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Development of μ/δ Opioid Ligands: Enkephalin Analogues Containing 4-Anilidopiperidine Moiety

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Introduction

It has been known that modulation of both μ and δ opioid receptors can give therapeutic benefit for the treatment of pain without the serious side effects caused by morphine [1–2]. Thus, our group developed a series of enkephalin analogues for the μ and δ receptors in which enkephalin-related tetrapeptide structures were linked to three different 4-anilidopiperidine moieties [3]. Among them, one analogue with a 2,6-dimethyltyrosine-containing tetrapeptide structure and *N*-phenyl-*N*-piperidin-4-yl propionamide moiety, **LYS540**, showed highly potent biological activities at both receptors (Table 1) (Figure 1) [3]. Further modifications of this ligand, mainly at the 2 and 4 positions of the tetrapeptide, were performed and found to increase both opioid activities in the binding and functional assays. We report here structure-activity relationships in detail, along with in-vivo assay results.

Results and Discussion

Synthetic enkephalin analogues were prepared by stepwise solution phase peptide synthesis using *N*^α-Boc chemistry starting from *N*-phenyl-*N*-piperidin-4-yl propionamide in 40–50% overall yields [3]. After chain elongation, crude peptides were isolated by preparative C₁₈ RP-HPLC (Vydac 218TP1010) with a gradient of 10–50% CH₃CN in aq. 0.1% TFA.

Opioid binding affinities were determined by radioligand competition analyses against [³H]DPDPE (δ) and [³H]DAMGO (μ) using membrane preparations from transfected HN9.10 cells. Agonist activities for opioid receptors were evaluated by classical assays in stimulated mouse vas deferens (MVD, δ) and guinea pig isolated ileum (GPI, μ) [4].

The ligands were designed to have good agonist activities for both μ and δ opioid receptors with increased potential cell permeability due to the lipophilic character of the C-terminal 4-anilidopiperidine moiety. Ligating the moiety to the enkephalin-related tetrapeptide structure increased their hydrophobicities from aLogP = -0.54 to 2.8 [5]. **LYS644** in which *D*-Ala² was replaced by *D*-Nle² increased opioid agonist activities in the MVD (IC₅₀ = 0.70, 3 fold) and GPI (IC₅₀ = 1.6, 5 fold) assays (Table 1). In our study, we observed that all truncation of Gly³, for example **LYS711**, decreased δ opioid activities in the binding and

functional assays, but nonetheless retained μ opioid activities. Shortening the backbone length might change the topographical structure of the essential two aromatic groups for the opioid activities and result in decreased δ opioid activity. Substitution on the phenyl ring with chlorine (**LYS707**) increased both opioid activities 2–3 fold in the binding and functional assays. The ligand showed very good antihyperalgesic and antiallodynic effects in in-vivo tests using radiant heat and von frey filaments, respectively, in L5/L6 SNL SD rats (Figure 2).

Acknowledgments

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Fig. 1.
Design of enkephalin analogues.

Fig. 2. Antiallodynic (left) and antihyperalgesic (right) effects of **LYS707** (i.th) in L5/L6 SNL SD rats.

Table 1

Bioactivities of the opioid ligands.

ligand	<i>K_i</i> (nM) ^{<i>a</i>}		<i>IC</i> ₅₀ (nM) ^{<i>b</i>}	
	hDOR [³ H]DPDPE	rMOR [³ H]DAMGO	MVD(δ)	GPI(μ)
LYS540 (Xxx = DAla; n = 1; X = H)	0.38	0.30	1.8	8.5
LYS644 (Xxx = DNle; n = 1; X = H)	0.18	0.39	0.70	1.6
LYS711 (Xxx = DAla; n = 0; X = H)	1.7	0.15	16	3.6
LYS707 (Xxx = DAla; n = 1; X = <i>p</i> -Cl)	0.14	0.14	0.70	2.6
H-Tyr-DAla-Gly-Phe-NH ₂	300	2.8	120	47

^{*a*} Competition analyses against radiolabeled ligand.^{*b*} Concentration at 50% inhibition of muscle contraction at electrically stimulated isolated tissues.