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Synthesis and receptor binding properties of chimeric peptides containing a μ-opioid receptor ligand and nociceptin/orphanin FQ receptor ligand Ac-RYYRIK-amide

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Abstract—Four chimera peptides composed of ORL1 receptor ligand Ac-RYYRIK-NH_2 and a μ-opioid receptor agonist dermorphin Y*A*FGYPS-NH_2 or Y*R*FB-NH_2, with a spacer linking the two pharmacophores, were synthesized and tested for their receptor binding properties. Chimera peptides with long spacers (a Lys and five or eight Gly residues) showed synergistically improved affinity for both the μ-opioid receptor and ORL1 receptor, while the chimera peptides with short spacers (Lys residue only) showed decreased or similar affinity compared to the monomeric receptor ligands. Chimera peptides containing long spacers may prove to be useful tools for studying ORL1 receptor/μ-opioid receptor heterodimers.
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Nociceptin (NOC)[1] (also known as orphanin FQ[2]) is a heptadecapeptide, FGGFTGARKSARKLANQ, isolated from brain as endogenous ligand for the opioid-receptor-like 1 (ORL1) receptor, one of the G-protein coupled receptors (GPCR), which is highly homologous to the traditional opioid receptors. Although NOC structurally resembles the opioid peptide dynorphin A, its pharmacological effects differ from those of opioids. The system consisting of the ORL1 receptor and its endogenous ligand NOC is involved in a variety of physiological functions, including analgesia or pain modulation.[3] NOC has unique pharmacological properties: it elicits analgesia in the spinal cord and hyperalgesia or anti-opioid analgesic effects in the brain.[1–9] Dooley et al. have recently identified some hexapeptides with high ORL1 receptor affinities similar to NOC.[10] These hexapeptides have shown to be partial agonists in the stimulation of [^{35}S]GTPγS binding and inhibition of forskolin-stimulated cAMP accumulation in CHO cells expressing human ORL1 receptor.[10] Berger et al. have revealed that one of these peptides Acetyl-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2 (Ac-RYYRIK-NH_2) behaves as antagonist for G protein activation in rat brains and inhibits the chronotropic effect evoked by NOC.[11] These results suggest that the hexapeptide amide is a possible lead molecule for more potent NOC antagonists or analgesic drugs.

It is well established that a number of GPCR, including opioid receptors, interact with each other to form homodimers or heterodimers, and that this is essential for their activation. Pan et al. have recently demonstrated that ORL1 receptor can form heterodimers with μ-opioid receptors to form a receptor complex with a unique binding selectivity profile.[12] The study of compounds that can activate ORL1/μ-opioid receptor heterodimers is interesting as it allows us to investigate the organization of such heterodimers and to develop compounds with novel biological properties. In this study, we designed and synthesized four chimera peptides by combining the NOC antagonist Ac-RYYRIK-NH_2 with potent μ-opioid receptor agonistic peptides, dermorphin Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 (Y*A*FGYPS-NH_2)[13] and Tyr-D-Arg-Phe-βAla-NH_2 (Y*R*FB-NH_2).[14] As shown in Fig. 1, the two pharmacophore peptides were linked tail to tail by a Lys spacer (short spacer, analogs **1** and **3**) or by a Lys and five or eight Gly residues (long spacer, analogs **2** and **4**), with Ac-RYYRIK at the α-amino group and opioid agonistic ligands at the ε-amino group of the Lys residue. The spacer length employed in analogs **2** and **4** was similar

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Analog **1**: YAFGYPS ┐
Ac-RYYRIK-K-NH_2

Analog **2**: YAFGYPS-GG ┐
Ac-RYYRIK-GGG-K-NH_2

Analog **3**: YRFB ┐
Ac-RYYRIK-K-NH_2

Analog **4**: YRFB-GGGGG ┐
Ac-RYYRIK-GGG-K-NH_2

Figure 1. Synthetic chimera peptides.

to those reported to be sufficient to cross-link ORL1 or μ-opioid receptor homodimers, as suggested by recent papers.[15,16] We describe here the synthesis and novel receptor binding properties of the chimera peptides.

The chimera peptides were synthesized by a Fmoc-based solid-phase method according to a procedure described previously.[17] Starting with a Fmoc-Lys(Alloc)-NH-SAL-resin, the sequence of the Ac-RYYRIK pharmacophore was constructed at the α-amino group of the Lys residue to yield N^{α}-acetylated protected hexapeptide-Lys(Alloc)-NH-SAL-resin. Then, after removal of the Alloc group with $(Ph_3P)_4Pd$/PhSiH system,[18] the sequence of μ-agonistic ligands was constructed at the ε-amino group of Lys. Deblocking and cleavage from the resin of fully protected peptide resin by TFA-5% phenol and purification on preparative HPLC, as described previously,[17] afforded highly pure chimera peptides. The analytical data of synthetic analogs are shown in Table 1.[19]

The receptor binding properties of the synthetic peptides were determined by competition experiments with [^{3}H]DAMGO using rat brain homogenates (μ-opioid receptor) and with [^{3}H]NOC using cell membranes expressing human ORL1 receptor expressed in HEK-293 cells (ORL1 receptor), according to procedures described previously.[15] As shown in Table 2, dermorphin and YRFB exhibited high μ-opioid receptor affinities, with IC_{50} values of 0.538 and 1.18 nM, respectively,

Table 2. Receptor binding affinity of chimera peptides and monomeric receptor ligands

Peptide	IC_{50} ± SEM (nM)	
	μ-Opioid receptor [a][^{3}H]DAMGO	ORL1 receptor [b] [^{3}H]NOC
Ac-RYYRIK-NH_2	>10000	0.767 ± 0.267
Dermorphin (YAFGYPS-NH_2)	0.538 ± 0.294	>10000
YRFB	1.18 ± 0.272	>10000
Analog **1**	0.150 ± 0.076	11.6 ± 3.00
Analog **2**	0.00236 ± 0.00121	0.0464 ± 0.0097
Analog **3**	2.76 ± 0.781	0.789 ± 0.293
Analog **4**	0.0477 ± 0.0228	0.0803 ± 0.0403

[a] Using rat brain homogenate.
[b] Using cell membrane expressing human ORL1 receptor in HEK-293 cells.

whereas these μ-opioid ligands had no intrinsic affinity for the ORL1 receptor. Ac-RYYRIK-NH_2 possessed a high ORL1 receptor affinity, with an IC_{50} value of 0.767 nM, but no substantial affinity for μ-opioid receptors.

Analog **1**, which contains Ac-RYYRIK and dermorphin YAFGYPS pharmacophores with a short spacer, showed high μ-opioid receptor affinity, comparable to that of dermorphin itself. This analog also showed a potent affinity for ORL1 receptor, although its affinity was one order of magnitude lower than that of Ac-RYYRIK-NH_2. Interestingly, analog **2**, which contains a long spacer composed of a Lys and five Gly residues, showed greatly increased affinity for both receptors compared with the corresponding monomeric ligand, with a 200-fold increase in affinity for the μ-opioid receptor and a 17-fold increase in affinity for the ORL1 receptor. However, analog **3**, in which Ac-RYYRIK is linked to another μ-opioid receptor ligand, YRFB, by a short spacer, showed a 5-fold reduction in affinity for the μ-opioid receptor and a high ORL1 receptor affinity, comparable to Ac-RYYRIK-NH_2. Analog **4** was designed to have a similar peptide length to that of analog **2** by inserting five Gly residues at the ε-side chain of the spacer Lys residue. This compound showed affinities for both the μ-opioid receptor and the ORL1 receptor that were improved by an order of magnitude as compared to the monomeric ligands.

Table 1. Analytical data of synthetic peptides

Analog	$[\alpha]_D$[a](°)	HPLC[a] t_R(min)	ESI-MS		Amino acid analysis[a]								
			Calcd[b]	Found	Ser	Gly	Ala	Ile	Tyr	Phe	Lys	Arg	Pro
1	−43.3	21.67	927.083 $[M+2H]^{2+}$	927.60	0.86 (1)	1.01 (1)	0.93 (1)	1.02 (1)	3.72 (4)	1.08 (1)	2.02 (2)	1.95 (2)	1.05 (1)
2	−8.0	20.59	713.478 $[M+3H]^{3+}$	713.40	0.89 (1)	6.08 (6)	1.04 (1)	0.99 (1)	3.98 (4)	1.03 (1)	2.01 (2)	1.85 (2)	1.04 (1)
3	−6.7	16.61	802.965 $[M+2H]^{2+}$	803.60	—	—	—	1.02 (1)	2.85 (3)	1.39[c] (2)	2.01 (2)	2.92 (3)	—
4	−1.3	14.49	687.784 $[M+3H]^{3+}$	688.50	—	7.33 (8)	—	1.13 (1)	2.35 (3)	1.31[c] (2)	1.91 (2)	3.00 (3)	—

[a] See Ref. 19 for conditions.
[b] Average mass.
[c] βAla was detected at the same elution position as Phe using an analyzer (Hitachi L-8500) and calculated as Phe.

Dimeric opioid ligands often show high receptor binding and biological activity compared to those of the monomeric ligand.[16,20–23] The present study demonstrates that Ac-RYYRIK-NH_2/μ-opioid receptor agonist chimera peptides have high binding affinities for both ORL1 and μ-opioid receptors. It is interesting that the receptor binding affinities of the analogs containing long spacers (**2** and **4**) were synergistically increased, showing 60- and 240-fold improvements, compared to the corresponding short-spacer analogs (**1** and **2**). Since two closely located pharmacophores may each interfere with the binding of the other to its receptor site, the addition of long spacers (**2** and **4**) should serve to prevent such interference. This study also revealed that spacer length in the cross-linking of two pharmacophores is an important element in binding with both receptors. The high-affinity ligands (**2** and **4**) may serve as useful tools for investigating ORL1 receptor/μ-opioid receptor heterodimers which probably exist in opioid systems. These compounds also may have a potential as analgesics with novel biological properties.

References and notes

1. Meunier, J. C.; Mollereau, C.; Toll, L.; Suaudeau, C.; Moisand, C.; Alvinerie, P.; Butour, L. L.; Guilemot, J. C.; Ferrara, P.; Monsarrat, B.; Mazarguil, H.; Vassart, G.; Parmentier, M.; Costentin, J. *Nature* **1995**, *377*, 532.
2. Reinscheid, R. K.; Nothacker, H. P.; Bourson, A.; Ardati, A.; Henningsen, R. A.; Bunzow, J. R.; Grandy, D. K.; Langen, H.; Monsma, F. J., Jr.; Civelli, O. *Science* **1995**, *270*, 792.
3. Calo', G.; Guerrini, R.; Rizzi, A.; Salvadori, S.; Regoli, D. *Br. J. Pharmacol.* **2000**, *229*, 1261.
4. Munier, J.-C. *Eur. J. Pharmacol.* **1997**, *340*, 1.
5. Henderson, G.; Macknight, A. T. *Trends Pharmacol. Sci.* **1997**, *18*, 293.
6. Rossi, G.; Leventhal, L.; Bolan, E. A.; Pasternak, G. W. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 858.
7. Tian, J. H.; Xu, W.; Fang, Y.; Mogil, J. S.; Grisel, J. E.; Grandy, D. K.; Han, J. S. *Br. J. Pharmacol.* **1997**, *120*, 676.
8. King, M.; Chang, A.; Pasternak, G. W. *Biochem. Pharmacol.* **1998**, *55*, 1537.
9. Kolesnikov, Y. A.; Pasternak, G. W. *Life Sci.* **1999**, *64*, 2021.
10. Dooley, C. T.; Spaeth, C. G.; Berzetei-Gurske, I. P.; Craymer, K.; Adapa, I. D.; Brandt, S. R.; Houghton, R. A.; Toll, L. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 735.
11. Berger, H.; Albrecht, E.; Wallukat, G.; Bienert, M. *Br. J. Pharmacol.* **1999**, *126*, 555.
12. Pan, Y.-X.; Bolan, E.; Pasternak, G. W. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 659.
13. Broccardo, M.; Erspamer, V.; Erspamer, G. F.; Improta, G.; Linari, G.; Melchiorri, P.; Montecucchi, P. C. *Br. J. Pharmacol.* **1981**, *73*, 625.
14. Sasaki, Y.; Ambo, A.; Suzuki, K. *Chem. Pharm. Bull.* **1991**, *39*, 2313.
15. Kohara, H.; Ambo, A.; Sasaki, Y. In *Peptide Sciences 2002*; Yamada, T., Ed.; Japanese Peptide Society, 2003; pp 217–218.
16. Portoghese, P. S. *J. Med. Chem.* **2001**, *44*, 2259.
17. Sasaki, Y.; Chiba, T. *J. Med. Chem.* **1995**, *38*, 3995.
18. Thieriet, N.; Aisina, J.; Giralt, E.; Guibe, F.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 7275.
19. Optical rotation was measured in 1% AcOH ($c = 0.5$) at 20 °C. Analytical HPLC was performed on a YMC-pack ODS-AM (150 × 4.5 mm) column using following solvent systems: A, 0.06% TFA; B, 0.06% TFA in 80% CH_3CN. A linear gradient elution from 10% to 50% B over 40 min was used at a flow rate of 1 ml/min, with monitoring of the eluate at 220 nm. All synthetic peptides had a purity of more than 96% on the HPLC. Amino acid analysis was carried out after 6 N HCl hydrolysis of peptides at 110 °C for 24 h. βAla was eluted at the same position as Phe.
20. Shimohigashi, Y.; Costa, T.; Chen, H. C.; Rodvard, D. *Nature* **1982**, *297*, 333.
21. Shimohigashi, Y.; Ogasawara, T.; Koshizaka, T.; Waki, M.; Kato, T.; Izumiya, N.; Kurono, M.; Yagi, K. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 1109.
22. Costa, T.; Shimohigashi, Y.; Krumins, S. A.; Monson, P. J.; Rodbard, D. *Life Sci.* **1982**, *31*, 1625.
23. Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Yim, C. B.; Ronsisvalle, G.; Tam, S. W.; Takemori, A. E. *J. Med. Chem.* **1986**, *29*, 1855.