Bioorganic & Medicinal Chemistry Letters 27 (2017) 4626–4629

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# A potent neuromedin U receptor 2-selective alkylated peptide

Naoki Nishizawa *, Yoko Kanematsu-Yamaki, Masaaki Funata, Hiroaki Nagai, Ayako Shimizu, Hisashi Fujita, Junichi Sakamoto, Shiro Takekawa, Taiji Asami *

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

ARTICLE INFO

*Article history:*
Received 22 July 2017
Revised 4 September 2017
Accepted 8 September 2017
Available online 9 September 2017

*Keywords:*
Neuromedin U (NMU)
NMU-8
Polyethylene glycol (PEG)
Diabetes
Obesity

ABSTRACT

Neuromedin U (NMU) mediates various physiological functions via NMUR1 and NMUR2 receptors. NMUR2 has been considered a promising treatment option for diabetes and obesity. Although NMU-8, a shorter peptide, has potent agonist activity for both receptors, it is metabolically unstable. Therefore, NMU-8 analogs modified with long-chain alkyl moieties via a linker were synthesized. An octadecanoyl analog (**17**) with amino acid substitutions [αMePhe$^{19}$, Nle$^{21}$, and Arg(Me)$^{24}$] and a linker [Tra-γGlu-PEG (2)] dramatically increased NMUR2 selectivity, with retention of high agonist activity. Subcutaneous administration of **17** induced anorectic activity in C57BL/6J mice. Owing to its high metabolic stability, **17** would be useful in clarifying the physiological role and therapeutic application of NMU.



Feeding is one of the most fundamental activities in animals, and it is tightly regulated by neuroendocrine signals. However, abnormal feeding behavior is a critical cause of obesity. Obesity-related disorders have become one of the most serious social problems facing the world currently.[1] Therefore, the importance of controlling obesity has been pointed out in recent years. As obesity develops owing to an imbalance between ingestion and consumption of energy, the control mechanism of feeding has been studied and still needs to be elucidated. A major part of feeding behavior is centrally regulated by the hypothalamus via a complex network of neuropeptides and hormones produced in the stomach and intestinal tract.

Neuromedin U (NMU), a neuropeptide isolated from porcine spinal cord, is characterized for its ability to induce uterine muscle contraction.[2] NMU and its related peptides are C-terminal amidated peptides comprising 23 amino acid residues (NMU-23) in rodents and 25 amino acid residues (NMU-25) in humans and swines.[3] An N-terminally truncated peptide with 8 amino acids (NMU-8), which was produced by cleavage at a pair of basic residues (Arg-Arg), was identified in dogs and swines.[4] Neuromedin S (NMS), a member of the NMU peptide family comprising 36 amino acid residues was isolated from the rat brain.[5] The C-terminal amide structure and the highly conserved seven-amino-acid sequence of the NMU peptide family play a crucial role in various physiological functions in mammals.[6]

NMU, which is widely distributed in the central nervous system (CNS) and gastrointestinal tract (GIT), is involved in various physiological functions, such as reproduction, stress response, and cardiac function.[3] NMU activates two receptor subtypes: NMUR1 (GPR66/FM3),[7–11] which is expressed in the peripheral tissues (e.g., the GIT), and NMUR2 (TGR1/FM4),[10,12–14] which is expressed in the CNS, including the hypothalamus and spinal cord. NMU knockout mice were found to show hyperphagia and obesity. However, intracerebroventricular administration of NMU exerted anorectic effect. These findings on the effects of NMU on feeding behavior and energy consumption attracts the attention of researchers.[15,16] In humans, NMU gene mutation has been considered to cause obesity.[17] Nevertheless, the direct effect of NMU administration has not been investigated in clinical trials.

C-terminal NMU peptides comprising eight and nine amino acid residues (NMU-8 and NMU-9) are the shortest peptides functioning as potent agonists.[3] The sequences of C-terminal NMU peptides are highly conserved across species; the C-terminal heptapeptide is completely conserved in mammals. Comprehensive

*Abbreviations:* Ac, acetyl; Arg(Me), $N^{\omega}$-methylarginine; Cha, 3-cyclohexylalanine; CNS, central nervous system; DIO, diet-induced obese; DMF, *N*,*N*-dimethylformamide; FLIPR, fluorometric imaging plate reader; Fmoc, 9-fluorenylmethoxycarbonyl; GIT, gastrointestinal tract; γGlu, γ-glutamic acid; HPLC, high performance liquid chromatography; HSA, human serum albumin; αMePhe, α-methylphenylalanine; Nal(2), 3-(2-naphthyl)alanine; Nle, norleucine; NMU, neuromedin U; PEG, polyethylene glycol; PEG(2), *O*-(3-aminopropyl)-*O*′-(*N*-diglycolyl-3-aminopropyl)diethyleneglycol; PEG(4), 15-amino-4,7,10,13-tetraoxapentadecanoic acid; PipAc, piperazin-1-ylacetyl; TFA, trifluoroacetic acid; Tra, tranexamic acid.

* Corresponding authors.
*E-mail addresses:* naoki.nishizawa@takeda.com (N. Nishizawa), taiji.asami.1@gmail.com (T. Asami).

http://dx.doi.org/10.1016/j.bmcl.2017.09.019
0960-894X/

**Table 1**
Structures and agonist activities of neuromedin U analogs. N-ter-linker-$AA^{12}$-$AA^{13}$-$AA^{14}$-$AA^{15}$-$AA^{16}$-$AA^{17}$-Tyr-$AA^{19}$-Leu-$AA^{21}$-Arg-Pro-$AA^{24}$-Asn-$NH_2$.

| Compound | Structure | | | | | | | | | | | Agonist activity, $EC_{50}$ (nM)[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N-ter | linker | $AA^{12}$ | $AA^{13}$ | $AA^{14}$ | $AA^{15}$ | $AA^{16}$ | $AA^{17}$ | $AA^{19}$ | $AA^{21}$ | $AA^{24}$ | Human | | Mouse | |
| | | | | | | | | | | | | NMUR1 | NMUR2 | NMUR1 | NMUR2 |
| NMU-8 | H | – | – | – | – | – | – | – | Phe | Phe | Arg | 0.45 | 0.43 | 0.81 | 0.43 |
| **2** | PEG20k | PipAc | – | – | – | – | – | – | Nal(2) | Phe | Arg | >1000 | 8.8 (5.8–13) | >1000 | 1.0 (0.80–1.4) |
| **3** | H | PipAc | – | – | – | – | – | – | Nal(2) | Phe | Arg | 9.5 (6.8–13) | 0.20 (0.15–0.25) | 9.6 (6.9–13) | 0.11 (0.074–0.15) |
| **4** | Hexadecyl | PipAc | – | – | – | – | – | – | Nal(2) | Phe | Arg | 100 (73–150) | 11 (7.8–15) | 170 (130–220) | 45 (22–95) |
| **5** | Hexadecyl | PipAc | – | – | – | – | – | Gly | Nal(2) | Phe | Arg | 67 (40–110) | 9.4 (6.7–13) | 100 (72–150) | 9.7 (6.9–14) |
| **6** | Hexadecyl | PipAc | – | – | – | – | Arg | Gly | Nal(2) | Phe | Arg | 48 (32–72) | 6.2 (3.8–10) | 110 (79–150) | 3.7 (2.1–6.7) |
| **7** | Hexadecyl | PipAc | – | – | – | Ser | Arg | Gly | Nal(2) | Phe | Arg | 45 (29–70) | 3.5 (2.1–5.7) | 80 (51–130) | 1.5 (1.1–2.0) |
| **8** | Hexadecyl | PipAc | – | – | Gln | Ser | Arg | Gly | Nal(2) | Phe | Arg | 34 (27–44) | 4.2 (2.4–7.3) | 94 (68–130) | 1.9 (1.5–2.5) |
| **9** | Hexadecyl | PipAc | – | Ser | Gln | Ser | Arg | Gly | Nal(2) | Phe | Arg | 42 (32–55) | 3.6 (2.3–5.5) | 70 (44–110) | 1.5 (1.3–1.9) |
| **10** | Hexadecyl | PipAc | Ala | Ser | Gln | Ser | Arg | Gly | Nal(2) | Phe | Arg | 34 (28–43) | 3.3 (2.1–5.2) | 76 (52–110) | 1.6 (1.2–2.0) |
| **11** | Hexadecanoyl | PEG(4) | – | – | – | – | – | – | Nal(2) | Phe | Arg | 52 (25–110) | 5.8 (3.3–10) | 300 (250–350) | 7.5 (4.8–12) |
| **12** | Hexadecanoyl | PEG(4)-PEG(4) | – | – | – | – | – | – | Nal(2) | Phe | Arg | 25 (19–33) | 3.8 (1.9–7.6) | 110 (77–150) | 1.7 (1.1–2.8) |
| **13** | Hexadecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | Nal(2) | Phe | Arg | 32 (25–40) | 3.0 (1.7–5.4) | 59 (36–97) | 1.4 (1.0–1.8) |
| **14** | Octadecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | Nal(2) | Phe | Arg | 49 (26–93) | 7.3 (4.7–11) | 290 (240–350) | 3.5 (2.1–6.0) |
| **15** | Octadecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | Cha | Nle | Arg | 6.3 (3.8–11) | 2.0 (1.4–2.9) | 67 (39–120) | 0.88 (0.55–1.4) |
| **16** | Octadecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | Cha | Nle | Arg(Me) | 31 (22–43) | 1.9 (1.5–2.6) | >1000 | 0.75 (0.49–1.2) |
| **17** | Octadecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | αMePhe | Nle | Arg(Me) | >1000 | 1.5 (1.1–2.1) | >1000 | 1.6 (1.0–2.3) |
| **18** | Hexadecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | αMePhe | Nle | Arg(Me) | >1000 | 0.80 (0.54–1.2) | >1000 | 2.8 (1.6–5.2) |
| **19** | Dodecanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | αMePhe | Nle | Arg(Me) | >1000 | 1.8 (1.3–2.9) | >1000 | 10 (7.5–14) |
| **20** | Hexanoyl | Tra-γGlu-PEG(2) | – | – | – | – | – | – | αMePhe | Nle | Arg(Me) | >1000 | 8.4 (5.7–12) | >1000 | 63 (36–110) |
| **21** | Ac | Tra-γGlu-PEG(2) | – | – | – | – | – | – | αMePhe | Nle | Arg(Me) | >1000 | 9.3 (6.3–14) | >1000 | 88 (55–140) |

[a] $EC_{50}$ values [nM (95% confidence interval)] of agonist activities were determined as concentrations of peptide analogs that gave half-maximum [$Ca^{2+}$] mobilizing activities. The $EC_{50}$ values of NMU-8 were the averages of 15 tests (for human NMUR1/2) and 13 tests (for mouse NMUR1/2).