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Opioid and Melanocortin Receptors: Do They Have Opioid Overlapping Pharmacophores?

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Abstract

We have identified compound **1** as a novel ligand for opioid and melanocortin (MC) receptors, which is derived from the overlapping of a well known structure for the δ opioid receptor, 2,6-dimethyltyrosine (Dmt)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), and a small molecule for the MC receptor, Tic-DPhe(p-Cl)-piperidin-4-yl-N-phenyl-propionamide. Ligand **1** showed that there is an overlapping pharmacophore between opioid and MC receptors through the Tic residue. The ligand displayed high biological activities at the δ opioid receptor ($K_i = 0.38$ nM in binding assay, $EC_{50} = 0.48$ nM in GTP- γ -S binding assay, $IC_{50} = 74$ nM in MVD) as an agonist instead of an antagonist and showed selective binding affinity ($IC_{50} = 2.3$ μ M) at the MC-3 receptor rather than at the MC-5 receptor. A study of the structure-activity relationships demonstrated that the residues in positions 2, 3, and the C-terminus act as a pharmacophore for the MC receptors, and the residues in positions 1 and 2 act as a pharmacophore for the opioid receptors. Thus, this structural construct can be used to prepare chimeric structures with adjacent or overlapping pharmacophores for opioid and MC receptors.

Keywords

opioid receptor; melanocortin receptor; anti-opioid effect; multi-target drug; overlapping pharmacophores; antinociception; side effect; Dmt-Tic; fentanyl

INTRODUCTION

Morphine is one of the most important drugs for the treatment of pain, even though its long term administration is accompanied by serious side effects such as constipation, tolerance, and dependence, features shared by drugs from the opioid class. Recent studies have shown that melanocortin (MC) receptor agonists, mainly acting via the MC-4 receptor, are involved in the modulation of nociception and function as anti-opioids,^{1–10} and the MC-1 receptor also has been implicated in pain.^{11,12} Starowicz et al. and other groups indicated that pain can be controlled by blockade of the MC-4 receptor because of functional interactions between the MC and opioid systems.^{1–4} It also was documented that **SHU9119**, a well known MC-4 receptor antagonist, can modulate nociception and thus enhance morphine analgesic effects.

⁵⁻⁷ These facts suggest that targeting both the MC-4 and the opioid receptors in a single molecule can generate a novel treatment for chronic pain states.

Our group has been developing novel ligands for the treatment of pain, which target more than one receptor based on our new paradigm.¹³⁻¹⁶ Neuropathic pain states and the side effects of opioids result from changes in the central nervous system to multiple receptors, and our new approach in pain pathway is to address this issue by targeting multiple receptors. Earlier, we developed novel bifunctional ligands which can act as agonists for μ and δ opioid receptors and antagonists for cholecystokinin (CCK)-1 and -2 receptors¹⁴⁻¹⁶ and in vivo assay results of these ligands demonstrated the validity of our working hypothesis.¹³ Herein we describe our efforts to find whether in such design of ligands to interact with more than one receptor, it is possible to design ligands with overlapping pharmacophores for opioid and MC receptors that eventually will be of benefit for rational drug design for the treatment of pain.

MATERIALS AND METHODS

N^α-Boc amino acids and coupling reagents were purchased from Novabiochem (Sandiego, CA), Bachem (Torrance, CA), and RSP (Shirley, MA). The materials for bioassays were purchased from Perkin-Elmer (Wellesley, MA), American Peptide Company (Louisville, KY), Gibco (Langley, OK), and Sigma (St. Louis, MO). Peptides were synthesized by stepwise solution-phase synthesis using *N*^α-Boc chemistry. None of functional groups except *N*^α-amino group were protected. Analytical HPLC was performed on a Hewlett-Packard 1090 (C-18, Vydac, 4.6 mm × 250 mm, 5 μ m) and preparative RP-HPLC was performed on a Hewlett-Packard 1100 (C-18, Vydac, 10 mm × 250 mm, 10 μ m). Mass spectra were taken in the positive ion mode using ESI methods.

Coupling Method

Amino component (1.0 equiv), *N*^α-Boc amino acid (1.1 equiv), BOP (1.1 equiv), and HOBT (1.1 equiv) were dissolved in DMF (1.5 mL/mmol). To the stirred mixture was added NMM (2.2 equiv) at 0°C. After stirring for 30 min at 0°C, the mixture warmed up to room temperature and stirred for additional 3–5 h until the disappearance of starting amino component (monitored by TLC). The reaction mixture was concentrated under reduced pressure and diluted with EtOAc. The organic layer was washed consecutively with 5% NaHCO₃ solution (three times), 5% citric acid (two times), brine, and water. The combined organic layer was dried under anhydrous Na₂SO₄ and concentrated under reduced pressure. The residual oil was triturated with appropriate organic solvent, usually diethyl ether, to afford >90% pure compound as a white powder.

Deprotection Method

N^α-Boc amino acids and peptides (5 mmol) were treated with cold TFA (10 mL) at 0°C for 20 min. The reaction mixture was evaporated under vacuo and co-evaporated with toluene to oil. The residual oil was isolated by triturating with diethyl ether (or petroleum ether) to give >90% pure compound as a white powder.

Synthesis of Ligands 1, 3, 4, and 6–10

N-phenyl-*N*-piperidin-4-yl-propionamide **2** was synthesized by the method previously described.¹⁷ Peptide ligands were synthesized by stepwise attachment of respective *N*^α-Boc amino acids to **2** using the coupling and deprotection methods described earlier (Scheme 1). After chain elongations were completed, the crude compounds were purified by RP-HPLC (10–60% acetonitrile in 20 min for **3** and **4**, 20–60% acetonitrile in 20 min for **6–10**, 30–45% acetonitrile in 22 min for **1**) to give >98% pure ligands as white solids (for the analytical data, see Table I).

Bioassays

All bioassays for the opioid and MC receptors were performed using methods previously described.^{13,16-19}

RESULTS AND DISCUSSION

We sought to design and synthesize a single ligand that binds to both opioid and MC receptors. Our initial design was inspired by the common structural features between MC-4 receptor agonist, discovered by Merck, and fentanyl, a potent μ opioid receptor agonist (Figure 1).²⁰ Both structures have a piperidine scaffold appended with aromatic moieties. Ligand **6** was designed by the replacement of the 4-cyclohexyl-4-[1,2,4]-triazolylmethyl piperidinyl moiety of the Merck compound with the 4-(phenyl-propionyl-amino)-piperidinyl of fentanyl. Ligand **6** showed binding to all MC receptors with moderate selectivity for MC-5 receptor ($IC_{50} = 58$ nM) over the other MC receptors. Ligand **6** also showed binding affinities in the micromolar range at both δ ($K_i = 8.4$ μ M) and μ ($K_i = 3.0$ μ M) opioid receptors. Inspired by these initial and promising results, we next investigated the possibility of designing and synthesizing ligands with equally high binding affinities at the opioid and the MC receptors in one molecule. We systematically modified ligand **6** and performed structure-activity relationship (SAR) studies at both receptors and their subtypes. All ligands were synthesized by stepwise solution-phase peptide synthesis using N^α -Boc chemistry starting from **2**, and were purified by preparative RP-HPLC to afford >98% pure ligands in >65% overall yields.

Opioid binding affinities were determined by radioligand competition analysis using [³H]DPDPE for the δ opioid receptor and [³H]DAMGO for the μ opioid receptor. Opioid agonist efficacy was examined by [³⁵S]GTP- γ -S binding. Classical functional assays were performed in the GPI (μ) and MVD (δ) to evaluate opioid agonist activities. Competition binding assays and second message cAMP assays for the MC-1, -3, -4, and MC-5 receptors were carried out using HEK-293 cells as previously described.^{18,19}

First, the Tic residue was truncated to examine its role in binding at both the opioid and MC receptors. Ligand **3** lacking the Tic residue lost its binding affinities for the δ opioid and all the MC receptors, while it retained weak binding affinity (6700 nM) at the μ opioid receptor (Table II). Inversion of the chirality of the *D*-Phe(*p*-Cl) residue, ligand **4**, did not lead to significant improvement in binding affinities at the δ and μ opioid receptors. It was known that the dipeptide moiety, *D*-Tic-*D*-Phe(*p*-Cl), acts as an address element for the MC-4 receptor, and both analogs with *R*- and *S*-configurations in the Tic residue were similar in binding affinities.²¹ Thus, in the pursuit of a MC receptor binding, *R*-configuration of the residue was preserved and three different amino acid derivatives, 3,4-dehydroproline, 4-hydroxyproline, and histidine, were attached to the ligand **3**, giving tripeptide ligands **7–9**, respectively. Ligands **7** and **8** showed similar poor biological profiles as ligand **3** at the opioid receptors and the MC receptors (Table III). Ligand **9** at the MC receptors were very different in selectivity and potency, as it showed the micromolar range of binding affinities (0.17–3.5 μ M) for all MC receptors except the MC-5 where it showed no binding. Comparing ligand **7** and **8**, basicity or the aromaticity of ligand **9**'s imidazole must play an important role in binding at the MC receptors. This is consistent with biological profile of ligand **6** at the MC receptors where the Tic residue provides the aromaticity.

To improve the binding at the opioid receptors, the Tic residue was retained and 2,5-dimethyltyrosine (Dmt) was introduced resulting to ligand **10**. It was well known that the replacement of tyrosine with Dmt dramatically increases the opioid activities in most cases.²² Ligand **10**, where the Phe(*p*-Cl) residue was replaced by the Dmt, showed increased binding at the δ and μ opioid receptors compared to ligand **6**, but there were no differences in functional assays. It is likely that ligands **3–4** and **6–10** do not have the appropriate topography to activate

the opioid receptors but their low micromolar range of binding affinities at these receptors might be attributed to the C-terminal moiety, *N*-phenyl-*N*-piperidin-4-yl-propionamide. In most cases, these ligands showed μ opioid selectivity in the binding and the functional assays.

When Dmt was attached to the N-terminal of ligand **6**, the resulting ligand **1** retained the high potency of Dmt-Tic at the δ opioid receptor and showed selective binding affinity at the MC-3 receptor. Ligand **1** binds to the MC-3 receptor in the micromolar range ($IC_{50} = 2.3 \mu M$), whereas it did not bind to any other MC receptors at concentration up to $10 \mu M$. Binding affinities at the δ opioid receptor were in the sub nanomolar range ($K_i = 0.38 nM$, $EC_{50} = 0.48 nM$) in both assays with strong agonist activity ($IC_{50} = 74 nM$) in the MVD assay. For the μ opioid receptor, no functional activity was observed in the GPI assay, though **1** binds to the μ opioid receptor in the nanomolar range ($K_i = 22 nM$, $EC_{50} = 6.2 nM$). It is not clear why the opioid binding and functional activities did not correlate with each other. It is notable that there was no opioid antagonist activity observed in the MVD or GPI assays for **1**.

From the SAR results, the tripeptide moiety of ligand **6** serves mainly as an MC pharmacophore with some opioid receptor binding while Dmt-Tic, a well known δ opioid antagonist, of ligand **1** plays a significant role in opioid receptor activity. These suggest that the Tic residue, present in both ligand **1** and **6**, acts as a common, overlapping pharmacophore for the opioid and MC receptors (Figure 2). We have not yet achieved the ideal biological profiles at the opioid and MC receptors and further studies are needed. However, ligand **1** can serve as a lead compound where it can be further modified to change the opioid antagonist properties to agonist properties and increase the binding at the MC receptors. The modifications of Dmt-Tic at the C-terminal are known to result in changes in functional activities; from an δ antagonist to an δ agonist, or μ agonist, or mixed agonist/antagonist.²² Thus, we anticipate that fine tuning of ligand **1** at the C-terminal would be a bifunctional ligand that can bind to the opioid and MC receptors.

CONCLUSIONS

A possible site for overlapping pharmacophores for opioid and MC receptors was identified in a single molecule (ligand **1**) which incorporates Dmt-Tic and a tripeptide with *N*-phenyl-*N*-piperidin-4-yl-propionamide moiety. The ligand showed high biological activities at the δ opioid receptor as an agonist, but weak binding affinity at the MC-3 receptor demonstrating that the opioid and MC pharmacophores in this molecule can productively bind to their respective receptors. On the basis of the fact that activation of the MC-4 receptor can negatively modulate opioid antinociception, the identification of a possible site of overlapping pharmacophores will provide a good starting point for rational drug design that may lead to therapeutic benefits by synergistic effects for the treatment of pain. More SAR studies will follow and will guide the design of potent MC-4 receptor selective compounds that retain potent opioid activities.

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SCHEME 1.

Reagents and conditions: (i) BOP, HOBT, NMM, DMF, 3–5 h, rt. (ii) TFA, 20 min, 0°C.

FIGURE 1.
Ligands for structure-activity relationships study.

FIGURE 2.
Design of overlapping pharmacophore for opioid and MC receptors.

Table I

Analytical Data for Ligands

No	Molecular Formula	LowMS ^a		HRMS ^b		HPLC (<i>t_R</i> , min) ^c	
		Observed		Observed	Calcd.	(A)	(B)
1	C ₄₄ H ₅₀ ClN ₅ O ₅	764.4		764.3632	764.3579	23.0	10.6
3	C ₂₃ H ₂₈ ClN ₃ O ₂	414.1		414.1949	414.1948	17.3	6.1
4	C ₂₃ H ₂₈ ClN ₃ O ₂	414.3		414.1932	414.1948	17.4	6.1
6	C ₃₃ H ₃₇ ClN ₄ O ₃	573.2		573.2620	573.2633	21.9	9.4
7	C ₂₈ H ₃₃ ClN ₄ O ₃	509.6		509.2326	509.2319	19.3	7.4
8	C ₂₃ H ₃₅ ClN ₄ O ₄	527.6		527.2419	527.2425	18.2	6.6
9	C ₂₉ H ₃₅ ClN ₆ O ₃	551.1		551.2516	551.2537	16.1	6.0
10	C ₃₅ H ₄₂ N ₄ O ₄	583.1		583.3277	583.3284	16.9	6.0

^a (M — TFA + H)⁺, ESI method [Finnigan, Thermoelectron, LCQ classic].

^b (M — TFA + H)⁺, FAB-MS (JEOL HX110 sector instrument) or MALDI-TOF.

^c Performed on a Hewlett Packard 1100 [C-18, Microsorb-MV™, 4.6 mm × 250 mm, 5 μm]. (A) 10–90% of acetonitrile containing 0.1% TFA within 40 min and up to 100% within additional 5 min, 1 mL/min, (B) 25–65% of acetonitrile containing 0.1% TFA within 20 min and up to 100% within additional 5 min, 1 mL/min.

Table II
In Vitro Biological Activities of the Bifunctional Ligands at δ/μ Opioid Receptors

[³⁵ S]GTP-γ-S Binding							
No	hDOR ^a [³ H]DPDPE ^b K _i (nM)	rMOR ^a [³ H]DAMGO ^c K _i (nM)	hDOR ^e		rMOR ^e		GPI(μ) IC ₅₀ (nM) ^d
			EC ₅₀ (nM)	E _{max} (%) ^f	EC ₅₀ (nM)	E _{max} (%) ^f	
1	0.38	22	0.48	18	6.2	16	0% at 1 μM
3	nr	6700	nr		nr		0% at 1 μM
4	2000	3300	n/d		n/d		3% at 1μM
6	8400	3000	n/d		n/d		8% at 1 μM
7	nr	4000	nr		nr		7% at 10 μM
8	nr	4800	nr		nr		2% at 1 μM
10	5600	570	nr		nr		4% at 1 μM

^aCompetition analyses were carried out using membrane preparations from transfected HN9.10 cells that constitutively expressed the respective receptor types.

^bK_D = 0.50 + 0.10 nM.

^cK_D = 0.85 + 0.20 nM.

^dConcentration at 50% inhibition of muscle contraction at electrically stimulated isolated tissues. nr, no response at 10⁻⁵ M; n/d, not determined.

^eExpressed in CHO cells.

^fNet total bound/basal binding $\times$ 100 + SEM.

Table III
In Vitro Binding Affinities at Human Melanocortin Receptors

No	hMC1R		hMC3R		hMC4R		hMC5R	
	IC ₅₀ ^a (nM)	EC ₅₀ ^b (nM)	IC ₅₀ ^a (nM)	EC ₅₀ ^a (nM)	IC ₅₀ ^a (nM)	EC ₅₀ ^a (nM)	IC ₅₀ ^a (nM)	EC ₅₀ ^a (nM)
1	nb	na	2300	na	nb	na	nb	na
3	nb	na	nb	na	nb	na	nb	na
6	310	na	860	na	1500	na	58	7800
7	nb	na	nb	na	nb	na	nb	na
8	nb	na	nb	na	nb	na	nb	na
9	170	>4000	3500	>5000	730	>1000	nb	na

^aConcentration of ligand at 50% specific binding.

^bEffective concentration of ligand to generate 50% maximal intracellular cAMP accumulation. nb, no binding at 10⁻⁵ M; na, no cAMP activity.