Bioorganic & Medicinal Chemistry Letters 27 (2017) 3829–3832

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potent antiobesity effect of a short-length peptide YY-analogue continuously administered in mice

Naoki Nishizawa, Ayumu Niida, Yusuke Adachi, Yasushi Masuda, Satoshi Kumano, Kotaro Yokoyama, Tomoko Asakawa, Hideki Hirabayashi, Nobuyuki Amano, Shiro Takekawa, Tetsuya Ohtaki, Taiji Asami *

Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd., Fujisawa 251-8555, Japan

ARTICLE INFO

Article history:
Received 9 May 2017
Revised 18 June 2017
Accepted 21 June 2017
Available online 23 June 2017

Keywords:
Peptide YY (PYY)
Y2 receptor (Y2R)
Antiobesity
Antidiabetes
Anorectic effect
Short-length PYY analogue

ABSTRACT

The gastrointestinal peptide, peptide YY_{3-36} (PYY_{3-36}) and its shorter peptide analogues have been reported to reduce appetite by activating the neuropeptide Y2 receptor (Y2R), which is associated with obesity and other metabolic diseases. A 14-amino acid PYY analogue, Ac-[D-Pro^{24},$Cha^{27,28,36}$,Aib^{31}]PYY (23–36) (**3**), showed high binding affinity and agonist activity for the Y2R, similar to that of PYY_{3-36}, but had weak anorectic activity upon continuous administration in lean mice. Three amino acid substitutions [$Pya(4)^{26}$, Aib^{28}, Lys^{30}], which contributed to the decreased hydrophobicity of **3**, efficiently increased its anorectic activity. The compound containing these three amino acids, Ac-[D-Pro^{24},$Pya(4)^{26}$,$Cha^{27,36}$,$Aib^{28,31}$,Lys^{30}]PYY(23–36) (**22**), exerted more potent and durable food intake suppression than that by PYY_{3-36} in lean mice, as well as excellent Y2R agonist activity (EC_{50}: 0.20 nM) and good subcutaneous bioavailability (66.6%). The 11-day continuous administration of **22** at 1 mg/kg/day successfully produced antiobese and antidiabetic effects, with more than 20% body weight loss in obese and Type 2 diabetes ob/ob model mice.

Appetite and feeding are regulated by complex networks of a variety of molecules. Among these molecules, gastrointestinal peptides, such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), are thought to send satiety signals to the brain on a short-term basis.[1–3] Peptide YY is an anorectic hormone, which is secreted from the L cells of the duodenum, ileum, and colon after a meal.[4–6] Two physiologically active forms of PYY, PYY_{1-36} and PYY_{3-36}, were identified as endogenous peptides; PYY_{1-36} is processed by plasma dipeptidyl peptidase IV to produce PYY_{3-36}.[7,8]

Peptide YY_{3-36} reduced hunger more potently than did PYY_{1-36}.[9] Intraperitoneal administration of PYY_{3-36} inhibited food intake and increased c-Fos expression in the arcuate nucleus of the hypothalamus,[10] suggesting the activation of neuronal pathways. The anorectic effect of PYY_{3-36} is elicited when this hormone binds to the neuropeptide Y Y2 receptor (Y2R). The activation of Y2R by peripheral administration of PYY_{3-36} suppresses the neuropeptide Y (NPY)/agouti-related peptide neuron in the hypothalamus, which facilitates the stimulation of POMC neurons to reduce food intake and body weight. Abdominal vagotomy abolished the anorectic effect elicited by the peripheral administration of PYY_{3-36} in rats, suggesting that the function of PYY_{3-36} is mediated via the afferent vagus nerve.[10] Plasma PYY concentration and its increase after a meal are lower in morbidly obese than in non-obese patients.[11] Therefore, the anorectic peptide PYY and Y2R are of interest for possible antiobesity therapeutics.

Peptide YY_{3-36} and a 12-amino acid PYY analogue, benzoyl-[$Cha^{27,28,36}$,Aib^{31}]PYY(25–36) (**1**),[a] were found to suppress food intake after intraperitoneal bolus administration at doses of 250 and 500 nmol/kg (ca. 0.43 and 0.86 mg/kg for **1**) in C57BL/6J mice.[12] In addition, PYY_{3-36} induced body weight reduction, as well as food intake inhibition, after its continuous subcutaneous administration at 1 mg/kg/day in C57BL/6J diet-induced obese (DIO) mice.[13] Conversely, **1** was ineffective when continuously administered at the same dose for 2 weeks. The relatively hydrophobic peptide **1** had a

Abbreviations: Aib, 2-aminoisobutyric acid; BA, bioavailability; Cha, 3-cyclohexylalanine; CHO, Chinese hamster ovary; DIO, diet-induced obese; Hyp, *trans*-4-hydroxyproline; Leu(Me), γ-methylleucine; MAT, mean absorption time; MRT, mean residence time; RP-HPLC, reversed-phase high performance liquid chromatography; Pya(4), 3-(4-pyridyl)alanine; PYY, peptide YY; TFA, trifluoroacetic acid.

* Corresponding author.
E-mail address: taiji.asami@takeda.com (T. Asami).

[a] Abbreviations used for amino acids and designation of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature.[16,17] Amino acid symbols denote the L-configuration unless indicated otherwise

http://dx.doi.org/10.1016/j.bmcl.2017.06.055
0960-894X/

long mean absorption time (MAT) value of 2.72 h for such a short-length peptide, which indicated a slow absorption rate from subcutaneous tissues. The pharmacokinetic profile of **1** affected the discrepancy of anorectic effects observed between the intraperitoneal and subcutaneous dosing. The *N*-terminal elongation and the amino acid substitution at position 24 of **1** resulted in a hydrophilic 14-amino acid peptide, Ac-[D-Hyp^{24},Cha^{27,28,36},Aib^{31}]PYY(23–36) (**2**), which showed improved in vitro activities and a potent anorectic effect after continuous dosing when compared to the effects of PYY_{3-36}.[13] The MAT value of **2** was low (0.14 h) because of its hydrophilic property. The subcutaneous bioavailability (BA) of **2** was higher than that of **1** (18.6% vs. 5.9%, respectively). The antiobesity effects of **2** and PYY_{3-36} were comparable (7.4% and 8.3% body weight loss, respectively) after their continuous administration to DIO mice at 1 mg/kg/day for 7 days. These results suggested the existence of small-sized PYY_{3-36} analogues without any bulky modification (e.g., PEG, albumin, and long alkyl chain), which would form a new option of potent anorectic peptides with the potential for sustained-release formulation. However, there is still a considerable interest to develop analogues that are more potent by improving pharmacokinetic profiles. The aim of this study was to provide a preclinical proof-of-concept for a sustained-release formulation of PYY analogues.

The results of in vitro and in vivo screenings are shown in Table 1. Compound **3**, an analogue of **2** with a substitution of D-Pro for D-Hyp at position 24, showed a high affinity and potent agonist activity for Y2R (Table 1). The moderate anorectic activity of **3** was observed during its continuous dosing at 1 mg/kg/day for 3 days in lean mice. Thus, **3** was selected as the lead compound to discover more potent analogues with decreased hydrophobicity, by exploring hydrophobic residues substitutions at positions 27, 28, and 30, namely at Cha^{27}, Cha^{28}, and Leu^{30} of **3**.

Hydrophilic amino acid replacement with Ser (**4**) at position 30 slightly decreased binding affinity. The substitution of 3-(4-pyridyl)alanine^{30} [Pya(4)^{30}] (**5**), a relatively hydrophilic amino acid with aromaticity and basicity, also deteriorated Y2R affinity. Similar in vitro activities were found when replacing Leu^{30} with norleucine (Nle) (**6**). The substitutions of Lys (**7**), ornithine (Orn) (**8**), and Arg (**9**) residues, all having a linear methylene side chain as observed in Nle, were also beneficial to maintain potent in vitro activities. Among these peptides, **7** and **9** remarkably potentiated anorectic activity compared to that by **3** [79% (**7**), 59% (**9**), and 33% (**3**) food intake inhibition at 1 mg/kg/day, respectively].

Regarding position 28, the substitution of γ-methylleucine [Leu(Me)] (**10**) maintained potent in vitro activities and potentiated anorectic activity (59% inhibition). Conversely, replacement with

Table 1
Biological activities of peptide YY (PYY) analogues substituted at the N-terminus and at positions 26, 27, 28, and 30.

R-AA23-AA24-Arg-AA26-AA27-AA28-Asn-AA30-Aib-ThrArg-Gln-Arg-Cha-NH_2

Compound	Structure							Binding affinity	Agonist activity	% Food intake inhibition[c]			
	R	AA23	AA24	AA26	AA27	AA28	AA30	IC_{50} (nM)[a]	EC_{50} (nM)[b]	Continuous dosing (mg/kg/day)			
										1	0.3	0.1	0.03
PYY_{3-36}								0.57	0.28	100	48	33	5
1	benzoyl	–	–	His	Cha	Cha	Leu	7.4 (4.3–13)	2.0 (1.2–3.3)	ND	ND	ND	ND
2	Ac	Ser	D-Hyp	His	Cha	Cha	Leu	0.70 (0.61–0.80)	0.20 (0.13–0.29)	79	47	18	ND
3	Ac	Ser	D-Pro	His	Cha	Cha	Leu	1.5 (1.2–1.9)	0.61 (0.38–0.98)	33	ND	ND	ND
4	Ac	Ser	D-Pro	His	Cha	Cha	Ser	2.3 (1.9–2.7)	0.60 (0.39–0.92)	ND	ND	ND	ND
5	Ac	Ser	D-Pro	His	Cha	Cha	Pya(4)	3.0 (2.4–3.7)	0.48 (0.38–0.60)	ND	ND	ND	ND
6	Ac	Ser	D-Pro	His	Cha	Cha	Nle	1.3 (1.1–1.6)	0.21 (0.13–0.36)	ND	ND	ND	ND
7	Ac	Ser	D-Pro	His	Cha	Cha	Lys	0.55 (0.42–0.74)	0.20 (0.11–0.33)	79	ND	ND	ND
8	Ac	Ser	D-Pro	His	Cha	Cha	Orn	1.2 (0.94–1.5)	0.068 (0.026–0.18)	22	ND	ND	ND
9	Ac	Ser	D-Pro	His	Cha	Cha	Arg	0.60 (0.52–0.68)	0.16 (0.081–0.30)	59	ND	ND	ND
10	Ac	Ser	D-Pro	His	Cha	Leu(Me)	Leu	0.94 (0.75–1.2)	0.41 (0.32–0.53)	59	ND	ND	ND
11	Ac	Ser	D-Pro	His	Cha	His	Leu	2.8 (2.0–3.8)	1.0 (0.76–1.3)	ND	ND	ND	ND
12	Ac	Ser	D-Pro	His	Cha	Pya(4)	Leu	2.5 (1.9–3.2)	0.75 (0.55–1.0)	41	ND	ND	ND
13	Ac	Ser	D-Pro	His	Cha	Aib	Leu	1.4 (1.1–1.8)	0.66 (0.50–0.86)	59	ND	ND	ND
14	Ac	Ser	D-Pro	His	Aib	Cha	Leu	2.6 (1.9–3.5)	1.7 (1.1–2.7)	ND	ND	ND	ND
15	Ac	Ser	D-Pro	His	Tyr	Cha	Leu	5.3 (4.3–6.5)	2.5 (1.9–3.4)	ND	ND	ND	ND
16	Ac	Ser	D-Pro	His	His	Cha	Leu	11 (8.1–14)	3.8 (3.3–4.3)	ND	ND	ND	ND
17	Ac	Ser	D-Pro	His	Pya(4)	Cha	Leu	6.0 (5.2–6.9)	1.8 (1.6–2.0)	ND	ND	ND	ND
18	Ac	Ser	D-Pro	His	Gln	Cha	Leu	8.7 (6.4–12)	4.4 (3.7–5.2)	ND	ND	ND	ND
19	Ac	Ser	D-Pro	Gln	Cha	Cha	Leu	1.1 (0.89–1.3)	0.42 (0.31–0.56)	55	ND	ND	ND
20	Ac	Ser	D-Pro	Ser	Cha	Cha	Leu	0.97 (0.81–1.2)	0.33 (0.23–0.47)	54	ND	ND	ND
21	Ac	Ser	D-Pro	Pya(4)	Cha	Cha	Leu	0.49 (0.43–0.57)	0.19 (0.15–0.24)	72	ND	ND	ND
22	Ac	Ser	D-Pro	Pya(4)	Cha	Aib	Lys	0.92 (0.75–1.1)	0.20 (0.11–0.37)	ND	112	80	38

ND: not determined.

[a] IC_{50} values [nM (95% confidence interval)] are the concentrations required to displace radiolabel binding by 50%.

[b] EC_{50} values [nM (95% confidence interval)] of agonist activities were determined as the concentrations of peptide analogues that induce 50% of the maximum [^{35}S]GTPγS binding.

[c] Percentage inhibition of food intake after the continuous administration of peptide analogues at 0.03, 0.1, 0.3, or 1 mg/kg/day, during 3 days, compared to that after the injection of PYY_{3-36} at 1 mg/kg/day (defined as 100% food intake inhibition) in 12-week-old male C57BL/6J mice (n = 5 per group).