPPS	
SEQ ID NO. 6	CA-GEGTFTSDLSRQMEEEAVRLFIEWAAHSQGTFTSDYSKYLD	
SEQ ID NO. 7	CA-SQGTFTSDYSRYLDEEAVRLFIEWLMNTK	
SEQ ID NO. 8	CA-SQGTFTSDLSRQLEEEAVRLFIEWLMNK	
SEQ ID NO. 9	CA-GQGTFTSDYSRYLDEEAVXLFIEWLMNTKRNRNNIA	
SEQ ID NO. 10	CA-SQGTFTSDYSRQMEEEAVRLFIEWLMNGGPSSGAPPPSK	
SEQ ID NO. 11	CA-GEGTFTSDLSRQMEEEAVRLFIEWAAHSQGTFTSDYSRYLDK	
SEQ ID NO. 12	CA-SQGTFTSDYSRYLDGGGHGEGTFTSDLSKQMEEEAVK	
SEQ ID NO. 13	CA-SQGTFTSDYSRYLDXEAVXLFIEWLMNTK	
SEQ ID NO. 14	CA-GQGTFTSDYSRYLDEEAVXLFIXWLMNTKRNRNNIA	
SEQ ID NO. 15	CA-GQGTFTSDYSRYLDEEAVRLFIXWLMNTKRNRNNI	

	A	
SEQ ID NO. 16	CA-SQGTFTSDLSRQLEGGGHSQGTFTSDLSRQLEK	
SEQ ID NO. 17	CA-SQGTFTSDYSRYLDEEAVRLFIEWIRNTKRNRNNI A	
SEQ ID NO. 18	CA-SQGTFTSDYSRYLDEEAVRLFIEWIRNGGPSSGAP PPSK	
SEQ ID NO. 19	CA-SQGTFTSDYSRYLD**E**EAV**K**LFIEWIRNTKRNRNNI A	Ring Formation
SEQ ID NO. 20	CA-SQGTFTSDYSRYLD**E**EAV**K**LFIEWIRNGGPSSGAP PPSK	Ring Formation
SEQ ID NO. 21	CA-SQGTFTSDYSRQLEEEAVRLFIEWVRNTKRNRNNI A	
SEQ ID NO. 22	DA-SQGTFTSDYSKYLD**E**KRA**K**EFVQWLMNTK	Ring Formation
SEQ ID NO. 23	HAibQGTFTSDYSKYLDEKRAKEFVCWLMNT	
SEQ ID NO. 24	HAibQGTFTSDYSKYLDEKRAKEFVQWLMNTC	
SEQ ID NO. 25	HAibQGTFTSDYSKYLD**E**KRA**K**EFVQWLMNTC	Ring Formation
SEQ ID NO. 26	HAibQGTFTSDYS**K**YLD**E**KRAKEFVQWLMNTC	Ring Formation
SEQ ID NO. 27	HAibQGTFTSDYSKYLD**E**QAA**K**EFICWLMNT	Ring Formation
SEQ ID NO. 28	HAibQGTFTSDYSKYLDEKRAKEFVQWLMNT	
SEQ ID NO. 29	CA-AibQGTFTSDYSKYLD**E**KRA**K**EFVQWLMNTC	Ring Formation
SEQ ID NO. 30	HAibQGTFTSDYAKYLD**E**KRA**K**EFVQWLMNTC	Ring Formation
SEQ ID NO. 31	YAibQGTFTSDYSKYLD**E**KRA**K**EFVQWLMNTC	Ring Formation

In Table 1, amino acids in bold and underlined in each of SEQ IN NOS: 19, 20, 22, 25, 26, 27, 29, 30, and 31, taken together, form a ~~represent~~ ring ~~formation~~, and amino acids represented by X mean a non-native amino acid, alpha-methyl-glutamic acid. In addition,

CA represents 4-imidazoacetyl, and DA represents desamino-histidyl.

Example 2-2: Test on in vitro activity of oxyntomodulin derivatives

In order to measure anti-obesity efficacies of the oxyntomodulin derivatives synthesized in Example 2-1, cell activity was measured in vitro using the cell lines prepared in Examples 1-1 and 1-2.

The cell lines were those prepared by transfecting CHO (Chinese Hamster Ovary) to express human GLP-1 receptor gene and glucagon receptor gene, respectively. Thus, they are suitable to measure GLP-1 and glucagon activities. Therefore, the activity of each oxyntomodulin derivative was measured using each transformed cell line.

Specifically, each cell line was sub-cultured twice or three time a week, and aliquoted in each well of a 96-well plate at a density of 1 X 10^5, followed by cultivation for 24 hours.

The cultured cells were washed with KRB buffer and suspended in 40 ml of KRB buffer containing 1 mM IBMX, and left at room temperature for 5 minutes. Oxyntomodulin (SEQ ID NO. 1) and oxyntomodulin derivatives (represented by SEQ ID NOs. 2-6, 8, 10-13, 17, 18, 23-25, 27-30 and 31) were diluted from 1000 nM to 0.02 nM by 5-fold serial dilution, and each 40 mL thereof was added to the cells, and cultured at 37°C for 1 hour in a CO2 incubator. Then, 20 mL of cell lysis buffer was added for cell lysis, and the cell lysates were applied to a cAMP assay kit (Molecular Device, USA) to measure cAMP concentrations. EC_{50} values were calculated therefrom, and compared to each other. EC_{50} values are shown in the following Table 2.

【Table 2】

Comparison of in vitro activities for GLP-1 receptor and glucagon receptor between oxyntomodulin and oxyntomodulin derivatives

SEQ ID NO.	EC_{50}(nM)	
SEQ ID NO.	CHO/GLP-1R	CHO/GCGR
SEQ ID NO. 1	50 - 210	10 - 43

SEQ ID NO. 2	51.8	12.8
SEQ ID NO. 3	>1,000	637.7
SEQ ID NO. 4	5.5	>1,000
SEQ ID NO. 5	5.9	>1,000
SEQ ID NO. 6	500.1	>1,000
SEQ ID NO. 8	419.6	>1,000
SEQ ID NO. 10	>1,000	>1,000
SEQ ID NO. 11	>1,000	>1,000
SEQ ID NO. 12	>1,000	>1,000
SEQ ID NO. 13	>1,000	>1,000
SEQ ID NO. 17	97.9	>1,000
SEQ ID NO. 18	96.3	>1,000
SEQ ID NO. 23	2.46	5.8
SEQ ID NO. 24	1.43	6.95
SEQ ID NO. 25	1.9	1.3
SEQ ID NO. 27	2.8-5.5	3.1-5.6
SEQ ID NO. 28	3.1	0.3
SEQ ID NO. 29	14.25	17.3
SEQ ID NO. 30	2.20	80.2
SEQ ID NO. 31	12.5	1.0

As shown in Table 2, there were oxyntomodulin derivatives showing excellent in vitro activities and different ratios of activities on GLP-1 receptor and glucagon receptor, compared to native oxyntomodulin of SEQ ID NO. 1.

It is known that oxyntomodulin activates both the GLP-1 receptor and glucagon receptor to suppress appetite, facilitate lipolysis, and promote satiety, thereby showing anti-obesity effects. The oxyntomodulin derivatives according to the present invention show higher in vitro activities on both the GLP-1 receptor and glucagon receptor than the wild-type oxyntomodulin, and therefore can be used as a therapeutic agent for obesity with higher efficacies than the known oxyntomodulin.

Example 3. Test on in vivo activity of oxyntomodulin derivatives

In order to measure in vivo therapeutic activity of oxyntomodulin derivatives, changes in food intake by administration of oxyntomodulin derivatives were examined in ob/ob mouse using native oxyntomodulin as a control.

Specifically, obese diabetic ob/ob mice, commonly used to test the efficacies of therapeutic agents for obesity and diabetes, were fasted for 16 hours, and administered with 1 or 10 mg/kg of oxyntomodulin, or 0.02, 0.1, 1 or 10 mg/kg of the oxyntomodulin derivative of SEQ ID NO. 2. Then, food intake was examined for 2 hours (FIG. 1). FIG. 1 is a graph showing changes in food intake according to administration dose of oxyntomodulin or oxyntomodulin derivative. As shown in FIG. 1, administration of 1 mg/kg of oxyntomodulin derivative showed more excellent inhibitory effects on food intake than administration of 10 mg/kg of oxyntomodulin.

Taken together, the oxyntomodulin derivatives of the present invention have much higher anti-obesity effects than the wild-type oxyntomodulin, even though administered at a lower dose, indicating improvement in the problems of the wild-type oxyntomodulin that shows lower anti-obesity effects and should be administered at a high dose three times a day.

[Claims]

[Claim 1]

A peptide comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 23 to 28, and 30. ~~of following Formula 1:~~

~~R1-X1-X2-GTFTSD-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21-X22-X23-X24-R2 (Formula 1)~~

~~wherein, R1 is histidine;~~

~~X1 is Aib(aminoisobutyric acid);~~

~~X2 is glutamic acid or glutamine;~~

~~X3 is leucine or tyrosine;~~

~~X4 is serine or alanine;~~

~~X5 is lysine or arginine;~~

~~X6 is glutamine or tyrosine;~~

~~X7 is leucine or methionine;~~

~~X8 is aspartic acid or glutamic acid;~~

~~X9 is glutamic acid, serine, alpha-methyl-glutamic acid or is deleted;~~

~~X10 is glutamine, glutamic acid, lysine, arginine, serine or is deleted;~~

~~X11 is alanine, arginine, valine or is deleted;~~

~~X12 is alanine, arginine, serine, valine or is deleted;~~

~~X13 is lysine, glutamine, arginine, alpha-methyl-glutamic acid or is deleted;~~

~~X14 is aspartic acid, glutamic acid, leucine or is deleted;~~

~~X15 is phenylalanine or is deleted;~~

~~X16 is isoleucine, valine or is deleted;~~

~~X17 is alanine, cysteine, glutamic acid, lysine, glutamine, alpha-methyl-glutamic acid or is deleted~~

~~X18 is tryptophan or is deleted;~~

~~X19 is alanine, isoleucine, leucine, serine, valine or is deleted;~~

~~X20 is alanine, lysine, methionine, glutamine, arginine or is deleted;~~

~~X21 is asparagine or is deleted;~~

~~X22 is alanine, glycine, threonine or is deleted;~~

~~X23 is cysteine, lysine or is deleted;~~

~~X24 is a peptide having 2 to 10 amino acids consisting of combinations of alanine, glycine and serine, or is deleted; and~~

~~R2 is KRNRNNIA(SEQ ID NO. 32), GPSSGAPPPS(SEQ ID NO. 33), GPSSGAPPPSK(SEQ ID NO. 34), HSQGTFTSDYSKYLD(SEQ ID NO. 35), HSQGTFTSDYSRYLDK(SEQ ID NO. 36), HGEGTFTSDLSKQMEEEAVK(SEQ ID NO. 37) or is deleted.~~

[Claim 2]

The peptide according to claim 1, wherein the peptide is an oxyntomodulin derivative capable of activating GLP-1 receptor and glucagon receptor.

[Claim 3]
The peptide according to claim 1, wherein the peptide has anti-obesity effects.

~~[Claim 4]~~
~~The peptide according to claim 1, wherein X19 is leucine, X20 is methionine, X21 is asparagine, and X22 is threonine in the amino acid sequence of Formula 1.~~

[Claim ~~5~~4]
The peptide according to claim 1, wherein one or more amino acid pairs at positions ~~10 and 14, 12~~ and 16, or 16 and 20~~, 20 and 24, and 24 and 28~~ of the amino acid sequence ~~of Formula 1 are substituted with glutamic acid or lysine which can~~ form a ring.

~~[Claim 6]~~
~~The peptide according to claim 5, wherein one or more amino acid pairs at positions 10 and 14, 12 and 16, 16 and 20, 20 and 24, and 24 and 28 of the amino acid sequence of Formula 1 are substituted with glutamic acid or lysine to form a ring.~~

~~[Claim 7]~~
~~The peptide according to claim 1, wherein the peptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs. 23 to 28, and 30.~~

~~[Claim 8]~~
~~The peptide according to claim 1, wherein X24 and R2 are deleted in the amino acid sequence of Formula 1.~~

~~[Claim 9]~~
~~The peptide according to claim 1, wherein X2 is glutamine in the amino acid sequence of Formula 1.~~

~~[Claim 10]~~
~~A polynucleotide encoding the peptide of claim 1.~~

[Claim ~~11~~5]
A pharmaceutical composition for the prevention or treatment of obesity, comprising the peptide of claim 1 as an active ingredient.

[Claim ~~12~~6]
The pharmaceutical composition according to claim ~~11~~5, further comprising a pharmaceutically acceptable carrier.

[Claim ~~13~~7]

The pharmaceutical composition according to claim ~~11~~5, wherein the composition is to be administered alone or in combination with other pharmaceutical formulations showing prophylactic or therapeutic effects on obesity.

[Claim ~~148~~]
The pharmaceutical composition according to claim ~~137~~, wherein the pharmaceutical formulation showing prophylactic or therapeutic effects on obesity is selected from the group consisting of a GLP-1 receptor agonist, a leptin receptor agonist, a DPP-IV inhibitor, a Y5 receptor antagonist, a Melanin-concentrating hormone (MCH) receptor antagonist, a Y2/3 receptor agonist, a MC3/4 receptor agonist, a gastric/pancreatic lipase inhibitor, a 5HT2c agonist, a β3A receptor agonist, an Amylin receptor agonist, a Ghrelin antagonist, and a Ghrelin receptor antagonist.