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Enkephalin antinociception in mice is mediated by δ_1- and δ_2-opioid receptors in the brain and spinal cord, respectively

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Pharmacological evidence for the existence of δ-opioid receptor subtypes has been reported. This study was conducted to determine which type of δ-opioid receptors was involved supraspinally and spinally when antinociception was induced by the natural enkephalins, [Leu^5]enkephalin and [Met^5]enkephalin. In the mouse tail flick assay, the antinociceptive ED_{50} values of both intracerebroventricularly (i.c.v.) administered [Leu^5]enkephalin and [Met^5]enkephalin (together with the peptidase inhibitors, bestatin and thiorphan) were significantly increased by 7-benzylidenenaltrexone (BNTX), a selective δ_1-opioid receptor antagonist but not by naltriben, a selective δ_2-opioid receptor antagonist. On the other hand, when the enkephalins were administered intrathecally (i.t.), the antinociceptive ED_{50} values of both enkephalins were significantly raised by naltriben but not by BNTX. β-Endorphin-induced (i.c.v.) antinociception was antagonized by naltriben administered i.t. or s.c. but not by BNTX administered i.t. or s.c. Different δ-opioid receptor subtypes appeared to be involved in supraspinal (δ_1) and spinal (δ_2) antinociception induced by endogenous δ-opioid receptor agonists, [Leu^5] and [Met^5]enkephalin. The antinociception produced by i.c.v. administered β-endorphin has been attributed to the release of [Met^5]enkephalin in the spinal cord and its antagonism by naltriben support the finding that enkephalins interact with δ_2-opioid receptors in the spinal cord to mediate antinociception. β-Endorphin may be interacting at receptors other than δ_1- or δ_2-opioid receptors in the brain, perhaps the putative ε receptors, to mediate their effects because neither i.c.v. administered BNTX nor naltriben inhibited its activity.

[Leu^5]enkephalin; [Met^5]enkephalin; Antinociception; δ_1-Opioid receptors; δ_2-Opioid receptors; BNTX (7-benzylidenenaltrexone); Naltriben; Spinal cord; Brain; β-Endorphin

1. Introduction

Recent reports have described the possible existence of δ-opioid receptor subtypes to mediate antinociception in mice in which [D-Pen^2, D-Pen^5]enkephalin and [D-Ala^2,D-Leu^5]enkephalin have been designated as δ_1- and [D-Ser^2,Leu^5,Thr^6]enkephalin and deltorphin II as δ_2-opioid receptor agonists (Sofuoglu et al., 1991a,b; Jiang et al., 1991; Mattia et al., 1991; 1992). In categorizing these peptides, the question arises as to the δ-opioid receptor subtype with which the endogenous enkephalins interact. This query has been difficult to answer because of the quick degradation of the enkephalins when administered and the very transient antinociception that is induced. This problem has been somewhat circumvented by the use of stabilized synthetic enkephalins such as [D-Pen^2,D-Pen^5]enkephalin and [D-Ser^2,Leu^5,Thr^6]enkephalin but it is not certain if these synthetic enkephalins truly reflect the activity of the natural enkephalins. Another approach to study this problem is the concomitant use of bestatin, an aminopeptidase inhibitor (Chaillet et al., 1983) and/or thiorphan, a neutral endopeptidase ('enkephalinase') inhibitor (Roques et al., 1980) along with the enkephalins. We have used the latter approach in the present study to assess the antinociceptive activity of [Leu^5] and [Met^5]enkephalin spinally and supraspinally. Also, we have used our highly selective, non-peptide δ_1- and δ_2-opioid receptor antagonists, 7-benzylidenenaltrexone (BNTX) (Portoghese et al., 1992) and naltriben (Portoghese et al., 1988), respectively to determine the δ-opioid receptor subtype with which the endogenous enkephalins interacted in

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the brain and the spinal cord. Because β-endorphin has been reported to induce antinociception by releasing [Met^5]enkephalin in the spinal cord (Tseng et al., 1986), the δ-opioid receptor subtype involved in this action also was investigated.

2. Materials and methods

2.1. Animals

Male Swiss-Webster mice (Sasco, St. Louis, MO) weighing 20–25 g were used in all experiments. They were housed in a temperature and humidity controlled room for at least 24 h before use. They were allowed free access to food and water. Each mouse was used only once.

2.2. Antinociceptive assay

An aminopeptidase and an 'enkephalinase' inhibitor, bestatin (10 mg/ml) and thiorphan (10 mg/ml), respectively were included in the aqueous injection solutions of the enkephalins in order to obviate their rapid degradation upon administration. Thus, 50 μg of each inhibitor was administered together with each injection of the enkephalins (Chaillet et al., 1983). The antinociceptive assay was the modified radiant heat tail flick test described by Tulunay and Takemori (1974). The data were made quantal by designating a positive antinociceptive response of an animal as those that increased their latency to tail flick (after drug treatment) by at least 3 S.D. of the mean latency of the whole group. At least three groups of ten mice were used to generate dose-response curves and to estimate ED_{50} values.

2.3. Statistics

The ED_{50} values, the ED_{50} ratios and their 95% confidence limits were estimated by the parallel line assay of Finney (1964) with the aid of a computer.

2.4. Drugs

Naltriben (Portoghese et al., 1988) and BNTX (Portoghese et al., 1991) were synthesized as described previously. [Leu^5]enkephalin, [Met^5]enkephalin, β-endorphin (human), DL-thiorphan and bestatin were bought from Sigma Chemical Co. (St. Louis, MO). Deltorphin II was bought from Bachem (Torrence, CA). Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections (5 μl) were performed by the methods of Haley and McCormick (1957) and Hylden and Wilcox (1980), respectively. Injections s.c. were given in a volume of 10 ml/kg.

3. Results

3.1. Effects of naltriben and BNTX on the antinociceptive activity of [Leu^5]enkephalin administered i.c.v. or i.t.

The i.c.v. administered peptidase inhibitors alone produced a significant increase in latency in 30% of the animals (three out of ten animals displayed significant antinociceptive effect). Their effects started to become apparent at 15 min (i.c.v.) after administration and quickly dissipated within 30 min. When given i.t., the peptidase inhibitors displayed no antinociceptive activity for up to 30 min. The antinociceptive activity of [Leu^5]enkephalin, together with the inhibitors, was measured at the peak activity of 10 min (i.c.v.) or 5 min (i.t.) after administration. The effects of the inhibitors alone were not discernible at these time periods.

The doses of BNTX and naltriben employed were the highest doses of these antagonist without losing selectivity or showing agonism. The antinociceptive effect of i.c.v. administered leu-enkephalin was inhibited significantly by BNTX (s.c.), but not by naltriben (table 1; fig. 1A, B). On the other hand, the antinociceptive activity of i.t. administered [Leu^5]enkephalin was inhibited significantly by naltriben (s.c.) but not by BNTX (s.c.). The antagonism by BNTX and naltriben of i.c.v. and i.t. administered [Leu^5]enkephalin, respectively caused rightward parallel shifts of the dose-response curve of [Leu^5]enkephalin (fig. 1A, B).

3.2. Effects of naltriben and BNTX on the antinociceptive activity of [Met^5]enkephalin administered i.c.v. or i.t.

The antinociceptive profile of [Met^5]enkephalin, with peptidase inhibitors, was similar to that of

TABLE 1

The effect of naltriben and BNTX on [Leu^5]enkephalin-induced antinociception [a].

Treatment [b]	ED_{50} (95% C.I.) (nmol)	ED_{50} ratio (95% C.I.)
[Leu^5]enkephalin, i.c.v.	4.1 (2.3–6.5)	
[Leu^5]enkephalin, i.c.v. + naltriben (1.2 μmol/kg, s.c.)	2.4 (1.4–3.9)	0.6 (0.3–1.2)
[Leu^5]enkephalin, i.c.v. + BNTX (1.3 μmol/kg, s.c.)	8.0 (6.6–9.7) [c]	1.9 (1.5–2.4) [c]
[Leu^5]enkephalin, i.t.	0.3 (0.2–0.4)	
[Leu^5]enkephalin, i.t. + naltriben (1.2 μmol/kg, s.c.)	0.8 (0.6–1.0) [c]	2.5 (1.7–3.7) [c]
[Leu^5]enkephalin, i.t. + BNTX (1.3 μmol/kg, s.c.)	0.5 (0.3–0.7)	1.4 (0.9–2.3)

[a] Antinociception assessed in the presence of 50 μg bestatin and 50 μg thiorphan. [b] Peak times; [Leu^5]enkephalin = 5 min; naltriben = 30 min; BNTX 30 min. [c] ED_{50} value significantly > that of control; ED_{50} ratio significantly > 1 ($P < 0.05$).

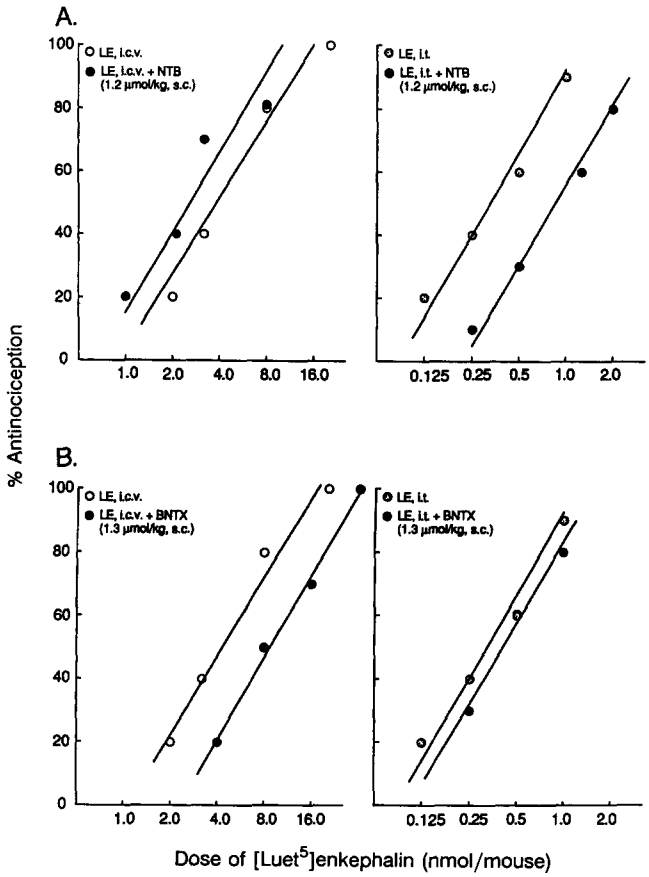

Fig. 1. Effect of naltriben (NTB), s.c. (A) and BNTX, s.c. (B) on the antinociceptive activity of [Leu^5]enkephalin (+50 μg bestatin and 50 μg thiorphan) in mice.

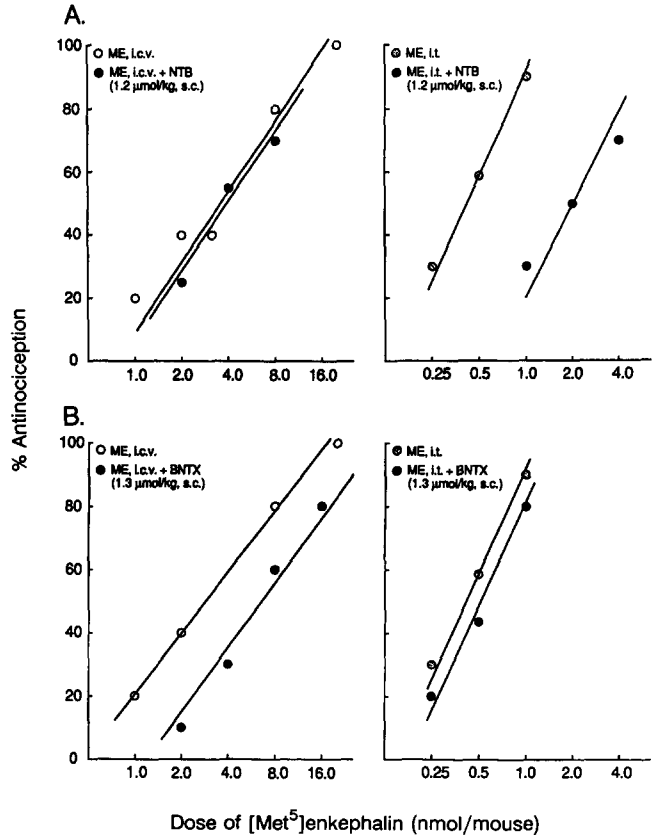

Fig. 2. Effect of naltriben (NTB), s.c. (A) and BNTX, s.c. (B) on the antinociceptive activity of [Met^5]enkephalin (+50 μg bestatin and 50 μg thiorphan) in mice.

[Leu^5]enkephalin. The antinociceptive potency of [Leu^5] and [Met^5]enkephalin was similar at supraspinal sites as well as spinal sites. Again, the antinociceptive activity of i.c.v. administered [Met^5]enkephalin was antagonized significantly by BNTX (s.c.), but not by naltriben (s.c.) (table 2; fig. 2A, B). In contrast, the antinociceptive activity of i.t. administered [Met^5]enkephalin was inhibited significantly by naltriben (s.c.)

TABLE 2

The effect of naltriben and BNTX on [Met^5]enkephalin-induced antinociception [a].

Treatment [b]	ED_{50} (95% C.I.) (nmol)	ED_{50} ratio (95% C.I.)
[Met^5]enkephalin, i.c.v.	3.1 (1.8–4.7)	
[Met^5]enkephalin, i.c.v. + naltriben (1.2 μmol/kg, s.c.)	4.0 (2.3–7.2)	1.3 (0.7–2.9)
[Met^5]enkephalin, i.c.v. + BNTX (1.3 μmol/kg, s.c.)	6.3 (3.9–11.5) [c]	2.0 (1.1–4.5)[c]
[Met^5]enkephalin, i.t.	0.4 (0.2–0.6)	
[Met^5]enkephalin, i.t. + naltriben (1.2 μmol/kg, s.c.)	2.0 (1.4–2.9) [c]	5.4 (3.3–9.8) [c]
[Met^5]enkephalin, i.t. + BNTX (1.3 μmol/kg, s.c.)	0.5 (0.4–0.7)	1.3 (0.8–2.3)

[a] Antinociception assessed in the presence of 50 μg bestatin and 50 μg thiorphan. [b] Peak times; [Met^5]enkephalin = 5 min; naltriben = 30 min; BNTX = 30 min. [c] ED_{50} value significantly > that of control; ED_{50} ratio significantly > 1 ($P < 0.05$).

TABLE 3

The effect of naltriben and BNTX on β-endorphin-induced antinociception.

Treatment [a]	ED_{50} (95% C.I.) (nmol)	ED_{50} Ratio (95% C.I.)
β-Endorphin, i.c.v.	0.08 (0.06–0.09)	
β-Endorphin, i.c.v. + naltriben (1.2 μmol/kg, s.c.)	0.43 (0.30–0.62) [b]	5.6 (3.5–10.1) [b]
β-Endorphin, i.c.v. + BNTX (1.3 μmol/kg, s.c.)	0.13 (0.09–0.20)	1.7 (0.9–3.3)
β-Endorphin, i.c.v. + naltriben (25 pmol, i.t.)	0.26 (0.22–0.31) [b]	3.7 (2.9–5.0) [b]
β-Endorphin, i.c.v. + BNTX (1 pmol, i.t.)	0.09 (0.03–0.19)	1.1 (0.3–5.4)
β-Endorphin, i.c.v. + naltriben (25 pmol, i.c.v.)	0.08 (0.07–0.09)	1.0 (0.9–1.3)
β-Endorphin, i.c.v. + BNTX (6.25 pmol, i.c.v.)	0.11(0.07–0.17)	1.4 (0.3–3.1)

[a] Peak times; β-Endorphin = 30 min; naltriben (s.c.) = 30 min; naltriben (i.t.) = 10 min; BNTX (s.c.) = 30 min; BNTX (i.t.) 10 min. [b] ED_{50} value significantly > that of control; ED_{50} ratio significantly > 1 ($P < 0.05$).

but not by BNTX (s.c.). The antagonism by BNTX and naltriben of i.c.v. and i.t. administered [Met^5]enkephalin, respectively was observed with rightward parallel shifts of the dose response curve of [Met^5]enkephalin (fig. 2A, B).

3.3. Effects of naltriben and BNTX on the antinociceptive activity of β-endorphin administered i.c.v.

β-Endorphin was studied because it has been reported that i.c.v. administered β-endorphin produces its antinociceptive effect by releasing [Met^5]enkephalin in the spinal cord (Tseng et al., 1986). The antinociceptive activity was inhibited significantly by naltriben administered s.c. or i.t. and produced rightward parallel shift of the dose-response curve of β-endorphin (table 3; fig. 3A, B). BNTX given either s.c. or i.t., did not alter the antinociceptive activity of β-endorphin (table 3; fig. 3A, B). However, when administered i.c.v., neither naltriben nor BNTX altered the antinociceptive activity of i.c.v. administered β-endorphin (table 3; fig. 3C).

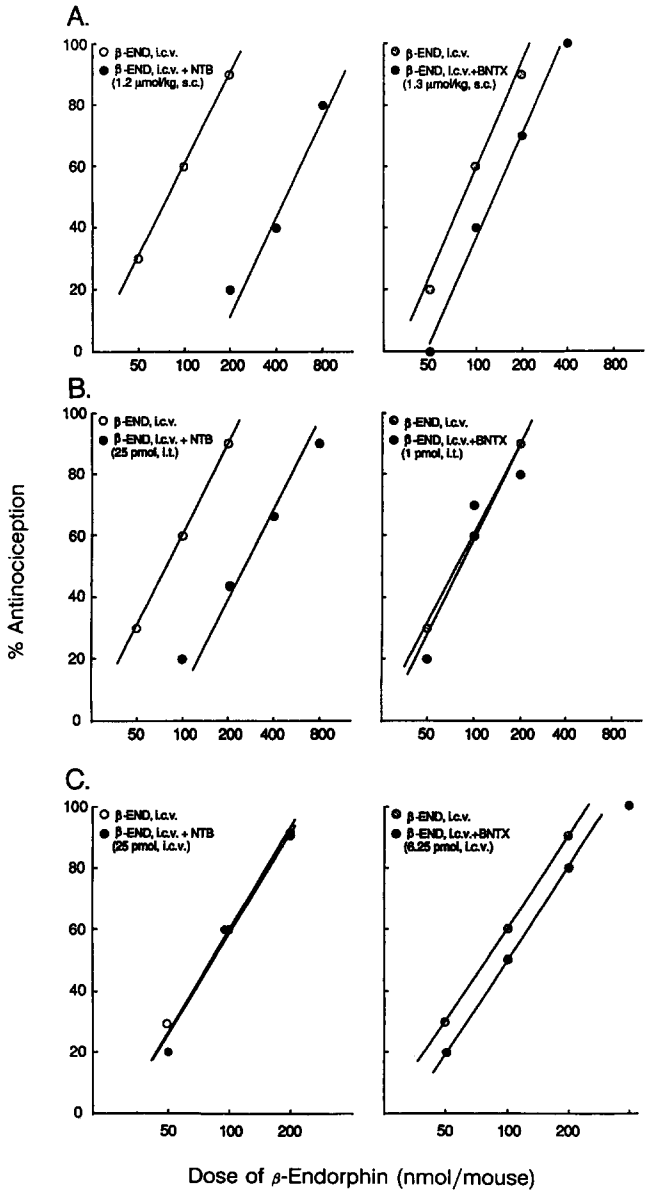

Fig. 3. Effect of naltriben (NTB) and BNTX given s.c. (A), naltriben and BNTX given i.t. (B) and naltriben and BNTX given i.c.v. (C) on the antinociceptive activity of β-endorphin administered i.c.v. in mice.

4. Discussion

The data in this study demonstrate that the natural enkephalins, [Leu^5] and [Met^5]enkephalin, possess antinociceptive activity at both supraspinal and spinal sites. In order to clearly illustrate this effect, the peptidase inhibitors, bestatin and thiorphan, had to be administered along with the enkephalins. These observations confirm the notion that the enkephalins are hydrolyzed very rapidly in vivo (Roques et al., 1980; Chaillet et al., 1983). The antinociceptive activity of peptidase inhibitors has been noted previously (Roques et al., 1980; Chaillet et al., 1983, Fournié-Zaluski et al., 1984; Al-Rodhan et al., 1990; Noble et al., 1992). However, these authors used either different assay procedures (hot plate and writhing) compared to our tail flick or different species (rat). In one study where the mouse tail flick was used (Schmidt et al., 1991), it was observed that the mixed peptidase inhibitor, kelatorphan possesses no significant antinociceptive activity in the tail flick assay although it shows activity in the hot plate and writhing assays. The antinociceptive activity of peptidase inhibitors may be related to their inhibitory potencies and pharmacokinetic properties (Schmidt et al., 1991). However, these results are not altogether surprising because it has been documented long ago that the intensity of the adverse stimuli in each of the antinociceptive assays are different (Hayashi and Takemori, 1971) and each of the assays detect the activity of different agonists (Tyers, 1980; Ward and Takemori, 1983). In the present study, the combination of bestatin and thiorphan produced a weak, fleeting antinociceptive response in the mouse tail flick assay when administered i.c.v. and showed no activity when administered i.t. At times when the antinociceptive activity of the enkephalins (+inhibitors) was measured (10 min, i.c.v.; 5 min, i.t.), no activity of the inhibitors alone was perceivable so one must assume that the antinociceptive activity that was observed was due to the administerted enkephalins.

The unexpected finding in this study was that the natural enkephalins appeared to interact with different δ-opioid receptor subtypes in the brain and spinal cord to mediate antinociception. The fact that BNTX, a highly selective δ_1-opioid receptor antagonist (Porto-

ghese et al., 1992), but not naltriben, a highly selective δ_2-opioid receptor antagonist (Sofuoglu et al., 1991), inhibited the antinociceptive effects of both [Leu^5] and [Met^5]enkephalin administered i.c.v., suggests that the enkephalins mediate their antinociceptive effects in the brain through interactions at δ_1-opioid receptors. In contrast, when the enkephalins were administered i.t., their antinociceptive activities were antagonized by naltriben but not by BNTX which suggests that in the spinal cord, the enkephalins interact with δ_2-opioid receptors to bring about antinociception. In retrospect, these findings may not be too surprising because there are biological precedents set for endogenous ligands to interact with several different types and subtypes of their respective receptors, e.g. acetylcholine, norepinephrine, dopamine, serotonin and histamine.

The antinociception produced by i.c.v. administered β-endorphin has been attributed to the release of [Met^5]enkephalin in the spinal cord (Tseng et al., 1986). In this study, the antinociceptive activity of i.c.v. administered β-endorphin was inhibited by naltriben, but not by BNTX, when given i.t. or s.c. which supports the finding that enkephalins interact with δ_2-opioid receptors in the spinal cord to produce antinociception. However, when administered i.c.v., both naltriben and BNTX failed to alter the antinociceptive activity of i.c.v. administered β-endorphin suggesting that β-endorphin may interact with receptors other than δ_1- or δ_2-opioid receptors, perhaps the putative ϵ receptors (Tseng et al., 1986), to mediate its effect. Earlier, Tseng's group has shown by employing selective opioid receptor antagonists that the antinociceptive activity of β-endorphin administered i.c.v. is not mediated by μ-, κ- or δ-opioid receptors and postulated the involvement of ϵ-opioid receptors Suh et al., 1988).

In summary, both [Leu^5] and [Met^5]enkephalin mediate antinociception supraspinally and spinally by interacting with δ_1- and δ_2-opioid receptors, respectively. β-Endorphin interacts with receptors other than δ_1- or δ_2-opioid receptors in the brain but its antinociceptive effect is antagonized in the spinal cord by a δ_2 selective opioid receptor antagonist.

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