Systemic and Spinal Analgesic Activity of a δ-Opioid-Selective Lanthionine Enkephalin Analog

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ABSTRACT

A lanthionine enkephalin derivative, Tyr-c[D-ValL-Gly-Phe-D-AlaL]-OH (DV$_5^{2}$DS$_5^{5}$LanEnk), where Val$_L$ and Ala$_L$ denote the lanthionine amino acid ends linked via a monosulfide bridge to form the lanthionine structure, was synthesized. It was found to possess selectivity for and potency at the δ versus μ opioid receptor as defined by binding studies and by its respective activity on the mouse vas deferens compared with the guinea pig ileum. The agent produced a potent analgesia after intrathecal and intraperitoneal delivery with ED$_{50}$ values being, respectively, 0.19 μg and 0.49 mg/kg. The effects of the agent were reversed by the δ-selective antagonist naltrindole. These analgesic actions occurred at doses that had no effect upon general behavior or motor function. These results suggest a potent δ-prefering agent suitable for development as a systemic δ opioid analgesic.

Agents that interact as agonists at the μ receptor produce a potent analgesia. Studies with intracerebral and intrathecal delivery have demonstrated that these effects are mediated by μ opioid binding sites in the brain and in the spinal cord in animals and in humans (Yaksh, 1997). Similarly, systemic delivery of agents known to cross the blood-brain barrier, e.g., the opioid alkaloids morphine and its congeners, will yield a potent antinociception that displays a pharmacology expected for μ receptors. The clinical relevance of this systemic activity is well appreciated and to date μ receptor agonists represent the mainstream of systemic pain therapy in humans. Comparable studies with δ opioid agonists have similarly demonstrated that δ receptor-prefering agonists can produce a potent analgesia after intrathecal delivery (Tung and Yaksh, 1982; Porreca et al., 1987). As the majority of δ opioids with selective affinity and efficacy for the δ site are peptides subject to poor blood-brain barrier penetration and relatively rapid degradation, the demonstration of the systemic activity of these agonists has been less rigorously approached. Recent efforts have been focused on developing δ opioid receptor agonists with an emphasis on nonpeptidic structures. Thus, molecules such as TAN-67 (Tseng et al., 1997), SNC-80 (Bilsky et al., 1995), and BW373U86 (Chang et al., 1993) have been reported to have selectivity and efficacy at the δ receptor and to show activity after systemic delivery. In our own work, after synthesizing a series of peptidomimetics containing enkephalin analogs and testing them for opioid activity and biostability (Baker et al., 2000; Goodman et al., 2001; Rew et al., 2002), we have arrived at a compound, Tyr-c[Val$_L$-Gly-Phe-D-Ala$_L$]-OH (DV$_5^{2}$DA$_5^{5}$LanEnk), that has significant δ selectivity and also in vivo activity. In this article, we report on the analgesic activities of this lanthionine enkephalin after systemic and intrathecal delivery.

Materials and Methods

These studies were carried out according to protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Diego (La Jolla, CA).

Drug Synthesis

DV$_5^{2}$DA$_5^{5}$LanEnk (Fig. 1) was synthesized via preparation of the linear peptide, including the lanthanide-bridge on solid phase, followed by cyclization in solution. The final product was purified and analyzed by reverse-phase high-performance liquid chromatography (Rew et al., 2002).
ployed were the nonspecific agent naloxone hydrochloride (Sigma-Aldrich, derived as trifluoroacetic salt; mol.wt. 584.67) and the opioid, DVL2DAL5LanEnk and [d-Pen1,D-Pen2]-enkephalin (DPDPE) were carried out using rat brain homogenates followed by the modified procedure (Wang, 1996) of existing assays (Berman et al., 1983). The parent Leu-enkephalin was employed as the reference molecule since the enzymatic stabilities in homogenates and plasma has been extensively reported (Hambrock et al., 1976; Marks et al., 1977; Hui et al., 1981; Shibanoiki et al., 1992).

Typically, 5 mM peptide substrate solution in 5 mM Tris - HCl buffer (pH 7.2), including 1.26 mM Cbz-Tyr-OH as an internal standard was prepared. To 1.80 ml of rat brain homogenates (130 mg/ml in 50 mM Tris - HCl buffer, pH 7.2), 200 ml of the peptide substrate solution was added, and the mixture was incubated at 37°C in a temperature-controlled, shaking water bath. Aliquots (250μl) were removed at different time intervals over 24 h, and enzymatic activity was quenched using 10 μl of trifluoroacetic acid. After centrifugation at 12,500 rpm for 15 min, the supernatant was removed and analyzed for the remaining intact peptide by high-performance liquid chromatography using a Vydac protein peptide C18 column. The column dimensions were 4.5 × 250 mm (90 Å silica, 5 μm), and UV detection was carried out at 220 nm. A binary system of water and acetonitrile, both containing 0.1% trifluoroacetic acid, was used throughout in a gradient elution. Concentration-time profiles were analyzed by exponential fitting, assuming a pseudo-first-order degradation process. Half-lives (t1/2) were calculated from the observed rate constant (kobs) as 0.693/kobs.

Results

In vitro analysis

The IC50 of these agents on the mouse vas deferens (β) and guinea pig ileum (μ) are presented in Table 1. A ratio for guinea pig ileum/mouse vas deferens >1 demonstrates a preference for the β opioid receptor site and <1 demonstrates a preference for the μ opioid receptor site. As indicated by the IC50 ratios, morphine is a μ-prefering agent, whereas DVL2DAL5LanEnk is comparatively potent as a β-prefering agonist.
In Vivo Antinociceptive Activity

Spinal Activity. The intrathecal injection of either morphine or \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \) resulted in an increase in the thermal escape latency (Fig. 2, top). The magnitude of the increase in response latency after i.t. injection was shown to be dose-dependent (Fig. 2, bottom). The ordering of potency for these two agents based on their calculated ED\(_{50}\) values (Table 2) indicates the \( \delta \)-agent to be approximately 18 times more potent than morphine after intrathecal delivery.

Systemic Activity. The intraperitoneal injection of either morphine and \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \) resulted in an increase in the thermal escape latency (Fig. 3, top). The magnitude of the increase in response latency after i.t. injection was shown to be dose-dependent (Fig. 3, bottom). The ordering of potency for these two agents based on their calculated ED\(_{50}\) values (Table 2) indicates them to be approximately equiactive after intraperitoneal delivery.

Duration of Action

As indicated, both drugs were able to produce a significant dose-dependent increase in the thermal escape latency. To compare the durations of action resulting from a given analgesic action, the area under the time-effect curve was plotted versus the peak effect observed for each dose after i.t. or i.p. injection. As indicated in Figs. 4 and 5, after both intrathecal and systemic delivery, the duration of action for a given antinociceptive effect for \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \) did not differ significantly from that observed with morphine. These observations suggested that the peptidomimetic possessed a sta-

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<td>In vitro opioid activity of morphine, ( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} ) and DPDPE. Part of the table has been published elsewhere (Rew et al., 2002).</td>
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<td>In vivo antinociceptive activity of morphine and ( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} ).</td>
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**Fig. 2.** Top, figure presents the thermal escape latency (seconds) plotted versus time (time effect curve) for the highest doses of morphine, \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \), or vehicle given intrathecally. Bottom, peak effect (%MPE) plotted versus dose (micrograms) for intrathecal morphine and \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \). ED\(_{50}\) calculations are presented in Table 2. Each point presents the mean and S.E.M. of four to eight rats.

**Fig. 3.** Top, figure presents the thermal escape latency (seconds) plotted versus time (time effect curve) for the highest doses of morphine, \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \), or vehicle given intraperitoneally. Bottom, peak effect (%MPE) plotted versus dose (micrograms) for intrathecal morphine and \( \text{DV}_L^2 \text{DA}_L^5 \text{LanEnk} \). ED\(_{50}\) calculations are presented in Table 2. Each point presents the mean and S.E.M. of four to eight rats.
Nociception is assessed as thermal escape latency time and antinociception expressed as peak %MPE observed with morphine. Importantly, these effects of the peptidomimetic were readily apparent. DVL$_2$DA$_5^5$LanEnk had no statistically significant effect upon the analgesic effects of morphine and DVL$_2$DA$_5^5$LanEnk given intrathecally. Conversely, i.t. CTOP (3 μg) reversed the effects of i.t. morphine but had no statistically significant effect upon the analgesic effects of DVL$_2$DA$_5^5$LanEnk. The effects of specific antagonists against systemic morphine were not examined.

**Behavioral Effects**

Over the range of the doses delivered intrathecally, neither agent produced a significant effect upon the motor function or general behavioral indices (placing, stepping, and righting). At the highest systemic doses employed, both morphine and DVL$_2$DA$_5^5$LanEnk reduced the pinnae and blink responses. After systemic delivery, morphine displayed increasing catalepsy at doses less than those required to produce the block of the thermal escape. In contrast, DVL$_2$DA$_5^5$LanEnk had no detectable effect upon the catalepsy endpoint (Table 4).

**Resistance of the Peptidomimetic Opioid to Metabolism**

DVL$_2$DA$_5^5$LanEnk, DPDPE, and Leu-enkephalin were incubated in rat brain homogenates solution (pH 7.2) at 37°C for 24 h. Leu-enkephalin displayed a rapid decline in concentration, with a $t_{1/2}$ = 6.7 min. In contrast, DVL$_2$DA$_5^5$LanEnk and DPDPE showed no detectable decline over this interval (Table 5).

**Discussion**

δ Opioid Receptor-Mediated Analgesia. Efforts to create a peptidomimetic structure with δ opioid properties has led to the synthesis of structures with significant δ opioid selectivity (Rew et al., 2002). In the present work, we examined the intrathecal and systemic activity of one of these structures. As indicated, after intrathecal delivery, consistent with the presence of δ opioid receptors in the dorsal horn and the ability of (Besse et al., 1990) δ opioid agonists to inhibit the firing of dorsal horn nociceptive neurons (Dickenson et al., 1987), this agent displayed a potent analgesic activity with a potency comparable to that of morphine.
and DPDPE are completely resistant to degradation in brain homogenates over the 24-h interval examined. The extraordinary resistance of these peptide analogs is likely due to the existence of the \(\beta,\beta\)-dimethyl group in the position 2 or position 5 that stabilizes the compound toward enzymatic degradation (Fig. 1). A previous study of enzymatic degradation for a similar lanthionine enkephalinamide (Wang et al., 1996) has shown that the lanthionine enkephalinamide, \(\text{DA}_{2}\text{L}_{5}\text{LanEnkNH}_2\), is approximately 5 times more stable than the corresponding disulfide analog \(\text{DC}^2\text{L}^2\text{LanEnkNH}_2\) toward degradation by rat brain homogenates. These two compounds do not have \(\beta,\beta\)-dimethyl groups in position 2 or position 5. Thus, the increase in biostability probably arises from the replacement of a cystine disulfide bridge by a lanthionine bridge. The relative systemic potency of \(\text{DV}_{2}\text{DA}_{2}\text{L}_{5}\text{LanEnk}\) after systemic and intrathecal delivery may thus be explained in part by the biostability of the lanthionine structure.

### \(\delta\) Opioid Analgesia

As noted in the Introduction, \(\delta\) opioid receptor agonists have been repeatedly shown after intrathecal delivery to produce a potent analgesia in animal models. Development of a systemically active \(\delta\) opioid agonist has potential virtues. Previous preclinical work has shown that in the face of tolerance with chronic \(\mu\) opioid delivery, the action of the \(\delta\) agonist is only marginally diminished, e.g., there is minimal cross-tolerance (Stevens and Yaksh, 1992). The \(\mu\) opioid agonists have a panoply of effects reflecting the association of \(\mu\) receptors with neural systems that regulate respiratory function, arousal, and gastrointestinal activity to name a few. Using available agents and central delivery, it has been argued that the \(\delta\) receptor may not play as important a role as the \(\mu\) receptor in modulating the other functions that reflect the side-effect profile of all \(\mu\) opioids. In humans, previous intrathecal studies with a \(\delta\)-preferring peptide (DADLE) has been shown to produce potent analgesia in cancer pain patients (Onofrio and Yaksh, 1983; Moulin et al., 1985), whereas metkephamid, a modified pentapeptide, was shown earlier to have efficacy in postoperative pain after systemic delivery (Bloomfield et al., 1983). The present studies afford initial data showing a systemically active \(\delta\)-preferring agonist with an analgesic potency comparable to that of morphine in this thermal escape model. These results provide an opportunity to assess the analogs therapeutic ratio for the \(\delta\) receptor compared with the \(\mu\) receptor and to consider additional development of a therapeutically useful \(\delta\) agonist.
References


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