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Opioid Peptide-Derived Analgesics

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ABSTRACT

Two recent developments of opioid peptide-based analgesics are reviewed. The first part of the review discusses the dermorphin-derived, cationic-aromatic tetrapeptide H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt[1]]DALDA, where Dmt indicates 2′,6′-dimethyltyrosine), which showed subnanomolar μ receptor binding affinity, extraordinary μ receptor selectivity, and high μ agonist potency in vitro. In vivo, [Dmt[1]]DALDA looked promising as a spinal analgesic because of its extraordinary antinociceptive effect (3000 times more potent than morphine) in the mouse tail-flick assay, long duration of action (4 times longer than morphine), and lack of effect on respiration. Unexpectedly, [Dmt[1]]DALDA also turned out to be a potent and long-acting analgesic in the tail-flick test when given subcutaneously (s.c.), indicating that it is capable of crossing the blood-brain barrier. Furthermore, little or no cross-tolerance was observed with s.c. [Dmt[1]]DALDA in morphine-tolerant mice. The second part of the review concerns the development of mixed μ agonist/δ antagonists that, on the basis of much evidence, are expected to be analgesics with a low propensity to produce tolerance and physical dependence. The prototype pseudopeptide H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (DIPP-NH_2[Ψ], where Tic indicates 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) showed subnanomolar μ and δ receptor binding affinities and the desired μ agonist/δ antagonist profile in vitro. DIPP-NH_2[Ψ] produced a potent analgesic effect after intracerebroventricular administration in the rat tail-flick assay, no physical dependence, and less tolerance than morphine. The results obtained with DIPP-NH_2[Ψ] indicate that mixed μ agonist/δ antagonists look promising as analgesic drug candidates, but compounds with this profile that are systemically active still need to be developed.

INTRODUCTION

The clinical treatment of severe pain relies heavily upon opioid analgesics, most of which act via μ opioid receptors. Morphine and other centrally acting μ opioid analgesics produce, in addition to the analgesic effect, several side effects—including inhibition of gastrointestinal motility, respiratory depression, tolerance, and physical dependence—that limit their use in pain treatment. Kappa opioid agonists have also been shown to be potent analgesics; however, it has long been recognized that centrally acting κ agonists have limited usefulness in humans because of their psychotomimetic and dysphoric effects.[1,2] Delta opioid agonists are known to produce analgesic effects when given intrathecally (i.th)[3,4] or intracerebroventricularly (i.c.v.).[4] There is evidence to indicate that they induce less tolerance and physical dependence than μ analgesics, no respiratory depression, and few or no adverse gastrointestinal effects.[5-7] Among the peptide δ opioid agonists, [D-Pen[2], D-Pen[5]]enkephalin (DPDPE) produced only a weak, centrally mediated analgesic effect after systemic administration, indicating that it does not cross the blood-brain barrier (BBB) to a significant extent.[8] Interestingly, the δ-selective and highly stable cyclic lanthionine enkephalin analog H-Tyr-c[D-Val_L-Gly-Phe-D-Ala_L]-OH was as active as morphine in producing centrally mediated analgesia when given intraperitoneally.[9] Several nonpeptide compounds showing potent and selective δ agonist activity in vitro were developed. These include TAN-67,[10] the racemic compound BW373U86,[11] and its chemically modified enantiomer SNC80.[12] These compounds produced analgesic effects when administered i.th. or i.c.v. but showed very low or no analgesic activity when given systemically. Furthermore, both BW373U86 and SNC80 produced convulsions in mice. Numerous compounds structurally derived from SNC80 were synthesized, and some were shown to be potent and selective δ agonists in in vitro assays.[13] However, analgesic data for these compounds have not yet been published. It remains to be seen whether δ opioid agonists that are efficacious enough to produce strong centrally mediated analgesic effects when given systemically or orally can indeed be developed.

Progress has been made in the development of peripherally acting κ and μ opioid agonists (for a review, see DeHaven-Hudkins and Dolle).[14] Since such compounds have no or very low ability to cross the BBB, they are expected not to produce the typical side effects mediated by central κ and μ opioid receptors (*vide supra*). There is evidence to indicate

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that peripherally restricted opioid analgesics produce antinociceptive effects under inflammatory conditions through local interaction with opioid receptors present on peripheral nerve terminals. Peripherally acting κ and μ opioid agonists may be effective as analgesics in various inflammatory pain states, for the treatment of visceral and postoperative pain, and as antipruritic agents. Most of the peripherally restricted opioids developed so far are still in the preclinical phase, and their promise as therapeutic agents needs to be confirmed in clinical trials.

In this paper, we review 2 recent developments of opioid peptide-based analgesics with novel activity profiles or with distinct physicochemical properties. The first part discusses the pharmacological characteristics of the μ opioid agonist tetrapeptide H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt^1]DALDA, where Dmt indicates 2′,6′-dimethyltyrosine), which has therapeutic potential for use in spinal analgesia and is capable of crossing the BBB. The second part discusses the development of opioid peptides that act as agonists at the μ opioid receptor and as antagonists at the δ receptor. Opioid compounds with such a mixed μ agonist/δ antagonist profile look promising as analgesics with a low propensity to produce tolerance and dependence.

[DMT^1]DALDA

The dermorphin-derived tetrapeptide H-Tyr-D-Arg-Phe-Lys-NH_2 (DALDA)[15] carries a net positive charge of 3+. In vitro it displayed μ agonist activity in the guinea pig ileum (GPI) assay, low nanomolar μ receptor binding affinity, and extraordinary μ receptor selectivity (Table 1). Replacement of the Tyr^1 residue in this peptide with 2′,6′-dimethyltyrosine (Dmt) led to a compound, [Dmt^1]DALDA, which showed 180-fold increased μ agonist potency in the GPI assay, subnanomolar μ receptor binding affinity, and still excellent μ receptor selectivity.[16] The high μ receptor binding affinity and excellent μ selectivity of this peptide were confirmed in another binding study that, furthermore, indicated some preference for μ_1 receptors (K_i = 0.05 ± 0.02 nM) over μ_2 receptors (K_i = 0.27 ± 0.13 nM).[17] When a calf striatal membrane binding assay was used, [^{3}H][Dmt^1]DALDA binding was shown to be far less sensitive than [^{3}H]H-Tyr-D-Ala-Gly-N^{2}MePhe-Gly-ol ([^{3}H]DAMGO) binding to the effects of divalent and sodium cations, and guanine nucleotides.[18] The observation that [Dmt^1]DALDA showed plateaus suggestive of a partial agonist in [^{35}S]GTPγS binding assays using brain and spinal cord membranes was in agreement with the demonstrated ability of [^{3}H][Dmt^1]DALDA to label agonist and antagonist conformations of μ receptors expressed in Chinese hamster ovary (CHO) cells. In the [^{35}S]GTPγS binding assay using CHO cells, [Dmt^1]DALDA showed high efficacy with the μ receptor and with several of its splice variants, but its potency (EC_{50}) varied markedly among some of the splice variants despite similar affinities in the receptor binding assays. In general, [Dmt^1]DALDA activated GTPγS binding more potently than did DAMGO. Taken together, these observations indicate that [Dmt^1]DALDA and DAMGO differ in the way they activate these receptors.

As expected on the basis of its structural characteristics, [Dmt^1]DALDA was found to be highly stable against enzymatic degradation when incubated in sheep blood.[19] When given i.th. to rats, [Dmt^1]DALDA was extremely active in producing an analgesic effect in the tail-flick assay: it was 3000 times more potent than morphine.[20] The same extraordinary antinociceptive potency of i.th. [Dmt^1]DALDA was also observed in the mouse tail-flick assay.[19] The observation that [Dmt^1]DALDA had only 7-fold higher μ receptor binding affinity than morphine (Table 1) suggested that this peptide may produce its extraordinarily potent antinociceptive effect via additional mechanisms. Interestingly, it was found that [Dmt^1]DALDA inhibited norepinephrine uptake in rat spinal cord synaptosomes with an IC_{50} of 4.1 μM.[20] Thus, i.th. administration of [Dmt^1]DALDA results in activation of both μ opioid receptors and α_2-adrenergic receptors in a synergistic manner known to potentiate the antinociceptive effect of μ opioid agonists.[21] This interpretation is in tune with the observation that the antinociceptive response to [Dmt^1]DALDA was attenuated by i.th. yohimbine, an α_2-adrenergic antagonist.[20] Furthermore, it

Table 1. In Vitro Opioid Activity Profiles of DALDA Peptides*[16]

	GPI	MVD	Receptor Binding	
Compound	**IC_{50}, nM†**	**IC_{50}, nM†**	**K_i^{μ}, nM†**	**K_i Ratio (μ/δ/κ)**
H-Tyr-D-Arg-Phe-Lys-NH_2 (DALDA)	254 ± 27	781 ± 146	1.69 ± 0.25	1/11 400/2500
H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt^1]DALDA)	1.41 ± 0.29	23.1 ± 2.0	0.143 ± 0.015	1/14 700/156
Morphine	29.3 ± 2.2	155 ± 31	1.00 ± 0.04	1/33/217

*Dmt indicates 2′,6′-dimethyltyrosine; GPI, guinea pig ileum; MVD, mouse vas deferens.
†Mean of 3–6 determinations ± standard error of the mean.

was shown that i.th. [Dmt[1]]DALDA in mice caused the release of dynorphin-like and met-enkephalin-like peptides in the spinal cord that then acted on κ and μ receptors, respectively, to potentiate the potency of i.th. [Dmt[1]]DALDA.[22] Thus, [Dmt[1]]DALDA may be regarded as a spinal analgesic with triple action. The duration of the antinociceptive effect of [Dmt[1]]DALDA (13 hours) in the rat tail-flick assay was 4 times longer than that of morphine (3 hours) when the 2 drugs were given i.th. at equipotent doses ($3 \times ED_{50}$).[20] The long duration of action of [Dmt[1]]DALDA may be due to not only its enzyme resistance but also the slow clearance from the spinal cord due to the peptide's high positive charge. Unlike morphine, [Dmt[1]]DALDA produced no respiratory depression after i.th. administration at a dose of $30 \times ED_{50}$, most likely because at the very low dose level required for producing the analgesic effect, significant rostral distribution of the drug to the medullary respiratory centers does not occur.[20] Because [Dmt[1]]DALDA has low propensity to produce respiratory depression after i.th. administration, it may be a candidate for a safe spinal opioid in patients. However, this peptide did produce quite profound tolerance after chronic i.th. administration.[23,24] When given i.c.v. to mice, [Dmt[1]]DALDA was 119 times more potent than morphine in the tail-flick test in one study[17] and 217 times more potent in another.[23]

Unexpectedly, [Dmt[1]]DALDA also produced a potent antinociceptive effect in the mouse tail-flick test when given subcutaneously (s.c.), being 218 times more potent than morphine in one study[17] and 36 times more potent in another.[23] These results indicate that this peptide is capable of crossing the BBB. The duration of the analgesic effect produced by s.c. [Dmt[1]]DALDA (12 hours) was again much longer than that determined for s.c. morphine (3 hours) at equipotent doses ($5 \times ED_{50}$). A pharmacokinetic study performed with sheep (intravenous infusion) revealed that the elimination half-life of [Dmt[1]]DALDA (118 minutes) was 4 times longer than that of morphine (30 minutes).[19] Both the metabolic stability and the long elimination half-life of this peptide may be responsible for its prolonged analgesic effect after systemic administration. In contrast to morphine-induced analgesia, [Dmt[1]]DALDA (s.c.) supraspinal analgesia was insensitive to several antisense probes targeting the different exons of MOR-1[17] and, unlike morphine, retained its analgesic actions in MOR-1 knockout mice.[25] Furthermore, various strains of mice showed differential sensitivities to morphine and [Dmt[1]]DALDA analgesia. These observations indicate that [Dmt[1]]DALDA is a μ opioid analgesic showing significant pharmacological differences in comparison with morphine. [Dmt[1]]DALDA did produce tolerance when chronically administered s.c. to mice[23]; however, little or no cross-tolerance was observed with this peptide given s.c. in morphine-tolerant mice.[17,26] The latter observation raises the interesting possibility that morphine-tolerant patients could be switched over to [Dmt[1]]DALDA for better pain relief.

The demonstrated ability of [Dmt[1]]DALDA to cross the BBB prompted studies examining whether this peptide could penetrate into Caco-2 cells.[27] Cellular uptake could be demonstrated using tritiated [Dmt[1]]DALDA in an incubation experiment, as well as the dansylated analog H-Dmt-Arg-Phe-Dap(dns)[28] in a confocal laser scanning microscopy study. Furthermore, it was demonstrated that [Dmt[1]]DALDA could translocate across Caco-2 cell monolayers.[27] These observations provide an explanation for this peptide's ability to penetrate into the central nervous system (CNS) after systemic administration and suggest that it may even have reasonable oral bioavailability. The mechanism of the cellular uptake of [Dmt[1]]DALDA remains to be elucidated; however, it has been established that it is not receptor mediated and does not involve a transporter or endocytosis.[27] [Dmt[1]]DALDA is a cationic-aromatic peptide consisting of alternating aromatic and basic amino acids, and it may enter cells via a local destabilization of the plasma membrane. Taken together, the results obtained with [Dmt[1]]DALDA demonstrate that potent and stable peptide drugs showing slow clearance and ability to cross the BBB can be developed.

MIXED μ AGONIST/δ ANTAGONISTS

Two studies have indicated that selective δ receptor blockade with a δ antagonist greatly reduced the development of morphine tolerance and dependence.[29,30] Several interesting observations in relation to this phenomenon have been made. Chronic morphine treatment was shown to result in an upregulation of δ binding sites in rats.[31] A study using different strains of mice demonstrated that the intensity of the withdrawal syndrome after chronic morphine treatment correlated with the level of δ binding sites.[32] Furthermore, the development of morphine tolerance and dependence following chronic morphine administration was blocked by an antisense oligodeoxynucleotide to the δ opioid receptor.[33] Finally, morphine was shown to retain its μ receptor mediated analgesic activity in δ opioid receptor knockout mice without producing tolerance upon chronic administration.[34] Very recently, δ receptor antagonists were shown to enhance morphine-mediated i.th. analgesia, possibly as a consequence of μ-δ receptor heterodimerization.[35] These various 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 an opioid compound acting 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. Furthermore, it has been shown

that the δ antagonist naltrindole reversed alfentanil (a μ agonist) induced respiratory depression[36] and enhanced colonic propulsion.[37] These results suggest that a mixed μ agonist/δ antagonist might also cause less respiratory depression and less inhibition of gastrointestinal transit than a μ agonist like morphine.

The first known compound with a mixed μ agonist/δ antagonist profile was the tetrapeptide amide H-Tyr-Tic-Phe-Phe-NH_2 (TIPP-NH_2).[38] This compound showed modest μ agonist potency in the GPI assay (IC_{50} = 1.70 ± 0.22 μM) and quite high δ antagonist potency in the mouse vas deferens (MVD) assay against the δ agonist DPDPE (K_e = 18.0 ± 2.2 nM). In agreement with the bioassay data, TIPP-NH_2 displayed relatively low affinity for μ receptors (K_i^μ = 78.8 ± 7.1 nM) and high affinity for δ receptors (K_i^δ = 3.0 ± 1.5 nM) in the rat brain membrane binding assays, indicating that it was quite δ-selective (K_i^μ/K_i^δ = 26.3). In an effort to strengthen the μ agonist component of TIPP-NH_2 without compromising its δ antagonist properties, the Tyr^1 residue was replaced with Dmt. The resulting compound, H-Dmt-Tic-Phe-Phe-NH_2 (DIPP-NH_2), showed much improved μ agonist potency in the GPI assay (IC_{50} = 18.2 ± 1.8 nM) and retained very high δ antagonist activity in the MVD assay (K_e = 0.209 ± 0.037 nM)[39] (Table 2). The receptor binding data were in agreement with these results and indicated that DIPP-NH_2 was still somewhat δ receptor-selective, as indicated by the ratio of the binding inhibition constants (K_i^μ/K_i^δ = 10.1). Reduction of the peptide bond between Tic^2 and Phe^3 of DIPP-NH_2 resulted in the highly stable pseudopeptide H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (DIPP-NH_2[Ψ]), which displayed further increased μ agonist potency in the GPI assay (IC_{50} = 7.71 nM) and retained very high δ antagonist activity (K_e = 0.537 nM) in the MVD assay.[39] DIPP-NH_2[Ψ] showed subnanomolar binding affinities for both μ and δ receptors and thus turned out to be a "balanced" μ agonist/δ antagonist (K_i^μ/K_i^δ = 2.11). In the rat tail-flick test, DIPP-NH_2[Ψ] given i.c.v. produced a potent analgesic effect (ED_{50} = 0.04 μg), being ~3 times more potent than morphine (ED_{50} = 0.11 μg).[39] At high doses (i.c.v.) it produced less acute analgesic tolerance than morphine but still a certain level of chronic tolerance. Unlike morphine, DIPP-NH_2[Ψ] produced no physical dependence upon chronic i.c.v. infusion at high dose levels (up to 4.5 μg/h) over a 7-day period.[39] Thus, the in vivo pharmacological behavior of DIPP-NH_2[Ψ] with regard to analgesic activity and the development of tolerance and dependence was, to a large extent, as expected for a mixed μ agonist/δ antagonist. However, DIPP-NH_2[Ψ] showed a limited ability to cross the BBB, and mixed μ agonist/δ antagonists capable of penetrating into the CNS and showing improved bioavailability in general have yet to be developed.

In DIPP-NH_2[Ψ] no clear distinction can be made between structural moieties that confer μ agonist properties to the molecule and moieties that are responsible for δ antagonist behavior. An alternative approach to the development of mixed μ agonist/δ antagonists is the design of compounds containing a known μ agonist and a known δ antagonist as distinct moieties. In an effort to develop a mixed μ agonist/δ antagonist capable of crossing the BBB, we recently synthesized a chimeric peptide containing [Dmt^1]DALDA and the potent and selective δ antagonist (inverse agonist) H-Tyr-TicΨ[CH_2NH]Cha-Phe-OH (TICP[Ψ]), where Cha indicates cyclohexylalanine[40] connected "tail-to-tail" via a short linker.[41] This peptide, H-Dmt→D-Arg→Phe→Lys-NH-CH_2-CH_2-NH-Phe←Cha[$NHCH_2$]ΨTic←Tyr←H ([Dmt^1]DALDA→CH_2CH_2NH←TICP[Ψ]) is expected to cross the BBB because it contains [Dmt^1]DALDA, which by itself effectively penetrates into the CNS (*vide supra*) and which likely will confer BBB crossing ability to the entire chimeric peptide construct. [Dmt^1]DALDA→CH_2CH_2NH←TICP[Ψ] showed high μ and δ receptor binding affinities in the opioid receptor binding assays (K_i^μ = 14 nM; K_i^δ = 4.8 nM) and the expected μ agonist/δ antagonist profile [IC_{50}(GPI) = 66 nM; K_e^δ(MVD) = 2.40 nM] (Table 2). In comparison with [Dmt^1]DALDA, the chimeric peptide has 47-fold lower μ agonist potency in the GPI assay

Table 2. In Vitro Opioid Activity Profiles of Mixed μ Agonist/δ Antagonists and Related Compounds*

	GPI	MVD	Receptor Binding†	
Compound	**IC_{50}, nM**	**K_e, nM‡**	**K_i^μ, nM**	**K_i^δ, nM**
H-Dmt-Tic-Phe-Phe-NH_2	18.2 ± 1.8	0.209 ± 0.037	1.19 ± 0.11	0.118 ± 0.016
H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (DIPP-NH_2[Ψ])	7.71 ± 0.31	0.537 ± 0.026	0.943 ± 0.052	0.447 ± 0.007
[Dmt^1]DALDA→CH_2CH_2NH→TICP[Ψ]	66.5 ± 5.4	2.40 ± 0.58	14.0 ± 1.5	4.79 ± 0.50
[Dmt^1]DALDA	1.41 ± 0.29	—	0.143 ± 0.015	2100 ± 310
TICP[Ψ]	Inactive	0.175 ± 0.025	1050 ± 10	0.259 ± 0.047

*DALDA indicates H-Dmt-D-Arg-Phe-Lys-NH_2; Dmt, 2′,6′-dimethyltyrosine; GPI, guinea pig ileum; MVD, mouse vas deferens; TICP[Ψ], H-Tyr-TicΨ[CH_2NH]Cha-Phe-OH, where Cha indicates cyclohexylalanine. Mean of 3–6 determinations ± standard error of the mean.
†Displacement of [^{3}H]DAMGO (μ-selective) and [^{3}H]DSLET (δ-selective) from rat brain membrane binding sites.
‡Determined against DPDPE.

and 98-fold lower μ receptor binding affinity. It has 14-fold lower δ antagonist potency than TICP[Ψ] in the MVD assay and 18-fold lower δ receptor binding affinity. Despite these reductions in potency, the chimeric peptide still has quite high μ agonist and δ antagonist potencies because of the extraordinarily high potencies of its 2 constituents. It is possible that the reduced potency of [Dmt^1]DALDA→CH_2 CH_2NH←TICP[Ψ] is due to some interference between the 2 components caused specifically by their "tail-to-tail" coupling. This chimeric peptide is currently being examined for its ability to produce a centrally mediated antinociceptive effect after administration by various routes.

Pyridomorphinans with a partial μ agonist/δ antagonist profile in vitro have been reported to produce a partial or full agonist effect in the warm-water tail-withdrawal assay after i.c.v. administration and a reduced level of tolerance.[42,43] The best compound of this class was 10-fold less potent than morphine. Taken together, the results obtained with DIPP-NH_2[Ψ] and with the pyridomorphinans indicate that mixed μ agonist/δ antagonists look promising as analgesic drug candidates; however, compounds with this profile that have higher μ agonist potency and higher efficacy and that are systemically active still need to be developed.

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