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Pharmacological characterization of the dermorphin analog [Dmt^1]DALDA, a highly potent and selective μ-opioid peptide

Claire L. Neilan [a], Thi M.-D. Nguyen [b], Peter W. Schiller [b], Gavril W. Pasternak [a,*]

[a] *Department of Molecular Neuropharmacology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA*
[b] *Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7*

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Abstract

The dermorphin-derived peptide [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2), labels μ-opioid receptors with high affinity and selectivity in receptor binding assays. In mouse, radiant heat tail-flick assay [Dmt^1]DALDA produced profound spinal and supraspinal analgesia, being approximately 5000- and 100-fold more potent than morphine on a molar basis, respectively. When administered systemically, [Dmt^1]DALDA was over 200-fold more potent than morphine. Pharmacologically, [Dmt^1]DALDA was distinct from morphine. [Dmt^1]DALDA displayed no cross-tolerance to morphine in the model used and it retained supraspinal analgesic activity in morphine-insensitive CXBK mice. Supraspinally, it also differed from morphine in its lack of sensitivity towards naloxonazine. Finally, in antisense mapping studies, [Dmt^1]DALDA was insensitive to MOR-1 exon probes that reduced morphine analgesia, implying a distinct receptor mechanism of action. Thus, [Dmt^1]DALDA is an interesting and extraordinarily potent, systemically active peptide analgesic, raising the possibility of novel approaches in the design of clinically useful drugs. © 2001 Published by Elsevier Science B.V.

Keywords: μ-Opioid peptide; Analgesia; Tail-flick assay; Radioligand binding; Splice variant; Enkephalin; Opioid; μ-Opioid receptor

1. Introduction

Opioid analgesics have remained the mainstay in the management of pain and μ-opioids are the most widely used. μ-Opioids comprise a wide range of drugs and structures. These agents are defined by their high affinity and selectivity for μ-opioid sites in traditional receptor binding assays and they share many pharmacological properties, such as tolerance, dependence, respiratory depression and inhibition of gastrointestinal transit. Yet, these drugs also display many differences, both clinically and in animal studies (Pasternak, 2001). Patients often respond better to one drug than another and the presence of incomplete cross-tolerance among these drugs has led to the use of opioid rotation in pain management. Animal studies confirm many differences among μ-opioid analgesics. A number of μ-opioids, including morphine-6β-glucuronide, heroin, 6-acetylmorphine and fentanyl, show incomplete cross-tolerance to morphine and retain their potencies in CXBK mice that are insensitive to morphine (Rossi et al., 1996). The analgesia elicited by various μ-opioids also involve different G-proteins (Standifer et al., 1996; Standifer and Pasternak, 1997). The cloning of a μ-opioid receptor, MOR-1, was soon followed by antisense studies revealing dramatic differences between morphine and other μ-opioids (Rossi et al., 1994, 1995a, 1997). In these experiments, antisense oligodeoxynucleotide probes, targeting various exons within the μ-opioid receptor gene (*Oprm*), differentially block the analgesic actions of either morphine or morphine-6β-glucuronide. A knockout model in which exon 1 of the MOR-1 receptor was disrupted, further separated the actions of morphine from both morphine-6β-glucuronide and heroin (Schuller et al., 1999). Like other knockout models disrupting the μ-opioid receptor gene (Loh et al., 1998; Matthes et al., 1996; Sora et al., 1997), morphine was totally inactive in these mice. Yet, morphine-6β-glucuronide, heroin and 6-acteylmorphine all

* Corresponding author. Department of Neurology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA. Tel.: +1-212-639-7046; fax: +1-212-794-4332.
E-mail address: pasterng@mskcc.org (G.W. Pasternak).

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retained full analgesic activity, although their potency was slightly diminished. Together, these observations illustrate the marked pharmacological differences among μ-opioid analgesics. These differences also raise the possibility of additional new μ-opioid drugs with unusual pharmacological profiles.

The tetrapeptide DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2), an analog of the tetrapeptide segment of the highly selective μ-opioid peptide dermorphin, possesses high selectivity for the μ-opioid receptor (Schiller et al., 1989). DALDA is highly polar and metabolically stable, with limited penetration of the blood–brain barrier (Samii et al., 1994) and the placental barrier (Holsey et al., 1999). Its inability to traverse these barriers, along with its metabolic stability, may lead to a potential utility as a peripheral analgesic, particularly in obstetrics.

More recently, in an effort to further increase the potency of DALDA, a number of analogues containing structural modifications of the tyrosine residue were synthesised. One such analog, [Dmt^1]DALDA ([2,6-dimethyl-Tyr]-D-Arg-Phe-Lys-NH_2), has superior potency and selectivity for the μ-opioid receptor (Schiller et al., 2000). Interestingly, [Dmt^1]DALDA produces systemic analgesia with a potency approximately 100-fold greater than morphine (Schiller, personal communication). This was clearly unexpected due to the very polar nature of the compound. The following study was undertaken to further characterize [Dmt^1]DALDA pharmacology.

2. Materials and methods

2.1. Drugs

All radioligands were purchased from DuPont NEN (Boston, MA), with the exception of [^{3}H]naloxone benzoylhydrazone, which was synthesized as previously described (Price et al., 1989). [Dmt^1]DALDA (MW 981) was synthesized in the laboratory of P.W.S. Morphine sulfate, morphine-6-glucoronide, naloxone, β-funaltrexamine, naloxonazine, and 3-methoxynaltrexone were obtained from the Research Technology Branch of the National Institute of Drug Abuse (Rockville, MD). Antisense oligodeoxynucleotides directed against regions of the MOR-1 clone were purchased from Midland Certified Reagent (Midland, TX).

Table 1
K_i values and Hill coefficients for [Dmt^1]DALDA binding as determined by competition binding assays
Values represent mean ± S.E.M. for at least three experiments performed in triplicate.

Receptor	K_i value (nM)	Hill coefficient
μ_1	0.05 ± 0.02	1.00
μ_2	0.27 ± 0.13	1.09
δ	115.7 ± 25.7	0.99
κ_1^a	11.5 ± 2.08	0.47
	2407 ± 1359	
κ_3^a	0.69 ± 0.31	0.59
	187.2 ± 81.8	

[a] These values were obtained by fitting to a two-site model.

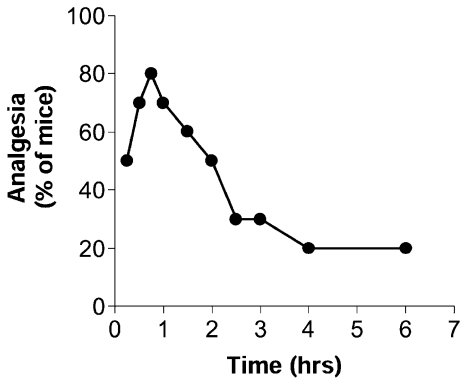

Fig. 1. Time-course of [Dmt^1]DALDA analgesia. A group of mice ($n \geq 20$) received a bolus dose of [Dmt^1]DALDA (90 μg/kg, s.c.) and was assessed at the indicated time in the radiant heat tailflick assay. Analgesia is expressed as percent of mice analgesic.

2.2. Competition binding assays

Membranes were incubated in 50 mM potassium phosphate buffer at 25°C for either 60 min (κ_1 and κ_3 binding) or 180 min (μ_1, μ_2, and δ binding), with radioligand and various concentrations of unlabelled ligand to give a total assay volume of 2 ml. The reaction was terminated by rapid filtration over glass fibre filters. Non-specific binding was determined with levallorphan (1 μM). μ Binding assays were performed using calf thalamus membranes with either [^{3}H]DADLE (0.7 nM) in the presence of DPDPE (10 nM) for μ_1 binding, or [^{3}H]DAMGO (1 nM) in the presence of DSLET (5 nM) for μ_2 binding. $MgCl_2$ (5 mM) was added to the buffer in order to increase levels of specific μ binding (Clark et al., 1988). For δ binding, calf frontal cortex membranes were used with [^{3}H]DPDPE (1 nM) and for κ_3 binding, calf striatal membranes were used with [^{3}H]naloxone benzoylhydrazone (1 nM) in the presence of EDTA (5 mM) to block μ binding (Clark et al., 1989). κ_1 Binding was performed in mouse brain membranes using [^{3}H]U69593 (1 nM) (Clark et al., 1989). K_i values and Hill coefficients were determined using GraphPad Prism, (San Diego, CA).

2.3. Analgesia

Male CD-1 mice were purchased from Charles River Breeding Laboratories (Raleigh, NC) and CXBK mice (20–24 g) from Jackson Laboratories. Animals were housed

Table 2
Analgesic activity of [Dmt[1]]DALDA and morphine in mice

Route of administration	ED_{50} value (95% confidence limits)		Ratio
	Morphine	[Dmt[1]]DALDA	
s.c.	4.8 μmol/kg (3.4–6.9)	22 nmol/kg (10–45)	218
i.c.v.	464 pmol (237–911)	3.9 pmol (2.0–7.2)	119
i.t.	314 pmol (154–644)	61 fmol (30–142)	5148
i.d.	14 nmol (7.5–26)	7.4 nmol (5.0–10.8)	1.9

in groups in a temperature-controlled room maintained on a 12-h light–12-h dark cycle. Food and water were available ad libitum until the time of the experiment. For in vivo experiments, each subject was tested only once and studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health. Antinociception, referred to as 'analgesia' in the current study, was assessed in the radiant heat tail-flick assay, as previously described (Ling et al., 1983; Pasternak et al., 1980). Baseline latencies typically ranged from 2 to 3 s. Analgesia was defined quantally as the doubling or greater of the baseline latency for an individual mouse. Analgesia was determined at peak effect: 5 min following intradermal injection, 10 min following i.t. injection, 15 min following i.c.v. injection, and 25 min following s.c. injection. ED_{50} values and 95% confidence limits were determined using the Litchfield–Wilcoxon method (Litchfield and Wilcoxon, 1949; Tallarida and Murray, 1987). Significance among single doses was determined using the Fisher Exact test.

2.4. Antisense oligodeoxynucleotides

Antisense studies were performed as previously reported (Rossi et al., 1995a, 1996). Groups of mice ($n \geq 10$) received i.c.v. injections of the indicated oligodeoxynucleotide (5 μg) on days 1, 3 and 5, and analgesia was assessed on day 6. Antisense sequences are based upon mouse MOR-1 sequences.

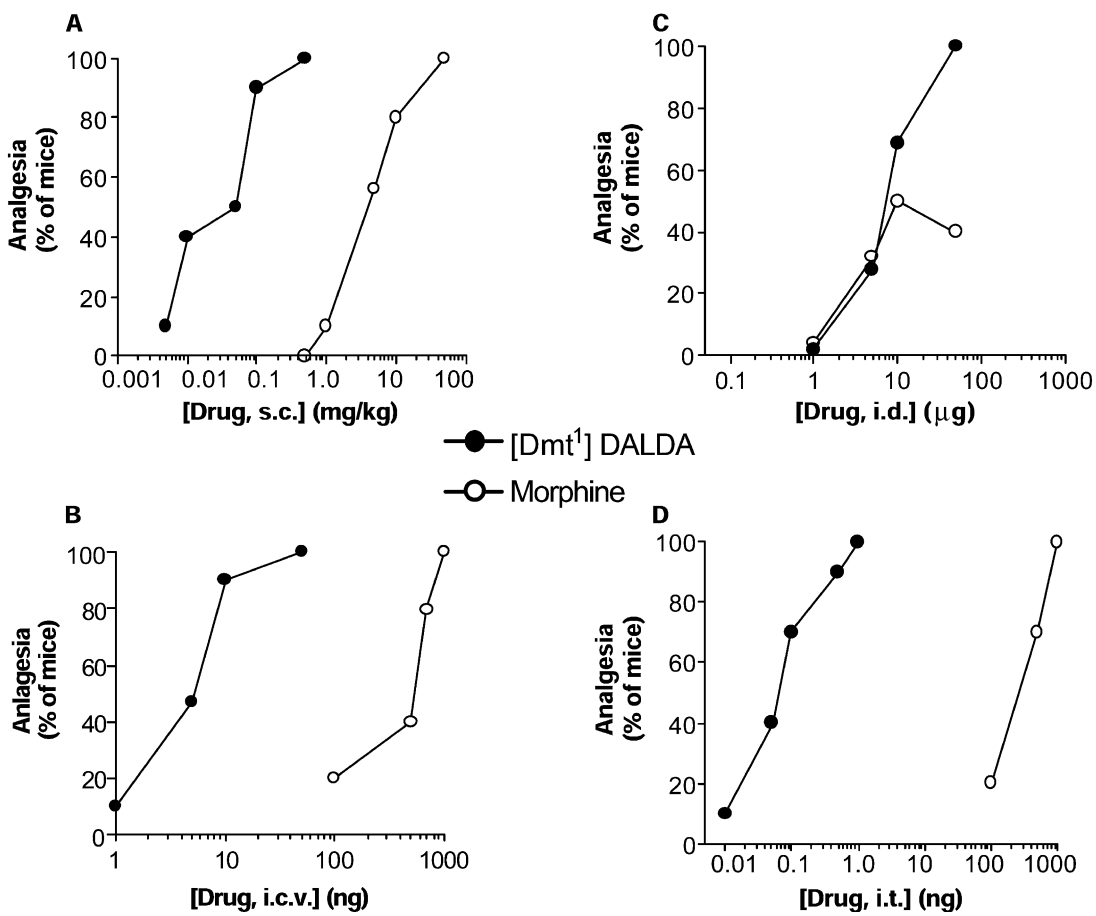

Fig. 2. Analgesic potency of [Dmt[1]]DALDA and morphine. [Dmt[1]]DALDA and morphine analgesic dose–response curves: (A) systemic, (B) supraspinal, (C) local/peripheral and (D) spinal administration. For this and each subsequent figure, analgesia was determined and expressed as percent of mice responding. Groups of ≥ 10 mice were used to determine each point with the exception of systemic administration where a cumulative dosing paradigm was employed.

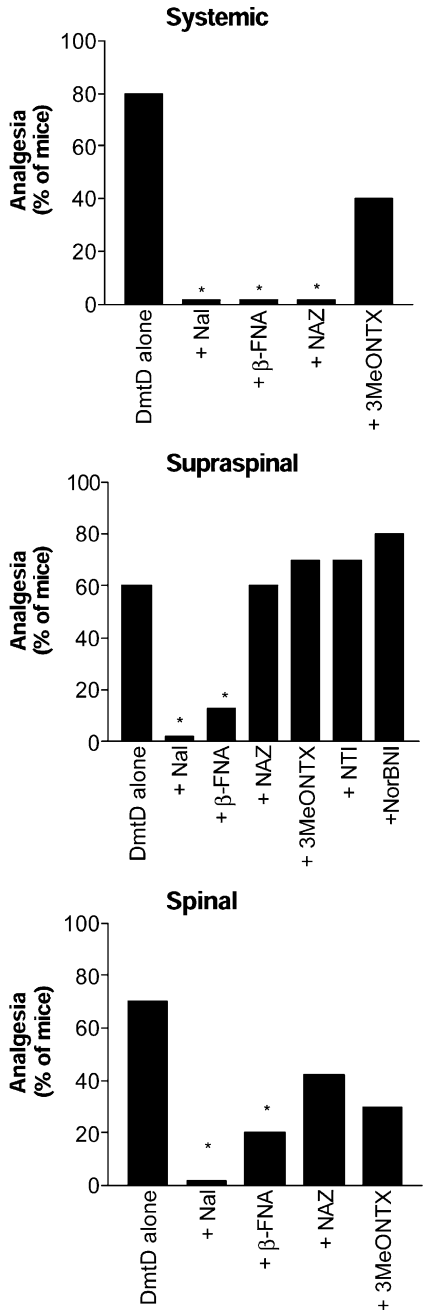

Fig. 3. Effect of selective antagonists on (A) systemic, (B) supraspinal, and (C) spinal [Dmt[1]]DALDA analgesia. All antagonists were administered s.c. prior to agonist: NX = naloxone, 1 mg/kg, 15 min; β-funaltrexamine, 40 mg/kg, 24 h; NAZ = naloxonazine, 35 mg/kg, 24 h; 3MeONTX = 3-methoxynaltrexone, 0.25 mg/kg, 15 min; NTI = naltrindole, 10 mg/kg, 15 min; NorBNI = norbinaltorphimine, 32 mg/kg, 24 h. Significant differences were determined using the Fisher Exact test, * $P < 0.05$.

2.5. Gastrointestinal transit

Mice were fasted for 18–24 h prior to experiment. Mice were treated with either saline or [Dmt[1]]DALDA s.c. and given a standard charcoal meal (0.5 ml) by gavage. The mice were sacrificed 30 min after administration of the charcoal meal and the distance the charcoal meal had traveled was measured. Statistically significant differences from control were assessed using Students' *t*-test.

3. Results

3.1. Radioligand binding

In competition binding assays, [Dmt[1]]DALDA bound with high selectivity to the μ receptor, affording K_i values in the low nanomolar range (Table 1) with somewhat greater selectivity for μ_1. Analysis of binding to κ_1 and κ_3 receptors revealed a best fit to a two-site curve. The higher affinity [Dmt[1]]DALDA binding component in the κ_3 receptor competition most likely represents competition of residual μ-opioid receptor binding in the assay (Clark et al., 1989). The selectivity ratio of [Dmt[1]]DALDA binding to μ_1, μ_2, δ, κ_1, and κ_3 receptors is 1:5:2300:230:3700, respectively.

3.2. Analgesia

[Dmt[1]]DALDA is a potent μ-opioid analgesic in the radiant heat tail flick assay. Following systemic administration (90 μg/kg, s.c.), this peptide produced profound analgesia with a peak effect at 30–45 min and some analgesia lasting up to 4 h (Fig. 1). Compared to morphine, [Dmt[1]]DALDA was well over 100 times more potent on a molar basis when administered either systemically or supraspinally (Fig. 2 and Table 2) (ED_{50} morphine 3.2 mg/kg or 4.8 μmol/kg, s.c., 310 ng or 464 pmol, i.c.v.; ED_{50} [Dmt[1]]DALDA 21.3 μg/kg or 22 nmol/kg, s.c. and 3.8 ng or 3.9 pmol, i.c.v.). In addition, [Dmt[1]]DALDA was 5000 times more potent than morphine at the molar level when administered i.t. (ED_{50} morphine 210 ng or 314 pmol, i.t., and [Dmt[1]]DALDA 60 pg or 61 pmol, i.t.). However, in the periphery, no major differences in potency

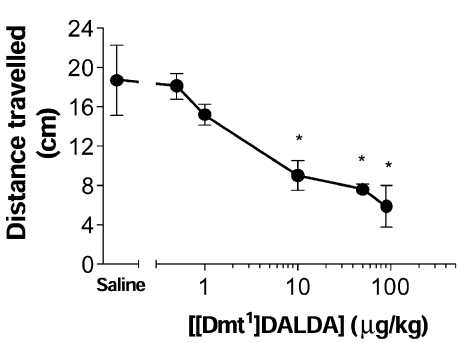

Fig. 4. Inhibition of gastrointestinal transit by [Dmt[1]]DALDA. Groups of mice ($n = 10$) received the indicated treatment followed by a charcoal meal and, transit determined as described in Section 2. Results are the means ± S.E.M. The [Dmt[1]]DALDA group was significantly different from the saline group ($P < 0.05$), as determined by Students' *t*-test.

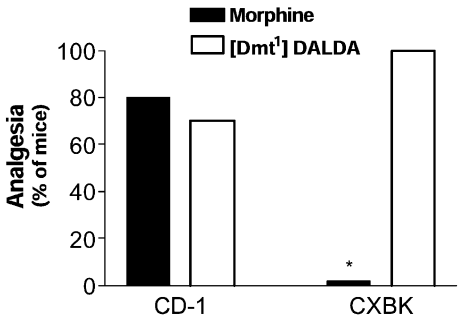

Fig. 5. Effects of morphine and [Dmt[1]]DALDA in CXBK mice. Groups of CD-1 mice ($n \geq 10$) or CXBK mice ($n = 5$) received either morphine (700 ng i.c.v.) or [Dmt[1]]DALDA (8 ng i.c.v), and were tested for analgesia in the tailflick assay 15 min later. Significant differences were determined using the Fisher Exact test, $^{*}P < 0.05$.

were seen. A localized intradermal (i.d.) injection of the peptide produced an analgesic effect that was equipotent but more efficacious than morphine (ED_{50} morphine 9.4 μg or 14 nmol, i.d.; [Dmt[1]]DALDA 7.3 μg or 7.4 nmol, i.d.).

In order to determine the selectivity of [Dmt[1]]DALDA analgesia, mice were pre-treated with antagonists at doses selective for their respective receptors (Paul et al., 1991) (Fig. 3). Pre-treatment with the non-selective antagonist naloxone fully inhibited all systemic, spinal, and supraspinal analgesia produced by the peptide. Similar results were observed with the μ-selective antagonist β-funaltrexamine. Pre-treatment with the μ_1-selective antagonist naloxonazine significantly inhibited systemic analgesia, but had no effect on supraspinal analgesia. Although a partial inhibition of spinal analgesia was observed, the effect was not significant. 3-Methoxynaltrexone, a modestly selective M6G antagonist (Brown et al., 1997), did not inhibit supraspinal analgesia, and the partial inhibition of systemic and spinal analgesia was not significant. The selective δ-antagonist naltrindole and the selective κ-antagonist nor-BNI were inactive against [Dmt[1]]DALDA-mediated supraspinal analgesia.

3.3. Gastrointestinal transit

In this experiment, the ability of [Dmt[1]]DALDA to inhibit the passage of a charcoal meal was determined. For mice pre-treated with saline, the average distance traveled

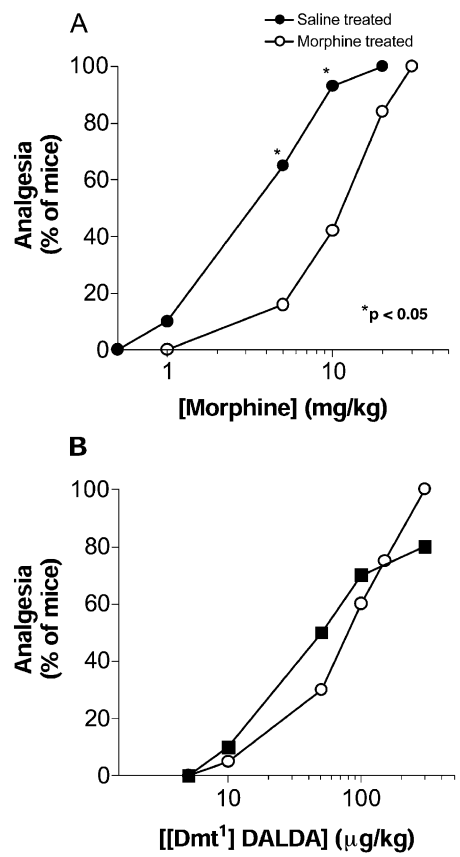

Fig. 6. [Dmt[1]]DALDA cross-tolerance with morphine. Groups of mice ($n \geq 20$) were made tolerant to morphine over a 4-day period. On days 1 and 2, mice were given either saline or 10 mg/kg of morphine twice daily, and on days 3 and 4, they were given either saline or 20 mg/kg of morphine twice daily. Analgesia was assessed on day 5. Significant differences between individual points were assessed using Fisher Exact test, and ED_{50} values were considered significantly different when 95% confidence limits did not overlap.

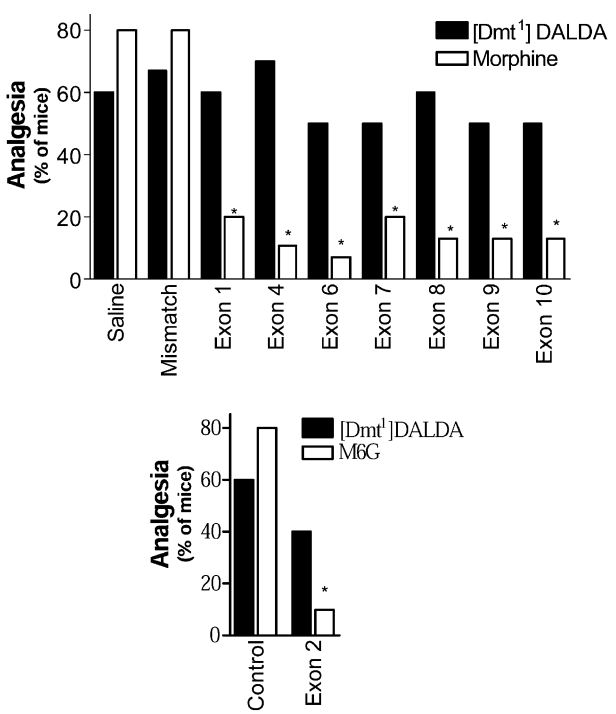

Fig. 7. Effect of MOR1 antisense oligodeoxynucletotides on supraspinal [Dmt[1]]DALDA and morphine analgesia. Groups of mice ($n = 20$) received either saline, the mismatch antisense (5 μg) or the indicated antisense (5 μg) targeting the indicated exon (Table 3) supraspinally, and were tested with either (A) morphine (700 ng, i.c.v.) or (B) M6G (12.5 ng, i.c.v.), as well as [Dmt[1]]DALDA (8 ng, i.c.v.). Significant differences were determined using the Fisher Exact test, $^{*}P < 0.05$.

was 18.7 ± 3.6 cm. [Dmt[1]]DALDA inhibited gastrointestinal transit in a dose-dependent manner, with an ED_{50} of 6 μg/kg, s.c. and a maximum effect at 10 μg/kg (Fig. 4).

3.4. Analgesia in CXBK mice

CXBK mice are insensitive to the analgesic effects of morphine administered systemically or supraspinally (Baron et al., 1975; Pick et al., 1993; Reith et al., 1981). Despite this, there are a number of opioids, including heroin and etonitazine, which retain their ability to elicit an analgesic response when administered i.c.v. (Connelly et al., 1994; Rossi et al., 1996). [Dmt[1]]DALDA (8 ng i.c.v., an ED_{80} dose in CD-1 mice) produced a full analgesic response in all of the CXBK mice tested. An equivalent analgesic dose of morphine, 700 ng, failed to produce any response (Fig. 5).

3.5. Cross-tolerance

We next examined cross-tolerance between morphine and [Dmt[1]]DALDA. Mice received daily injections of morphine for 4 days and analgesia was assessed on day 5. This treatment shifted the ED_{50} for morphine threefold, from 3.2 mg/kg (2.2–4.6) in naive animals to 10.4 mg/kg (6.9–15.7) in treated ones ($P < 0.05$, Fig. 6A). However, there was no significant shift in the ED_{50} for [Dmt[1]]DALDA in the morphine-treated mice [ED_{50} naive = 65.5 μg/kg (28.6–150.5); ED_{50} morphine-treated = 78.0 μg/kg (50.6–120.4); Fig. 6B].

3.6. Antisense studies

Our laboratory has reported a number of alternatively spliced isoforms of the μ-opioid receptor (Pan et al., 1999, 2000; Rossi et al., 1995a, 1997). Down-regulation of specific exons of MOR-1 using antisense oligodeoxynucleotides differentially inhibits morphine and M6G analgesia (Pan et al., 2000; Rossi et al., 1995a, 1997). In this study, mice were treated supraspinally with antisense oligodeoxynucleotides targeting various MOR-1 exons. None of the antisense oligodeoxynucleotides examined significantly inhibited supraspinal [Dmt[1]]DALDA analgesia despite their continued activity against either morphine (exons 1, 4, 5, 6, 7, 8, 9, 10) or M6G (exon 2) (Fig. 7, Table 3). In additional studies on spinal [Dmt[1]]DALDA analgesia, antisense probes targeting exons 1 or 2 also were inactive (data not shown).

Table 3
Antisense sequences targeting MOR-1

Exon	Sequence
1	CGCCCCAGCCTCTTCCTCT
Mismatch	CGCCCC**GA**CCTCTTCC**CT**T
2	TTGGTGGCAGTCTTCATTTTGG
4	GGGCAATGGAGCAGTTTCTG
6	GGCTCAAAGACAAGGGACAGGTCA
7	CCTGTAAAGATCTGGGCCACGC
8	GGGCCATCATCAGGAAGAAGG
9	GAAAGGCATCTTCCCTCTCGCT
10	CTTGCTGCCTTCGTAAGGACCTGG

4. Discussion

[Dmt[1]]DALDA is a highly potent and selective μ-opioid analgesic with a unique mechanism of action distinct from that of morphine. It was surprising to see the extraordinary analgesic potency of [Dmt[1]]DALDA both spinally and systemically, being 5000- and 200-fold, respectively, more potent than morphine. The activity of systemic [Dmt[1]]DALDA could not result solely from peripheral actions for a number of reasons. First, in a model of peripheral analgesia the potency of [Dmt[1]]DALDA intradermally was equivalent to morphine, a far different relative potency than that seen systemically. In addition, naloxone given either spinally or supraspinally blocked systemic [Dmt[1]]DALDA analgesia (data not shown). Although peripheral mechanisms may still be important, the naloxone results clearly implied that the peptide had central actions. The majority of opioid peptides are subject to rapid enzymatic degradation, particularly in blood, and for this reason, generally do not have time to traverse the blood–brain barrier. There are a number of systemically active peptides that cross the blood–brain barrier, including the dynorphin analog E-2078 (Nakazawa et al., 1991), the double-enkephalin compound biphalin (Abbruscato et al., 1996), the amphibian peptide [Lys_7]dermorphin (Negri et al., 1995) and [Dmt[1]]DPDPE (Hansen et al., 1992). Enhanced stability facilitates systemic activity. The D-amino acid at the two-position of [Dmt[1]]DPDPE, the parent compound of [Dmt[1]]DALDA, stabilizes it towards aminopeptidase, as originally observed with other enkephalin derivatives (Pert et al., 1976), including DALDA (Samii et al., 1994). Additionally, the carboxyl terminal amidation reduces susceptibility of the peptide to carboxypeptidase. The prolonged duration of action for systemic [Dmt[1]]DALDA is consistent with its metabolic stability. How it penetrates the brain remains unclear, but it has been suggested that DALDA slowly crosses the blood–brain barrier via lipid-mediated free diffusion (Samii et al., 1994). Recent studies also show the importance of P-glycoprotein in the blood–brain barrier (Schinkel et al., 1994) and it plays a role in opioid pharmacokinetics and entry into the brain (Chen and Pollack, 1998, 1999; Jonker et al., 1999; Letrent et al., 1999a,b; Schinkel et al., 1995; Thompson et al., 2000), raising questions regarding the potential role of P-glycoprotein in the systemic activity of [Dmt[1]]DALDA.

Radioligand binding studies confirmed a μ-opioid receptor selective profile for [Dmt[1]]DALDA with sub-

nanomolar K_i values for both μ_1 and μ_2 receptors. These affinities are similar to prior results looking at total μ binding (K_i: 0.14 nM) (Schiller et al., 2000). Like the earlier studies, [Dmt[1]]DALDA was far less active against κ or δ binding sites in our competition assays.

Selective antagonists further established the specificity of [Dmt[1]]DALDA actions in vivo for μ receptors. The μ-opioid-selective antagonist β-funaltrexamine blocked systemic, spinal and supraspinal analgesia, confirming a μ-opioid receptor mechanism of action. Like other μ-opioids, [Dmt[1]]DALDA potently inhibited gastrointestinal transit. Despite its activity at μ-opioid receptors, [Dmt[1]]DALDA analgesia was readily distinguished from morphine analgesia. Although the μ_1-selective antagonist naloxonazine was active against systemic [Dmt[1]]DALDA, it was ineffective when [Dmt[1]]DALDA was given supraspinally. In contrast, supraspinal morphine analgesia is readily reversed by naloxonazine treatment (Ling et al., 1986; Paul et al., 1989).

Cross-tolerance is a valuable approach to define common mechanisms of action. Although μ-opioids show cross-tolerance, it oftentimes is incomplete (Payne and Pasternak, 1992; Rossi et al., 1996). This is most easily detected with modest levels of morphine tolerance, as in the model employed in the current study. In this paradigm, [Dmt[1]]DALDA was not cross-tolerant to morphine, consistent with a distinct receptor mechanism. However, it is also possible that incomplete tolerance reflects differences in the efficacy of the two drugs.

Strain differences among mice also have proven helpful in elucidating receptor mechanisms of action. CXBK mice are insensitive to morphine given either systemically or supraspinally. However, a number of μ-opioids elicit supraspinal analgesia in CXBK mice, including heroin, etonitazine, fentanyl, and 6-acetylmorphine, implying the presence of a second μ-opioid mechanism of action distinct from that of morphine (Rossi et al., 1996). [Dmt[1]]DALDA maintained its supraspinal analgesic activity in CXBK mice with a potency similar to that observed in CD-1 mice, further distinguishing its actions from those of morphine.

At the molecular level, differences between morphine and other μ-opioids have been observed in both knockout (Schuller et al., 1999) and antisense models (Rossi et al., 1994, 1995a,b, 1997). Our laboratory has previously demonstrated the presence of a number of splice variants of the μ-opioid receptor (Pan et al., 1999, 2000; Rossi et al., 1995a). The use of antisense oligodeoxynucleotides can assess the importance of variants containing specific exons in producing analgesia. For example, down-regulation of variants containing exons 1 and 4 of MOR-1 diminish supraspinal morphine analgesia, while antisense probes targeting exons 2 and 3 do not (Pasternak and Standifer, 1995; Rossi et al., 1994, 1995a, 1997). In contrast, M6G analgesia is sensitive to probes targeting exons 2 and 3, but not 1 or 4 (Rossi et al., 1995a,b, 1997). In the current study, mice were pretreated with antisense probes targeting the different exons of MOR-1. Each antisense probe was active, down-regulating either the analgesic actions of M6G (exon 2) or morphine (exons 1, 4, 6, 7, 8, 9 and 10). Yet, none of them significantly blocked supraspinal [Dmt[1]]DALDA analgesia. The insensitivity of [Dmt[1]]DALDA analgesia to any of the antisense probes provides strong evidence at the molecular level that [Dmt[1]]DALDA is distinct from all the other μ-opioids examined in this paradigm, including morphine, fentanyl, heroin, 6-acetylmorphine and M6G. Indeed, the inactivity of any of the antisense probes raises the question of whether receptors encoded by MOR-1 mediate [Dmt[1]]DALDA analgesia despite its sensitivity towards μ-selective antagonists.

In conclusion, [Dmt[1]]DALDA is a most unusual and highly interesting peptide analgesic that exhibits a very unique pharmacology. It shows great promise as a systemically active peptide analgesic. Although its selectivity clearly fits with its classification as a μ-opioid, [Dmt[1]]DALDA proceeds via a very unusual mechanism of action. These findings raise important questions regarding the mode of action of μ-ligands that clearly warrant further study.

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