Opioid Ligands With Mixed μ/δ Opioid Receptor Interactions: An Emerging Approach to Novel Analgesics

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ABSTRACT

Opioids are widely used in the treatment of severe pain. The clinical use of the opioids is limited by serious side effects such as respiratory depression, constipation, development of tolerance, and physical dependence and addiction liabilities. Most of the currently available opioid analgesics exert their analgesic and adverse effects primarily through the opioid μ receptors. A large number of biochemical and pharmacological studies and studies using genetically modified animals have provided convincing evidence regarding the existence of modulatory interactions between opioid μ and δ receptors. Several studies indicate that δ receptor agonists as well as δ receptor antagonists can provide beneficial modulation to the pharmacological effects of μ agonists. For example, δ agonists can enhance the analgesic potency and efficacy of μ agonists, and δ antagonists can prevent or diminish the development of tolerance and physical dependence by μ agonists. On the basis of these observations, the development of new opioid ligands possessing mixed μ agonist/ δ agonist profile and mixed μ agonist/ δ antagonist profile has emerged as a promising new approach to analgesic drug development. A brief overview of μ - δ interactions and recent developments in identification of ligands possessing mixed μ agonist/ δ agonist and μ agonist/ δ antagonist activities is provided in this report.

KEYWORDS: Analgesics, Opioid Ligands, Mixed Mu/Delta agonists, Mixed Mu agonist/Delta antagonists, Peptides, Nonpeptides

INTRODUCTION

Opioid analgesics are the standard therapeutic agents for the treatment of moderate-to-severe pain. These drugs exert their analgesic activity through their interaction with the opioid μ , δ , or κ receptors as agonists. The clinical usefulness of μ opioid agonists such as morphine, however, is limited by significant side effects such as respiratory depression,

constipation, development of tolerance and physical dependence, and addiction potential. One approach to limit μ -receptor-mediated side effects is to selectively target δ and κ opioid receptors. This approach has been explored using agonist ligands selective for δ and κ opioid receptors but has seen only limited success. The δ agonists generally display limited analgesic efficacy and κ receptor agonists are limited to their use as peripheral analgesics owing to their psychotomimetic and dysphoric central effects. An alternative approach that is gaining considerable interest is the development of compounds that possess mixed opioid activity at the different opioid receptors.^{1,2}

Several lines of evidence indicate the existence of physical and functional interactions between the opioid receptors, particularly between the μ and δ receptors. Several biochemical and pharmacological studies using μ and δ receptor ligands gave an early indication of such interactions between the μ and δ receptors.³⁻⁶ The μ and δ opioid receptors exist on overlapping populations of neurons in pain-modulating regions of the central nervous system, and the presence of both μ and δ receptors within the same neuron has been demonstrated.⁷ In recent years, several studies have shown that μ and δ receptors form functionally distinct heterodimeric or hetero-oligomeric complexes.⁸⁻¹¹ The physiological and pharmacological significance of μ - δ interactions have been substantiated by recent studies using opioid receptor gene knockout animals.¹² The existence of intermodulatory effects between μ and δ receptors has spawned a new interest in the pursuit of ligands with a mixed interaction profile at the μ and δ receptors as a novel therapeutic approach for the treatment of pain. Presented herein is a brief overview of ligands that possess a mixed μ agonist/ δ agonist or μ agonist/ δ antagonist profile of activity.

MIXED μ AGONISTS / δ AGONISTS

Several early studies using coadministration of μ and δ agonist ligands demonstrated that both the potency and efficacy of μ agonists can be increased by δ agonists. Vaught and Takemori found that Leu⁵-enkephalin given at subantinociceptive doses could potentiate the analgesic actions of morphine.¹³ Several other studies extended these observations to synergistic antinociceptive effects between other μ agonists and δ agonists.¹⁴⁻¹⁶ The activation of δ opioid

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receptors has been reported to have synergistic effect on μ opioid functional activities in cells transfected with μ and δ receptors.^{7,8,17} Treatment of rats or mice for several days with μ agonists leads to translocation of δ opioid receptors to neuronal plasma membranes and enhances δ -receptor-mediated antinociception.¹⁸ These observations imply that addition of a δ agonist may allow for the treatment of pain with lower doses of μ agonists, and ligands possessing dual agonist activities at the δ and μ receptors may allow for the effective treatment of pain with lessened μ -receptor-mediated side effects.^{19,20}

Peptide ligands possessing μ and δ agonist activity

There have been several reports on peptide ligands that display high-affinity binding and agonist actions at both μ and δ receptors. Of particular interest among peptide ligands possessing dual agonist actions at μ and δ receptors are biphalin and biphalin analogs. Biphalin, (Tyr-D-Ala-Gly-Phe-NH)₂, binds to both μ and δ receptors with high affinity. It is a highly potent analgesic and is as potent as etorphine in the tail-flick test when it is administered by intracerebroventricular (icv) injection.^{21,22} This peptide also produces antinociceptive effects comparable to morphine after systemic injection and has been shown to produce less dependence than morphine on chronic use.^{23,24} Several explanations have been proposed for biphalin's high potency. Most of these focused on the presence of 2 pharmacophores in one molecule and on the possible synergistic interactions between the μ and δ receptors. Although a definitive explanation for the extraordinary potency of biphalin is still not available, it has been suggested that its high agonist activity at both μ and δ receptor may be a contributing factor.²⁵ Several biphalin analogs have been synthesized to understand the structural elements responsible for its high activity and to identify ligands with enhanced antinociceptive activity, blood brain barrier (BBB) penetration, and stability toward enkephalinases. Simplified fragment analogs of biphalin such as Tyr-D-Ala-Gly-Phe-NHNH←Phe and analogs with nonhydrazine linkers such the piperazine **1** (Figure 1) also display μ and δ bioactivity comparable to biphalin. Two cyclic biphalin analogs, **2** and **3**, have recently been synthesized through replacing the D-alanine residues in the 2,2' positions of biphalin with L- and D-cysteine and formation of intramolecular disulfide bond between the cysteine thiol groups. While the cyclic peptide **2** containing L-cysteine residues displayed reduced potencies, the cyclic peptide **3** containing the D-cysteine residues displayed potencies similar to that of biphalin in binding and functional bioassays in mouse vas deferens (MVD) and guinea pig ileum (GPI) smooth muscle preparations. In agonist efficacy determinations using [³⁵S]GTP γ S binding in cells expressing human δ opioid receptor and rat μ opioid receptor, compound **3** displayed δ and μ receptor activation capacities

Figure 1. Chemical Structures of Compounds **1-3**

($E_{\max}$ at δ = 100%; $E_{\max}$ at μ = 47%) higher than the activation levels displayed by biphalin ($E_{\max}$ at δ = 27%; $E_{\max}$ at μ = 25%).²⁶

Structural manipulations on peptides containing the Dmt-Tic (Dmt = 2',6'-dimethyltyrosine, -Tic = tetrahydroisoquinoline-3-carboxylic acid) pharmacophore have produced compounds possessing varying intrinsic activities including those that display agonist activity at both μ and δ receptors.²⁷ The tripeptide amide, H-Dmt-Tic-Gly-NHPh was one such compound that displayed mixed μ/δ agonist activity. It was found to be nearly equipotent as an agonist at δ (pEC_{50} = 8.52) and μ (pEC_{50} = 8.59) receptors in functional assays in smooth muscles.²⁸ Endomorphin-2 (Tyr-Pro-Phe-NH₂) is a peptide possessing μ agonist activity coupled with weak δ agonist activity. The replacement of the Tyr residue in this peptide with Dmt residue yielded a compound (Dmt-Pro-Phe-NH₂) endowed with high agonist activity at both μ (IC_{50} in GPI = 0.07 nM) and δ (IC_{50} in MVD = 1.87 nM) sites owing to simultaneous increase in agonist potency at both the receptors.²⁹ Some of the C-terminal arylamide analogs such as Dmt-Pro-Phe-NH-1-naphthyl also displayed mixed μ/δ agonist activities.³⁰ Another Dmt containing peptide that has recently been shown to possess mixed μ/δ agonist activity is the tetrapeptide H-Dmt-D-Arg(NO₂)-Phe-Lys-(Z)-NH₂. This compound displayed potent agonist activity in the GPI (IC_{50} = 0.509 nM) and MVD (IC_{50} = 1.69 nM) bioassays.³¹

Nonpeptide ligands possessing μ and δ agonist activity

The therapeutic potential of nonpeptide ligands with dual agonist activity at μ and δ receptors has also attracted attention in recent years. Since the identification of nonpeptide agonist ligands such as BW373U86 (**4**) and SNC80 (**5**) (Figure 2), a significant amount of research effort has been expended on the discovery and development of nonpeptide agonists of the δ receptors.^{32,33} Some of the studies using a combination of these nonpeptide δ agonists with μ agonists gave evidence supporting potential clinical advantages of a compound with combined μ and δ receptor agonist

Figure 2. Structures of δ agonists BN373U86(**4**), SNC 80(**5**), and nonpeptide ligands possessing mixed μ and δ agonist activity.

activities.³⁴ In studies with rats, additive analgesic effects were observed when the μ agonist fentanyl was administered in combination with the δ agonist BW373U86. Moreover, the coadministration was shown to diminish fentanyl-induced muscle rigidity (straub tail) and attenuate BW373U86-mediated seizures. In other studies it has been found that prolonged coadministration of morphine and BW373U86 attenuated the development and expression of morphine-induced dependence and tolerance in rats.³⁵ Coadministration of BW373U86 with alfentanil led to significant attenuation of alfentanil-induced respiratory depression.³⁶ On the basis of these results, suggesting that compounds with mixed μ and δ receptor agonist activity may be useful in producing analgesic effects with fewer adverse effects, nonpeptide ligands possessing a mixed agonist profile of activity have been pursued. Structural changes involving the placement of the diethylamide function of BW373U86 at the meta position and conversion of the diethylamide to *N*-methylanilide yielded compound **6** possessing mixed δ/μ agonist activity. A series of aryl ring-substituted analogs of **6** also displayed mixed μ/δ agonist activity similar to that of the parent compound.²⁰ From this series of compounds, the *m*-fluorophenyl compound **7**

(DPI-3290) has undergone extended evaluations. DPI-3290 displayed nonselective high-affinity binding to all 3 opioid receptors. In MVD it decreased electrically induced tension in a concentration-dependent manner with IC_{50} values of 1.0, 6.2, and 25 nM at δ , μ , and κ receptors, respectively. When administered intravenously to rats, it displayed potent dose-dependent antinociceptive activity with an ED_{50} value of 0.05 mg/kg in tail-pinch latency assay. The antinociceptive activity of this compound was partially blocked by the δ opioid receptor antagonist naltrindole and completely blocked by naloxone. It has been suggested that the antinociceptive actions of this compound result from its combined action at both δ and μ receptors.³⁷ In further studies it has been demonstrated that the respiratory depression profile of DPI-3290 was distinct from those observed with morphine and fentanyl, and DPI-3290 improved rather than added to the respiratory depression caused by alfentanil.³⁸

Among epoxymorphinan ligands investigated by Schidhammer and coworkers, several 14-alkoxy derivatives were found to display high affinity binding to all 3 opioid receptors with potent agonist activity in vitro and in vivo. Among these, the 14-ethoxymetopon derivative **8** possessing a phenylethyl group on N-17 retained high affinity binding at the μ ($K_i = 0.16$ nM) and δ ($K_i = 3.14$ nM) receptors with diminished binding affinity at the κ ($K_i = 83.2$ nM) receptor. This compound displayed agonist activity in the GPI ($IC_{50} = 1.9$ nM) and in the MVD ($IC_{50} = 9.7$ nM) bioassays. In antinociceptive evaluations, this compound was found to be 60-fold more potent than morphine in the hot-plate test. In its ability to induce constipation, however, its potency was equal to that of morphine.³⁹ Several morphinan compounds reported by Grundt and coworkers have displayed mixed μ/δ agonist profile of activity. For example, among a series of 3-hydroxy-4-methoxyindolomorphinans, compound **9** was found to display full or nearly full agonist activity at all 3 receptors in the [³⁵S]GTP γ S binding assay using Chinese hamster ovary (CHO) cells expressing the individual human opioid receptors. Its agonist potency at μ ($EC_{50} = 44.7$ nM) and δ ($EC_{50} = 42.6$ nM) receptors was higher than at κ receptors ($EC_{50} = 323$ nM).⁴⁰ The indolomorphinan **10** and **11** were also found to possess mixed μ/δ agonist activity. The 14-phenethylamino compound **11** was a potent μ agonist ($EC_{50} = 5.33$ nM, % stimulation = 91%) and partial δ agonist ($EC_{50} = 0.95$ nM, % stimulation = 33%). It has been suggested that the high potency and low efficacy of this compound at the δ receptors could translate into δ antagonist activity in vivo and might therefore display properties of a μ agonist/ δ antagonist in vivo.⁴¹

MIXED μ AGONISTS/ δ ANTAGONISTS

A large body of evidence indicates that δ receptor antagonists suppress tolerance, physical dependence, and related

side effects of μ agonists without affecting their analgesic activity. In studies using the δ receptor antagonist naltrindole, it was demonstrated that the δ antagonist greatly reduced the development of morphine tolerance and dependence in both the acute and chronic models without affecting the analgesic actions of morphine.^{42,43} Continuous infusion of the δ selective antagonist TIPP[ψ] by the icv route in parallel with continuous administration of morphine by the subcutaneous (sc) route to rats attenuated the development of morphine tolerance and dependence to a large extent.⁴⁴ The development of morphine tolerance and dependence following chronic morphine administration was blocked by antisense oligonucleotides to the δ opioid receptors.⁴⁵⁻⁴⁷ Furthermore, studies with δ receptor knockout mice have documented the critical role of δ receptors in the development of opioid tolerance. In contrast to wild-type mice, in which the analgesic response to a fixed morphine dose was lost within 5 days, the δ opioid knockout mice failed to develop tolerance following daily administration of 5 mg/kg of morphine, sc, for 8 days. After 10 days of chronic morphine dosing, cumulative dose-response curves revealed a significant 2.8-fold shift to the right of the morphine ED₅₀ in wild-type mice, whereas the potency of morphine in the δ receptor knockout mice remained unchanged following chronic morphine administration.⁴⁸ Recently, compelling evidence for the involvement of δ opioid receptors in the development of morphine-induced tolerance has been obtained through studies using knock-in mice in which the native μ receptors were replaced by mutant μ receptors (S196A), which confers μ agonism to the antagonist ligand naltrexone. In these animals, an analgesic response was observed with acute administration of naltrexone, and chronic administration of naltrexone did not result in tolerance to naltrexone itself or to morphine. This lack of tolerance in these animals was attributed to concurrent blockade of δ opioid receptors with activation of μ receptors. Further studies using wild-type and knock-in mice revealed that inhibition of δ opioid receptor must occur at the time of μ activation to prevent tolerance development. Tolerance development could therefore be prevented by δ receptor blockade but cannot be reversed once it has occurred.⁴⁹ These observations clearly indicate that δ opioid receptors play a major role in the development of morphine tolerance and dependence and provide a rationale for the development of opioid ligands that act as an agonist at the μ receptor and as an antagonist at the δ receptor. Such a mixed μ agonist/ δ antagonist would be expected to be an analgesic with low propensity to produce analgesic tolerance and physical dependence and might be of benefit in the management of chronic pain. Moreover, studies indicating that the δ antagonist naltrindole reversed alfentanil-induced respiratory depression⁵⁰ and enhanced colonic propulsion⁵¹ suggest that a mixed μ agonist/ δ antagonist

may be less prone to cause respiratory depression and gastrointestinal side effects.

Peptide ligands possessing mixed μ agonist/ δ antagonist activity

The first peptide ligand with μ agonist/ δ antagonist properties was reported by Schiller and coworkers.⁵² Among the analogs of the moderately μ selective β -casomorphin-5, H-Tyr-c[D-Orn-Phe-D-Pro-Gly-], replacement of the Phe³ residue by 2-naphthylalanine (2-Nal) gave the peptide H-Tyr-c[D-Orn-2-Nal-D-Pro-Gly-], which displayed high affinity for both μ and δ receptors. This compound was an agonist in GPI smooth muscle assay and a moderately potent antagonist against various δ agonists in the MVD assay.⁵² Ligands with a more balanced μ agonist/ δ antagonist profile of activity were discovered among analogs of the tetrapeptide amide H-Tyr-Tic-Phe-Phe-NH₂ (TIPP-NH₂). Substitution of Dmt for Tyr¹ in TIPP-NH₂ and reduction of the peptide bond between Tic² and Phe³ led to a compound, H-Dmt-Tic ψ [CH₂-NH]Phe-Phe-NH₂ (DIPP-NH₂[ψ]), which showed high μ agonist potency and very high δ antagonist activity in the GPI and MVD bioassays. When administered icv, DIPP-NH₂[ψ] produced a potent antinociceptive effect in the rat tail-flick test and produced less tolerance than morphine and no physical dependence on chronic administration at high dose levels, thus fulfilling to a large extent the expectations based on the mixed μ agonist/ δ antagonist concept with regard to analgesic activity and development of tolerance and dependence.⁵³ Recently, chimeric peptides containing a μ agonist fragment and δ antagonist fragment have also been synthesized and evaluated. The chimeric peptide resulting from linking the μ agonist H-Dmt-D-Arg-Phe-Lys-NH₂ ([Dmt¹]DALDA) and the δ antagonist H-Tyr-Tic ψ [CH₂-NH]Cha-Phe-OH (TICP[ψ]) (Cha = cyclohexylalanine) tail-to-tail using H₂N-CH₂-CH₂-NH₂ as the linker has been shown to display the expected μ agonist/ δ antagonist profile in the GPI and MVD bioassays. The δ antagonist and μ agonist potencies of this chimeric peptide, however, were lower than the potencies of DIPP-NH₂[ψ].⁵⁴

Ligands possessing mixed μ agonist/ δ antagonist profile have been found among several analogs containing the Dmt-Tic pharmacophore.²⁷ For example, the Dmt-Tic analog lacking the COOH function in the Tic moiety displayed relatively nonselective binding to the δ and μ receptors with high δ antagonism (pA₂ = 7.41) and modest μ agonism (pEC₅₀ = 6.31) with an E_{max} of 52% in smooth muscle bioassays.⁵⁵ Among N- and C-modified Dmt-Tic analogs, the N,N-dimethylated adamantylamide, N,N-(Me₂)Dmt-Tic-NH-1-adamantane, was found to display high affinity binding to δ and μ receptors (K_i at δ = 0.16 nM, K_i at μ = 1.12 nM) with potent δ antagonist (pA₂ = 9.06 in MVD) and

μ agonist ($IC_{50} = 16$ nM in GPI) activities.⁵⁶ The benzylamide peptide, H-Dmt-Tic-Gly-NH-CH₂-Ph, was also found to be a potent μ agonist ($pEC_{50} = 8.57$) and δ antagonist ($pA_2 = 9.25$) in the smooth muscle assays.²⁸ The heteroaryl analog, H-Dmt-Tic-NH-CH₂-Bid (Bid = 1*H*-benzimidazole-2-yl) similarly displayed potent δ antagonist activity coupled with weak μ agonist activity.⁵⁷

Nonpeptide ligands possessing mixed μ agonist/ δ antagonist activity

In a series of naltrindole analogs possessing phenyl-, phenoxy-, and benzyloxy substituents that were synthesized and evaluated for potential selective binding at the putative δ receptor subtypes, one of the compounds, the 7'-phenoxy-naltrindole (**12**, Figure 3) was found to display potent δ antagonist activity with a K_e of 0.25 nM in the MVD and weak μ agonist activity with an IC_{50} of 450 nM in the GPI.⁵⁸ The first nonpeptide ligand with a mixed δ antagonist/ μ agonist profile that was examined in some detail came from another series of compounds possessing the pyridomorphinan framework. The naltrexone-derived 4-chlorophenyl substituted pyridomorphinan **13** (SoRI 9409), displayed potent δ antagonist ($K_e = 0.66$ nM in the MVD) and moderate μ agonist ($IC_{50} = 163$ nM in the GPI) activities in the

smooth muscle preparations. In antinociceptive evaluations, this compound displayed partial agonist activity in the warm-water tail-withdrawal assay and full agonist activity in the acetic acid writhing assay after icv or intraperitoneal (ip) injections. Studies in mice with selective antagonists characterized this compound as a partial μ agonist/ δ antagonist. Significantly, in contrast to morphine, repeated icv injection of an A_{90} dose of this compound did not produce any significant antinociceptive tolerance.^{59,60} Paradoxically, however, when the profile of this compound was determined using [³⁵S]GTP γ S binding assays, it failed to display μ agonist activity in guinea pig caudate membranes as well as in cloned cells expressing human μ opioid receptors.⁶¹ In an effort to identify compounds with μ agonist/ δ antagonist activity in vitro and in vivo, an expanded series of pyridomorphinans derived from naloxone, oxymorphone, and hydromorphone framework were synthesized and evaluated. These efforts led to the identification of hydromorphone-derived pyridomorphinans **14** (SoRI 20411) and **15** (SoRI 20648) as compounds possessing μ agonist/ δ antagonist activity in both the [³⁵S]GTP γ S binding and smooth muscle functional assays. In analgesic activity evaluations, **14** was found to be the more efficacious compound with an A_{50} potency value of 42.8 nmol in the warm-water tail-withdrawal assay in mice. The antinociceptive activity of this compound was completely blocked by the μ selective antagonist β -FNA, confirming that the analgesic activity of this compound was indeed mediated through the μ receptors. In conformity with the expectations, this mixed μ agonist/ δ antagonist ligand did not produce any significant tolerance on repeated administration.⁶² Compounds possessing δ antagonist and weak μ agonist activity have been found in other C-ring annulated morphinans such as the pyrrolomorphinans. Among a limited number of such pyrrolomorphinans synthesized and evaluated, it was found that compound **16** possessing a 4-methylphenyl substituent on the pyrrole ring displayed δ antagonist activity with a K_e of 14.1 nM and partial μ agonist activity with an EC_{50} of 1360 nM and E_{max} of 34% in [³⁵S]GTP γ S binding assays in CHO cells expressing human δ and μ opioid receptors.⁶³

Portoghesi and coworkers have, for a long time, pursued the design of bivalent ligands to probe opioid receptor complexes.⁶⁴⁻⁶⁷ The recent demonstration of the existence of μ - δ receptor hetero-oligomeric complexes has spurred further interest in the design of such bivalent ligands that could potentially interact with these receptor complexes. Of particular interest among such series of bivalent ligands are the bivalent ligands **17**, which possess a μ agonist and a δ antagonist pharmacophore. These bivalent ligands possess the oxymorphone unit as the μ agonist pharmacophore and 7'-aminonaltrindole as the δ antagonist pharmacophore connected by spacers of length ranging from 19 to 25 Å. These compounds were evaluated in mice for acute

Figure 3. Structures of nonpeptide ligands possessing mixed μ agonist/ δ antagonist activity (**12-16**) and bivalent ligands possessing μ agonist and δ antagonist pharmacophores (**17**).

antinociception in tail-flick test and were found to be more potent than morphine and fully efficacious. These were also evaluated for their ability to induce tolerance and dependence. Morphine and the bivalent ligand with the shortest spacer (**17**, $n = 2$) promoted the development of tolerance and dependence. The bivalent ligands with slightly longer spacers (**17**, $n = 3$ and $n = 4$) produced tolerance but not dependence. Ligands with the longest spacers (**17**, $n = 5, 6$, and 7) did not produce tolerance or physical dependence.^{68,69}

CONCLUSIONS

The development of potent opioid analgesics devoid of the limiting side effects has been the goal of considerable research efforts. Accumulating evidence strongly support the existence of physical and functional interactions between the opioid μ and δ receptors. Recent studies using μ agonists along with δ agonists or δ antagonists have provided convincing evidence that there could be clear therapeutic advantages in combining the actions of μ agonists with that of a δ agonist or a δ antagonist. Recent successes in the identification of peptide and nonpeptide ligands possessing mixed μ agonist/ δ agonist and μ agonist/ δ antagonist profiles, and the encouraging results obtained in studies with these ligands is an exciting development in opioid drug discovery. It is likely that the pursuit of this new approach will lead to novel analgesics superior to those currently available for the treatment of pain.

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