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Potent in vivo antinociception and opioid receptor preference of the novel analogue [Dmt^1]endomorphin-1

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Abstract

[Dmt^1]Endomorphin-1 is a novel analogue of the potent μ-opioid agonist endomorphin-1. Given the physiological role of endomorphin-1 in vivo, this compound was investigated to determine if the antinociception occurred through systemic, supraspinal or in a combination of both neuronal pathways. This compound exhibited a potent dose-dependent effect intracerebroventricularly in both spinal and supraspinal regions, and was blocked by opioid antagonist naloxone, which verified the involvement of opioid receptors. Specific opioid antagonists characterized the apparent receptor type: β-funaltrexamine (μ_1/μ_2-irreversible antagonist) equally inhibited spinal- and central-mediated antinociception; on the other hand, naloxonazine (μ_1-subtype) was ineffective in both neural pathways and naltrindole (δ-selective antagonist) partially (26%), though not significantly, blocked only the spinal-mediated antinociception. Therefore, spinal antinociception was primarily triggered by μ_2-subtypes without involvement of μ_1-opioid receptors; however, although a slight enhancement of antinociception by δ-receptors cannot be completely ruled out since functional bioactivity indicated mixed μ-agonism/δ-antagonism. In terms of the CNS action, [Dmt^1]endomorphin-1 appears to act through μ_2-opioid receptor subtypes.

Keywords: Antinociception; Dimethyltyrosine; Endomorphin; Opioid receptor subtypes; Spinal; Supraspinal

1. Introduction

Endomorphin-1 (H-Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2) are highly selective ligands to the μ-opioid receptors (Zadina et al., 1997). Intracerebroventricular or intrathecal injection of these peptides produces potent antinociception that is selectively blocked by μ-opioid receptor antagonists, such as β-funaltrexamine, CTOP or the non-selective naloxone (Stone et al., 1997; Zadina et al., 1997; Narita et al., 1998; Tseng et al., 2000). Recent studies have shown that replacing Tyr^1 in the first position of endomorphin-2 by the more hydrophobic amino acid residue 2′,6′-dimethyl-L-tyrosine (Dmt) increased μ-opioid receptor affinity and associated functional bioactivity by 5- and 30-fold, respectively (Okada et al., 2003a), and elevated the in vivo activity in mice as measured by the classical pharmacologically defined tail-flick and hot-plate tests paradigms for measuring analgesia (Li et al., 2005). While endomorphin-2 interacts with both μ_1- and μ_2-opioid receptor subtypes and has no appreciable affinities for either δ- and κ_1-opioid receptors (Horvath, 2000), [Dmt^1]endomorphin-2 elevated receptor affinity and functional bioactivity toward δ-opioid receptors by factors of 327- and 583-fold, respectively, relative to endomorphin-2. Furthermore, receptor preference of [Dmt^1]endomorphin-2 in the tail-flick assay revealed that it induced spinal antinociception through the activation of δ-opioid receptors,

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as well as μ-opioid receptors, indicating the importance of Dmt in mediating δ-opioid receptor activity (Bryant et al., 2003; Jinsmaa et al., 2005); the supraspinal mechanism, on the other hand, only occurred through the activity of both μ_1-/μ_2-opioid receptor subtypes.

In spite of their close sequence identity between endomorphins-1 and -2, they differ in their biological profiles (Sakurada et al., 1999; Jinsmaa et al., 2005). In this study, we investigated whether incorporation of Dmt into endomorphin-1 plays a role on pain perception and modifies the inherent opioid receptor selectivity of endomorphin-1 using the strategy of differential receptor inhibition by opiate antagonists in mice after central injection.

2. Materials and methods

2.1. Animals

Male Swiss–Webster mice weighing 20–25 g (Taconic Farms, Germantown, NY) were housed in a 12 h light/dark- and temperature-controlled room with free access to food and water. All whole animal procedures were carried out according to protocols approved by the Animal Care and Use Committee (ACUC) at the National Institute of Environmental Health Sciences/NIH.

2.2. Drugs

Naloxone hydrochloride and loperamide hydrochloride were obtained from Sigma (St. Louis, MO, USA), and naltrindole hydrochloride, naloxonazine dihydrochloride, and β-funaltrexamine hydrochloride were purchased from Tocris Bioscience (Ellsville, MO, USA). All opiate receptor antagonists were injected subcutaneously (sc): naloxone (10 mg/kg) and naltrindole (3 mg/kg) were administered 30 min before the test compound, while naloxonazine (35 mg/kg) and β-funaltrexamine (40 mg/kg), were administered 24 h prior to testing (Paul et al., 1989). The dose of naloxone and naltrindole was selected based on the ability to completely block the effect of morphine (0.5 μg/mouse, icv) and deltorphin II (4.4 μg/mouse, icv), respectively. Radiolabelled [^{3}H]deltorphin II was a product of NEN (Bellirica, MA), and [^{3}H]DAMGO and GTPγ[^{35}S] were from Amersham (Buckinghamshire, UK). All cell culture media were obtained from Invitrogen. Penicillin–streptomycin solution, sodium pyruvate and HAT media supplement (hypoxanthine, aminopterin and thymidine), Bradford reagent, bovine serum albumin, GDP, GTPγS and dithiothreitol were purchased from Sigma (St. Louis, MO). Fetal bovine serum was obtained from HyClone (Logan, UT) and protease inhibitor cocktail tablets from Roche Diagnostics (Indianapolis, IN). Cells were from the ATCC (Manassas, VA).

2.3. Synthesis of [Dmt1]endomorphin-1

2.3.1. Synopsis

Dmt was synthesized according to Dygos (Dygos et al., 1992). [Dmt^1]endomorphin-1 was formed by segment condensation method in solution: Boc-Dmt-OH was coupled with H-Pro-OMe by using PyBop as the coupling reagent and the resultant Boc-Dmt-Pro-OMe was then treated with 1 N NaOH solution to give Boc-Dmt-Pro-OH. After deprotection of Boc-Trp-Phe-NH_2 with HCl/dioxane, the resulting H-Trp-Phe-NH_2 was condensed with Boc-Dmt-Pro-OH using PyBop as the coupling reagent. The final Boc protecting group was removed with HCl/dioxane in the presence of anisole, and the resulting peptide was purified by semipreparative RP-HPLC to >98% purity according to the detailed methods developed for the synthesis of [Dmt^1]endomorphin-2 (Okada et al., 2003a; Fujita et al., 2004; Li et al., 2005).

2.3.2. Analytical methods

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. TLC was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany). Optical rotations were determined with a DIP-1000 automatic polarimeter (Japan Spectroscopic Co.). Mass spectra were measured with a KRATOS MALDI-TOF analyses (matrix assisted laser desorption ionization time-of-flight mass spectrometry). Semi-preparative RP-HPLC and analytic RP-HPLC used a Waters Delta 600 with COSMOSIL C18 column (20 mm × 250 mm) and COSMOSIL C18 column (4.6 mm × 250 mm), respectively. The solvents for analytical HPLC were: A, 0.05% TFA in water; B, 0.05% TFA in CH_3CN. The column was eluted at a flow rate of 1 ml/min with a linear gradient: 90% A to 10% A in 30 min; the retention time was reported as t_R (min). 1H NMR spectra were measured on a Bruker DPX-400 spectrometer at 25 °C.

2.4. Opioid receptor competitive binding assays

The receptor binding assays were performed as described previously (Salvadori et al., 1997; Okada et al., 2003b; Li et al., 2005). Briefly, the receptor binding affinities were determined under equilibrium conditions (2.5 h at 22 °C) using rat brain synaptosomes (P_2 fraction) pre-equilibrated to remove endogenous opioids. δ- and μ-Opioid receptors were radiolabeled with [^{3}H]deltorphin II (45.0 Ci/mmol) and [^{3}H]DAMGO (50.0 Ci/mmol). Excess unlabeled peptide (2 μM) established the level of non-specific binding. Radiolabelled membranes were rapidly filtered onto Whatman GF/C glass fibre filters soaked in 0.1% polyethenimine and washed with 3 ml ice-cold 50 mM Tris HCl buffer (pH 7.5) containing 0.1% BSA, dried at 75 °C for an hour and radioactivity determined using EcoLume (ICN). All analogues were analyzed in duplicate using 5–8 dosages and 3–5 independent repetitions with different synaptosomal preparations to ensure statistical significance. The affinity constants (K_i) were calculated according to Cheng and Prusoff (Cheng and Prusoff, 1973).

2.5. Functional bioactivity in isolated tissue preparations

Bioactivity in vitro was determined using guinea-pig ileum for μ-opioid receptors and mouse vas deferens for δ-opioid activity. Both tissues, suspended separately in balanced salt solutions, were used for field stimulation with bipolar rectangular pulses of supramaximal voltage. Agonists were tested for their inhibition of the electrically evoked twitch and the results are expressed as the

IC_{50} values obtained from dose–response curves and represent the mean ± SE of 5–6 separate assays. [D-Ala^2]Deltorphin I and dermorphin were used as internal δ- and μ-opioid peptide standards for mouse vas deferens (MVD) and guinea-pig ileum (GPI), respectively (Okada et al., 2003b).

2.6. GTPγ[^{35}S] binding assay

2.6.1. Cell culture and membrane preparation

Cells were cultured as monolayers at 37 °C in a humidified atmosphere in 5% CO_2 and 95% air. SK-N-SH cells (passage 3–16) in Minimal Essential Medium with Earle's salt, non-essential amino acids, L-glutamine (2 mM) and sodium pyruvate (1 mM); NG108-15 cells (passage 5–12) in Dulbecco's Modified Eagle Medium supplemented with hypoxanthine, aminopterin and thymidine (HAT). Both culture media were supplemented with fetal bovine serum (10%), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were washed twice with ice cold phosphate buffered saline (PBS) and harvested in ice cold 50 mM Tris buffer pH 7.4 containing 0.2 mM EGTA, 1 mM $MgCl_2$ and protease inhibitor cocktail (assay buffer), centrifuged (1500 rpm×5 min at 4 °C). The cell pellet was resuspended in 10 ml ice cold Tris buffer, homogenized for 10 s (Ultra Turrax T8 homogenizer) and centrifuged (40,000 ×*g*, 20 min). Membranes were washed once with 10 ml Tris buffer and resuspended in the same solution. The concentration of protein was determined with the Bradford method using bovine serum albumin as the standard. The membrane suspension was divided into aliquots and stored until use at −80 °C.

2.6.2. GTPγ[^{35}S] binding assay

Membranes (50 μg protein) were incubated with varying concentrations of the opioid compounds in the Tris assay buffer containing NaCl (100 mM), $MgCl_2$ (5 mM), GDP (30 μM), GTPγ[S] (95 pM), dithiothreitol (0.2 mM), protease inhibitor cocktail, in a total volume of 0.5 ml for 60 min at room temperature (22 °C). Nonspecific binding was determined in the presence of unlabeled GTPγS (10 μM). Reaction was terminated by vacuum filtration on GF/B filters pre-soaked overnight in 1% bovine serum albumin. Filters were washed twice with ice cold 50 mM Tris buffer (pH 7.4). Bound GTPγ[^{35}S] was quantified by liquid scintillation using EcoLume (ICN).

2.7. Intracerebroventricular injection

A 25 μl Hamilton microsyringe, fitted with a disposable 27-gauge needle, was used for intracerebroventricular (icv) administration. The needle was inserted 2.3–3.0 mm deep as described (Laursen and Belknap, 1986). Briefly, the bregma was found by lightly rubbing the point of the needle over the skull until the suture was felt through the skin. The needle was inserted 2 mm lateral to the midline and 4 μl total volume injected. Immediately after testing, the animals were sacrificed according ACUC-approved protocols available at NIEHS: a slit was made along the midline of the scalp and mice exhibiting a needle tract 2 mm lateral from the bregma were counted as having been injected correctly.

2.8. Tail-flick test

Spinal effects were measured using a tail-flick instrument (Columbus Instruments, Columbus, OH). Radiant heat was applied on the dorsal surface of the tail and the latency for removal of the tail from the onset of the radiant heat is defined as the tail-flick latency. The baseline of tail-flick latency was adjusted between 2 and 3 s (pre-response time) and a cut off time was set at 8 s to avoid external heat-related damage. The analgesic response was measured 10 min following icv injection. The duration time for icv injection was 10 min, and the test was terminated when tail-flick latency was close to the pre-response time.

2.9. Hot-plate test

Supraspinal effect was measured using 55.0 ± 0.1 °C electrically heated plate (IITC MODEL 39D Hot Plate analgesia meter, Woodland Hills, CA). Ten minutes after icv administration of the opioid ligand, the hot-plate latency was measured as the interval between placement of mice onto the hot plate and observing movement consisting of either licking or shaking their hind paws with a baseline latency of 15 s and maximal cut off time of 30 s. The duration time for icv injection was 10 min, and the test was terminated when hot-plate latency was close to the pre-response time to prevent heat damage to the mice.

2.10. Statistical analysis

Statistical significance of the data was estimated by one-way analysis of variance (ANOVA) followed by Dunnett's test using the computer software program JMP (SAS Institute Inc., Cary, NC). The data were considered significant at $P<0.05$. The area under the time–response curve (AUC) was obtained by plotting the response time (s) on the ordinate and time (min) on the abscissa after administration of the compounds.

3. Results

3.1. Receptor affinity, selectivity and functional bioactivity in vitro

[Dmt^1]Endomorphin-1 interacted with μ- and δ-opioid receptors in rat brain membrane preparations with high affinity, particularly toward the μ-opioid receptor [K_iμ = 0.067 ± 0.02 nM

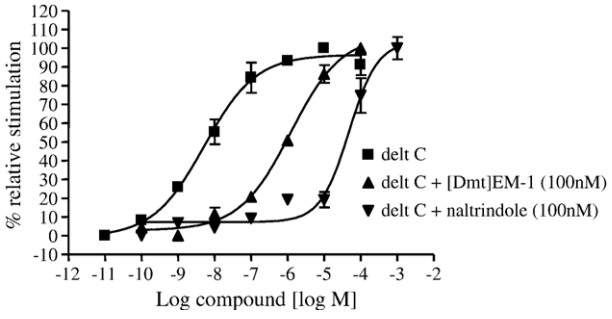

Fig. 1. Effect of [Dmt^1]endomorphin-1 (100 nM) and naltrindole (100 nM) on deltorphin C-stimulated GTPγ[^{35}S] binding in NG108-15 cell membranes. Each value presents the mean ± s.e.m.

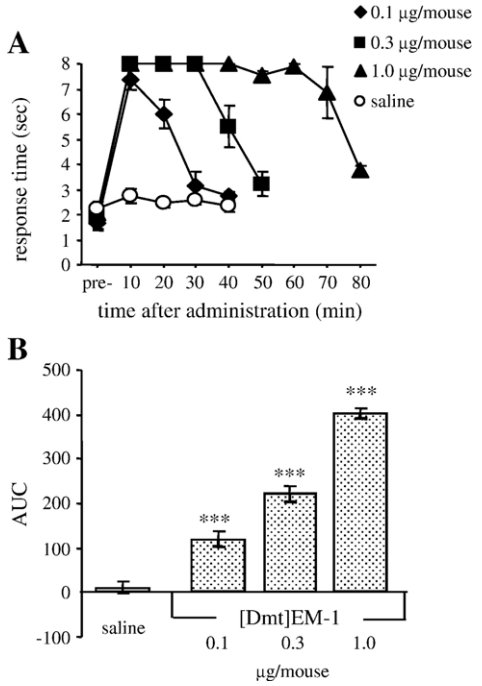

Fig. 2. The effect of intracerebroventricularly injected [Dmt[1]]endomorphin-1 ([Dmt[1]]EM-1) in the tail-flick test in mice. (A) Time course, (B) Area Under the dose response Curve (AUC). Each value is the mean±s.e.m. (n=5–6). ANOVA and Dunnett's test were used to statistically analyze the data. ***P<0.001 represents significant difference in the AUC values between opioid and saline-treated mice.

and $K_i\delta$=5.0±0.6 nM], such that the selectivity for μ-over δ-opioid receptors was relatively low ($K_i\delta/K_i\mu$=75).

In the GPI bioassay, [Dmt[1]]endomorphin-1 exhibited potent opioid agonist activity for μ-opioid receptors (IC_{50}=0.272±0.07 nM). The δ-opioid associated bioactivity, on the other hand, was quite remarkable: whereas at 10 μM [Dmt[1]]endomorphin-1 had very weak to negligible δ-agonist activity with only 21% inhibition of the contractions, it exhibited potent δ-opioid antagonism against deltorphin II with a pA_2=8.6.

In addition to functional bioassays, we performed GTPγ[^{35}S]-binding assay using SK-N-SH and NG108-15 cells constitutively expressing human μ- or mouse δ-opioid receptors, respectively. [Dmt[1]]Endomorphin-1 expressed μ-opioid agonist activity in SK-N-SH cells with an IC_{50}=1.065 nM; moreover, it increased the effect of the μ-opioid agonist loperamide, suggesting the compound is full agonist on μ-opioid receptors (data not shown). However, [Dmt[1]]endomorphin-1 shifted the effect of δ-opioid agonist deltorphin C in NG108-15 cells by several orders of magnitude (Fig. 1.), which confirmed the results of the MVD bioassays system that this compound exhibits δ-antagonism.

3.2. Antinociception of [Dmt[1]]endomorphin-1 and comparison of the activity to [Dmt[1]]endomorphin-2

Intracerebroventricularly injected [Dmt[1]]endomorphin-1 exhibited dose-dependent antinociception in the both tail-flick

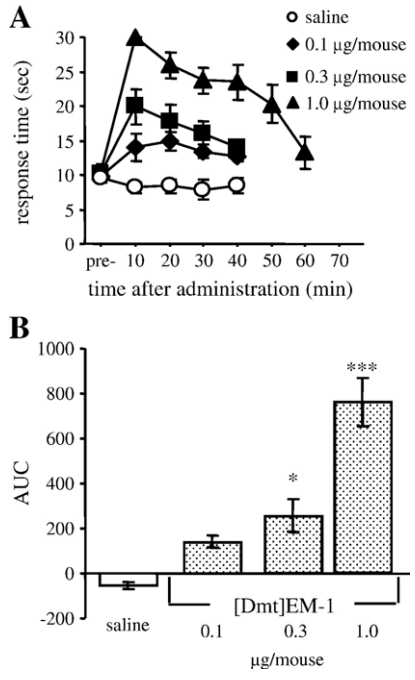

Fig. 3. The effect of intracerebroventricularly injected [Dmt[1]]endomorphin-1 ([Dmt]EM-1) in the hot-plate test in mice. (A) Time course, (B) Area Under the dose response Curve (AUC). Each value is the mean±s.e.m. (n=5–6). ANOVA and Dunnett's test were used to statistically analyze the data. ***P<0.001, *P<0.05 represent significant differences in the AUC values between opioid and saline-treated mice.

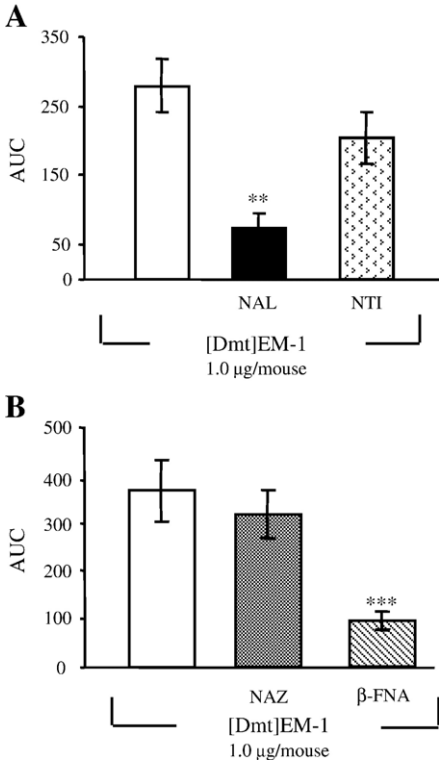

Fig. 4. The effect of (A) naloxone (NAL; 10 mg/kg sc) and naltrindole (NTI; 3 mg/kg sc), (B) naloxonazine (NAZ; 35 mg/kg sc) and β-funaltrexamine (β-FNA; 40 mg/kg sc) on [Dmt[1]]endomorphin-1([Dmt[1]]EM-1)-induced antinociception (1 μg/mouse, icv) in the tail-flick test in mice. Each value is the mean±s.e.m. (n=6–7). (*) Denotes values that are significantly different from [Dmt]EM-1-treated mice by Dunnett's test (***P<0.001, *P<0.05).

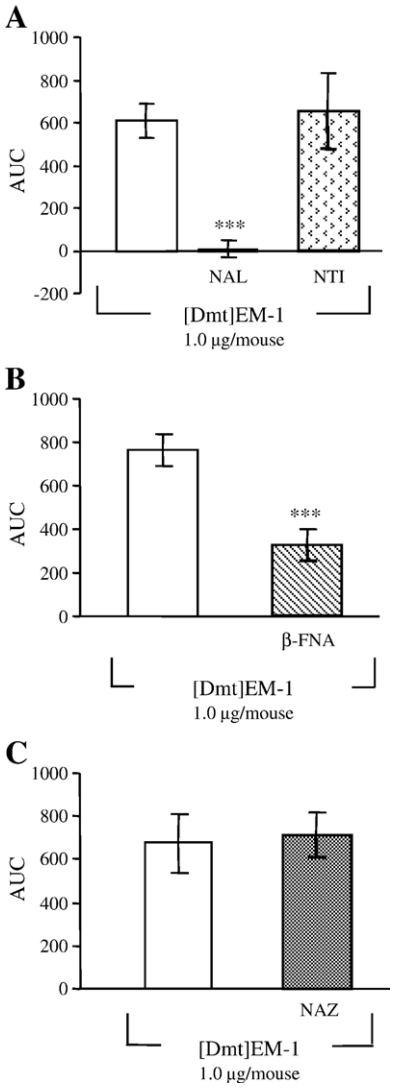

Fig. 5. The effect of (A) naloxone (NAL; 10 mg/kg sc) and naltrindole (NTI; 3 mg/kg sc), (B) β-funaltrexamine (β-FNA; 40 mg/kg sc) and (C) naloxonazine (NAZ; 35 mg/kg sc) on [Dmt^1]endomorphin-1([Dmt]EM-1)-induced antinociception (1 μg/mouse, icv) in the hot-plate test in mice. Each value is the mean ±s.e.m. (n=6–7). (*) Denotes values that are significantly different from [Dmt]EM-1-treated and control mice by Dunnett's test (***P<0.001).

and hot-plate tests with significant antinociceptive effects beginning at a dose of 0.1 and 0.3 μg/mouse, respectively (Fig. 2A and B, Fig. 3A and B). The time course of antinociceptive activity was significant within 10 min after injection and lasted over 1 h depending on the dose and measurement test; the compound was 1.6–2.3 and 1.5–1.6 times more potent than [Dmt^1]endomorphin-2 in tail-flick and hot-plate tests, respectively (Li et al., 2005).

3.3. Spinal antinociception

Nearly complete inhibition (88%) of spinal antinociception by [Dmt^1]endomorphin-1 occurred following subcutaneous injection of the non-specific opioid antagonist naloxone (10 mg/kg) (Fig. 4A). The δ-opioid antagonist, naltrindole (Portoghese et al., 1988), partially, but not significantly, blocked 26% of the total effect, while β-funaltrexamine exerted a strong inhibition (74%). Naloxonazine, whose mode of action is specifically limited to the μ_1-opioid receptor subtype, was inactive against [Dmt^1]endomorphin-1-induced analgesia (Fig. 4B). The κ-opioid receptor antagonist, nor-binaltorphimine was equally ineffective (data not shown). These opiate antagonists do not have any effect on the measured parameters when administered alone (data not shown).

3.4. Supraspinal antinociception

Inhibition of the supraspinal (CNS) antinociception observed with [Dmt^1]endomorphin-1 in combination with the various opioid antagonists differed from the inhibition observed by spinal-induced mechanisms (see, Section 3.3 above). For example, both naloxone and β-funaltrexamine were less effective in blocking the effect exerted by [Dmt^1]endomorphin-1, being only 62% and 57%, respectively (Fig. 5A and B). Furthermore, the application of naloxonazine and naltrindole failed to demonstrate any effect in this testing paradigm (Fig. 5C). The κ-opioid receptor antagonist nor-binaltorphimine was equally ineffective (data not shown).

4. Discussion

The novel endomorphin-1 analogue [Dmt^1]endomorphin-1 demonstrated relatively high affinity for both μ- and δ-opioid receptors. In fact, the affinity to δ-opioid receptors increased by orders of magnitude, far greater than that observed toward the μ-opioid receptor by endomorphin-1 (Zadina et al., 1997). Furthermore, the affinities for μ- and δ-opioid receptors were even higher than that of [Dmt^1]endomorphin-2 by 2- and 6-fold, respectively (Li et al., 2005).

Although, [Dmt^1]endomorphin-1 has a weak selectivity for μ- over δ-opioid receptors, as seen with [Dmt^1]endomorphin-2, its functional bioactivity determined in vitro with the classical pharmacologically-defined GPI and MVD assays were quite interesting and differed significantly from [Dmt^1]endomorphin-2 (Li et al., 2005). While [Dmt^1]endomorphin-1 exhibited μ-opioid agonism, it became a potent δ-opioid antagonist, a characteristic which was not detected with [Dmt^1]endomorphin-2 (Okada et al., 2003a; Fujita et al., 2005; Li et al., 2005). Therefore, Dmt not only affects the ligand interaction with the receptor, but simultaneously exhibits differential expression of agonist or antagonist activities depending on the specific receptor; i.e., the μ-opioid receptor responds by eliciting a functional agonist mode of activity, while the δ-opioid receptor is strongly inhibited.

The in vivo activity of [Dmt^1]endomorphin-1 also demonstrated that the compound was more potent than [Dmt^1]endomorphin-2, which exerts its bioactivity both spinally and supraspinally (Okada et al., 2003a; Fujita et al., 2005; Li et al., 2005). By testing a series of specific opiate receptor antagonists, such as β-funaltrexamine, naloxonazine and naltrindole, the results clearly indicated that different receptor subtypes are involved in and differentiated between the antinociceptive effects generated by these two structurally related opioid agonists. As

reported previously, studies on the role of opioid receptors in the production of supraspinal and spinal antinociception suggested that both μ- and δ-opioid receptors are involved in the spinal mechanism (Tung and Yaksh, 1982; Heyman et al., 1988); however, only μ_1-opioid receptors may be primarily responsible for supraspinal analgesia (Ling and Pasternak, 1983; Jinsmaa et al., 2004). Sakurada et al. reported that the antinociception induced by endomorphin-1 seems to be mediated by μ_2-opioid receptors, whereas endomorphin-2 may act, preferentially, through μ_1-opioid receptors (Sakurada et al., 1999). Our results with [Dmt^1] endomorphin-1-induced antinociception support the differential effect between these two endomorphin opioids. The data also suggested that due to the partial inhibition by naltrindole (Portoghese et al., 1988), which appears relatively insignificant, μ_2-opioid receptors may account for the bulk of spinal-mediated analgesia. In fact, since [Dmt^1]endomorphin-1 exhibits very weak δ-agonism and substantial δ-antagonism, we would not anticipate meaningful δ-opioid receptor-mediated agonism to occur.

In the CNS (supraspinal analgesia) on the other hand, [Dmt^1] endomorphin-1 appears to induce antinociception solely through the action of μ_2-opioid receptors without involvement of the μ_1-subtype or δ-opioid receptors whatsoever. These data revealed that the receptors involved in the antinociception of [Dmt^1]endomorphin-1 were different than those for [Dmt^1] endomorphin-2 in the CNS since [Dmt^1]endomorphin-2-induced antinociception was invoked by a combination of both μ_1- and μ_2-subtypes. Moreover, we failed to detect any involvement of κ-opioid receptors confirming previously reported data (Goldberg et al., 1998; Jinsmaa et al., 2005).

The majority of opioid peptides do not exert antinociception following peripheral administration in vivo because of the rapid degradation by proteolytic enzymes, and inherently low permeation and exclusion across epithelial barriers including the blood–brain barrier (Banks and Kastin, 1996). Moreover, it has been reported that the antinociception induced by endomorphin-2 was potentiated by the co-administration of dipeptidyl IV inhibitors, indicating that this enzyme plays a role the inactivation of endomorphin-2 in vivo (Shane et al., 1999) and may be an important peptidase responsible for terminating endomorphin-2-induced antinociception at the supraspinal level in mice (Sakurada et al., 2003). Only one other analogue of endomorphin-2, [endo1-β-Pro]endomorphin-2, is active centrally and peripherally following subcutaneous administration (Spampinato et al., 2003). [Dmt^1]endomorphin-1 had significantly potent antinociception after subcutaneous administration lasting over 2 h indicating either stability to protease activity or passage through endothelial membranes (data not shown). The antinociceptive effects determined by the tail-flick test are essentially peripheral in nature with only partial central activity, since the action of [Dmt^1]endomorphin-1 was effectively blocked (83%) by intraperitoneal injection of naloxone methiodide, an alkaloid opiate which does not readily cross the blood–brain barrier (Craft et al., 1995). Furthermore, icv injected naloxone, an antagonist which mainly interacts with central opioid receptors (Yeung and Rudy, 1980; Porreca et al., 1981), partially inhibited the peripheral action (26%) of [Dmt^1] endomorphin-1 (data not shown).

We conclude that the central and peripheral antinociceptive effects of [Dmt^1]endomorphin-1 stem through its action at μ_2-opioid receptors. Furthermore, this analogue could serve as a lead structure for analogues with C-terminal modifications (Fujita et al., 2004) to target reduction of opioid tolerance, yet provide specific peripheral analgesia suitable for clinical applications, such as treating acute and chronic pain that arises from various diseases, inflammation, surgery or injury (DeHaven-Hudkins and Dolle, 2004).

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