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Spinal δ_2-, but not δ_1-, μ-, or κ-opioid receptors are involved in the tail-flick inhibition induced by β-endorphin from nucleus raphe obscurus in the pentobarbital-anesthetized rat

Leon F. Tseng [a,*], Judy H.H. Tsai [a], Keith A. Collins [a], Philip S. Portoghese [b]

[a] *Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI, USA*
[b] *Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN, USA*

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Abstract

The antinociception induced by β-endorphin given supraspinally has been previously demonstrated to be mediated by the release of [Met^5]enkephalin acting on δ-opioid receptors in the spinal cord. The present study was designed to determine what type of opioid receptors in the spinal cord is involved in β-endorphin-induced antinociception in the rat. Antinociception was induced by β-endorphin (0.6 nmol) given into nucleus raphe obscurus and was assessed by the tail-flick test in pentobarbital-anesthesized rats. Naltriben (0.6–6.0 nmol), a selective δ_2-opioid receptor antagonist, given intrathecally dose-dependently attenuated β-endorphin-induced inhibition of the tail-flick response. On the other hand, 7-benzylidene naltrexone (2.1–64.3 nmol), CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2, 0.09–2.8 nmol), or nor-binaltorphimine (1.4–40.8 nmol), selective δ_1-, μ-, and κ-opioid receptor antagonists, respectively, did not block β-endorphin-induced antinociception. The results of present study in rats are consistent with previous experiments in mice indicating that spinal δ_2-, but not δ_1-, μ- or κ-opioid receptors are involved in β-endorphin-induced inhibition of the tail-flick response.

Keywords: β-Endorphin; Antinociception; δ_2-Opioid receptor; Naltriben; Tail-flick inhibition

1. Introduction

Accumulating evidence indicates that the antinociception induced by β-endorphin given supraspinally is mediated by the release of [Met^5]enkephalin and subsequent stimulation of opioid receptors in the spinal cord. This contention is based on the findings that β-endorphin given supraspinally releases [Met^5]enkephalin from the spinal cord (Tseng et al., 1985a,b, 1986) and the blockade of the opioid receptors in the spinal cord by intrathecal (i.t.) injection of naloxone antagonizes the antinociception induced by β-endorphin given intracerebroventricularly (i.c.v.) (Tseng and Fujimoto, 1984, 1985; Suh et al., 1989). The opioid receptors in the spinal cord involved in i.c.v. administered β-endorphin-induced antinociception have since been identified to be δ-opioid receptors, because ICI 174,864 and ICI 151,129, selective δ-opioid receptor antagonists, but not β-funaltrexamine or CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2), a selective μ-opioid receptor antagonists, or nor-binaltorphimine, a selective κ-opioid receptor antagonist, block the effect (Suh and Tseng, 1990; Xu et al., 1992).

Recent studies indicate that δ-opioid receptors can be further classified into δ_1 and δ_2-opioid receptors (Sofluoglu et al., 1991; Mattia et al., 1991; Jiang et al., 1991). The δ_1-opioid receptors are selectively stimulated by [D-Pen^{2,5}]enkephalin (DPDPE) and blocked by 7-benzylidene naltrexone whereas the δ_2-opioid receptors are selectively stimulated by [D-Ala^2]deltorphin II and blocked by naltriben. We have previously demonstrated in the mouse that δ_2-, but not δ_1-opioid receptors in the spinal cord are involved in i.c.v. administered β-endorphin-induced antinociception (Tseng et al., 1993). The present study was designed in an attempt to determine if the same results obtained in the mouse can also be found in the rat.

* Corresponding author. Medical College of Wisconsin, Anesthesiology, Medical Education Building – 462c, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA.

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β-Endorphin was administered i.c.v. for the production of antinociception in a previous experiment in the mouse (Tseng et al., 1993). Previous studies in rats using micro-intracerebral injection techniques have indicated multiple sites in the brain sensitive to β-endorphin for the production of antinociception (Tseng et al., 1980, 1990; Tseng and Wang, 1992). Nucleus raphe obscurus is one of the sensitive sites (Tseng et al., 1990). It becomes important to study the mechanisms of antinociceptive action of β-endorphin elicited specifically from a particular nucleus of the brain. Antinociception was induced by intracerebral (i.c.) injection of β-endorphin into nucleus raphe obscurus and the selective opioid receptor antagonist was injected intrathecally to selectively block the receptors in the spinal cord. We found that the inhibition of the tail-flick response induced by β-endorphin from nucleus raphe obscurus is mediated by the stimulation of δ_2-, but not δ_1-, μ- or κ-opioid receptors in the spinal cord.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Sasco, Omaha, NE, USA) weighing 350–380 g were used. Animals were housed 5 per group in a room maintained at 22 ± 0.5°C with an alternating 12 h light-dark cycle. Food and water were available ad libitum. Each animal was used only once.

2.2. Procedures for intracerebral (i.c.) and intrathecal (i.t.) drug injection and analgesiometric testing

The experiments were carried out in pentobarbital-anesthetized rats. This experimental model has been used in the previous experiments to study the brain sites sensitive to β-endorphin-induced antinociception and the release of [Met^5]enkephalin (Tseng et al., 1990; Tseng and Wang, 1992). Compared with the experiments done in conscious rats, which required surgical cannulation of a guide cannula for i.c. injection of the drug, the procedure allowed β-endorphin to be injected directly into i.c. sites using an injection cannula as described below. Rats were pretreated with methyl atropine bromide (2 mg/kg i.p.), anesthetized with pentobarbital sodium (50 mg/kg i.p.) and were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The anesthetic state was maintained by injecting 6–10 mg/kg of pentobarbital sodium every 45 min or as needed when animals showed whisker movement or any spontaneous motor movements. The tail-flick response to radiant heat stimulus was used as the antinociceptive test (D'Amour and Smith, 1941). Pentobarbital, at this anesthetic dose, did not affect latency of the tail-flick response. The intensity of the heat was adjusted so that the rat flicked its tail after 3.5–5 s. An injection cannula, made of 30-gauge stainless steel tubing connected to a 25-μl Hamilton microsyringe with a segment of PE-10 tubing, was prefilled with β-endorphin solution and injected stereotaxically into nucleus raphe obscurus (coordinates: AP, −3.8–4.2 mm posterior to the interaural line; lateral 0.0 mm and vertical, 9.5–10.0 mm from the skull surface at lambda). This region has been previously found to be one of the brain regions sensitive to β-endorphin for inhibiting the tail-flick response (Tseng et al., 1990; Tseng and Collins, 1991). An injection cannula made of 30-gauge stainless steel tubing connected to a segment of PE-10 tubing was inserted into the lumbar subarachnoid space for i.t. injection of the antagonists. The injection volume was 0.5 μl for i.c. injection of β-endorphin and 5 μl for i.t. injection of the receptor antagonist. Intrathecal injection of the drug was followed by injecting 10 μl of saline solution (0.9% NaCl sterile solution) to flush the i.t. tubing of its contents. The drug solution was injected at a constant speed over a 1 min period for both i.c. and i.t. injection. Rats given injection of saline served as controls.

Experiments were designed to study the effects of i.t. injection of selective opioid receptor antagonists on the inhibition of the tail-flick response induced by β-endorphin from nucleus raphe obscurus. β-Endorphin at a dose of 2 μg was injected stereotaxically into nucleus raphe obscurus and the tail-flick response was measured every 10 min. Selective opioid receptor antagonists at increasing doses were injected i.t. every 20 min after peak antinociception was reached by i.c. administered β-endorphin (30 min) or i.t. opioid agonist (20 min). The doses of antagonists are then presented as the cumulative doses in nmol injected. In the case that the first β-endorphin injection did not produce antinociception, a second injection of β-endorphin at the same dose was administered into a different site in nucleus raphe obscurus (0.2 mm posterior or anterior to the first injection site) at least 1 h after the first injection. At the end of the experiments, 0.5 μl of methylene blue solution (2% solution in distilled water) was injected into the brainstem site which had been injected with β-endorphin. Next, 5 μl of methylene blue solution followed by 10 μl of saline to flush the cannula was injected into the i.t. site. The brain and the spinal cord were removed and immersed in 10% formaldehyde. The brainstem was sectioned sagittally with a microtome and the injection sites were identified under a binocular microscope. The stereotaxic atlas of rats by Paxinos and Watson (1986) was used as a guide for the identification of the anatomic structure. Location of i.t. injection areas was verified

visually by examining the spinal cord for dye distribution. Data obtained from rats in which the i.c. injection site was not within the nucleus raphe obscurus region or i.t. injection site was not in the i.t. space were discarded.

2.3. Data analyses

Changes of the tail-flick latency response were expressed as percent maximum possible effect (% MPE) which was calculated as: % MPE = [(Post-opioid latency–Baseline latency)/(Cut-off time–baseline latency)] × 100, in which cut-off time was 10 s. The antagonism of the i.c. β-endorphin-induced tail-flick inhibition by i.t. injection of antagonist was expressed as Percent Reversal, which is calculated as: Percent Reversal = [% MPE in rats injected i.c. with β-endorphin and i.t. saline]–[% MPE in rats injected i.c. with β-endorphin and i.t. antagonist].

2.4. Drugs

Drugs used were: human β-endorphin, [D-Pen^{2,5}]enkephalin (DPDPE), [D-Ala^2,*N*-Me-Phe^5,Gly-ol]enkephalin (DAMGO), CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2), [D-Ala^2]deltorphin II (Peninsula Laboratory, Belmont, CA, USA); *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzene-acetamide methane sulfonate (U50, 488H), *nor*-binaltorphimine HCl (Research Biochemical, Natick, MA, USA); 7-benzylidene naltrexone and naltriben (Portoghese, 1991; Portoghese et al., 1992).

3. Results

3.1. Effects of naltriben, 7-benzylidene naltrexone, nor-binaltorphimine or CTOP given i.t. on the inhibition of the tail-flick response induced by β-endorphin given into nucleus raphe obscurus

The tail-flick latencies before the injection of β-endorphin into nucleus raphe obscurus were found to be 3.87 ± 0.07 s (N = 18). β-Endorphin, at a dose of 2 μg (0.58 nmol) given into nucleus raphe obscurus, developed inhibition of the tail-flick response in 10–20 min. The inhibition reached a maximum at 20–30 min and remained inhibited for about 90–120 min. As shown in Fig. 1, i.t. injection of increasing doses of naltriben (0.6–6 nmol) given at 20 min after β-endorphin caused a dose-dependent reversal of the tail-flick inhibition induced by β-endorphin. 7-Benzylidene naltrexone at a high dose (64 nmol) but not at lower doses (2.1–21 nmol), caused only a slight reversal of the β-endorphin-induced inhibition. Intrathecal injection of saline did not reverse β-endorphin-induced antinociception. Naltriben (0.6–6 nmol) or 7-benzylidene naltrexone (2.1–64 nmol) injected i.t. alone did not affect the baseline tail-flick latencies even at highest doses (data not shown). Intrathecal injection of increasing doses of nor-binaltorphimine (1.4–40.8 nmol) or CTOP (0.09–2.8 nmol) caused a slight reversal of the i.c.v. β-endorphin-induced inhibition at higher doses but not at low doses (Fig. 2).

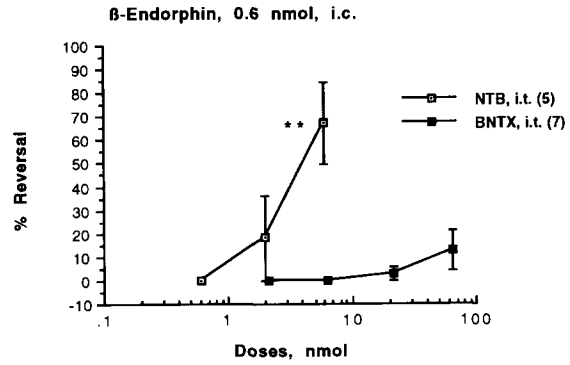

Fig. 1. Effects of i.t. injection of various doses of naltriben and 7-benzylidene naltrexone on the inhibition of the tail-flick response induced by β-endorphin injected into nucleus raphe obscurus in pentobarbital-anesthetized rats. Naltriben (NTB, open squares) and 7-benzylidene naltrexone (BNTX, closed squares) were cumulatively injected i.t. every 20 min starting 30 min after i.c. injection of β-endorphin. The tail-flick response was measured 10 min after each i.t. injection. The number in parentheses denotes the number of rats and the vertical line indicates S.E.M. $^{**}P < 0.01$, compared with rats injected with i.t. saline.

3.2. Effects of naltriben or 7-benzylidene naltrexone given i.t. on the inhibition of the tail-flick response induced by [D-Ala^2]deltorphin II or DPDPE given i.t.

To ascertain if the doses of naltriben and 7-benzylidene naltrexone used in the above study selectively

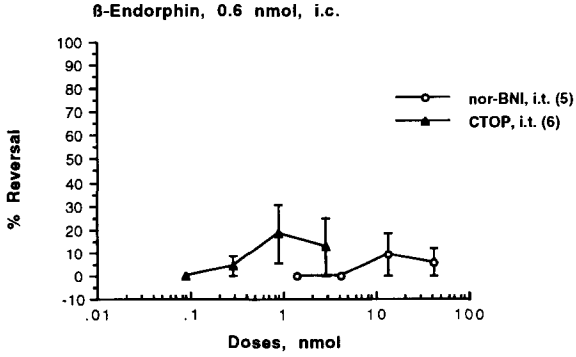

Fig. 2. Effects of i.t. injection of various doses of nor-binaltorphimine and CTOP on the inhibition of the tail-flick response induced by β-endorphin injected into nucleus raphe obscurus in pentobarbital-anesthetized rats. Nor-binaltorphimine (nor-BNI, open circles) and CTOP (closed triangles) were cumulatively injected i.t. every 20 min starting 30 min after i.c. injection of β-endorphin. The tail-flick response was measured 10 min after each i.t. injection. The vertical line indicates S.E.M. The number in parentheses denotes the number of rats.

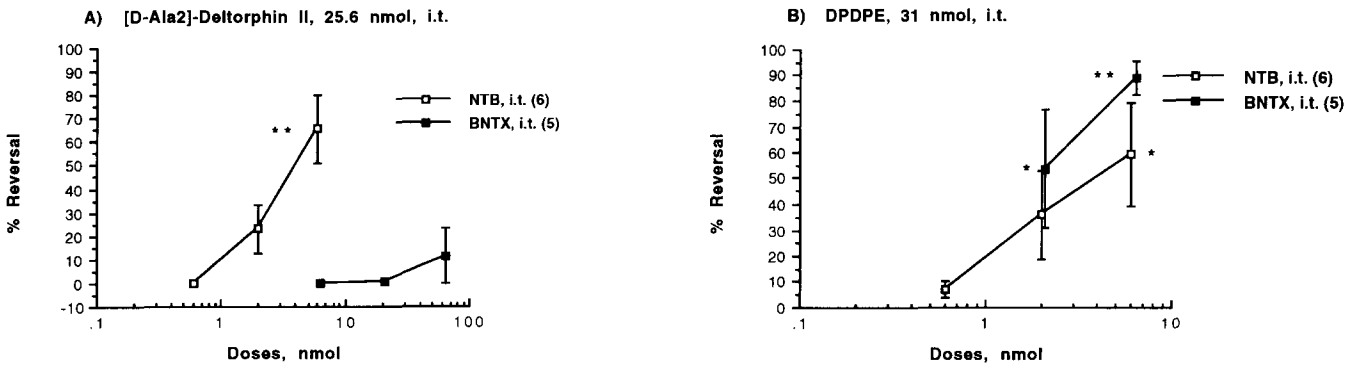

Fig. 3. Effects of i.t. injection of various doses of naltriben and 7-benzylidene naltrexone on the inhibition of the tail-flick response induced by i.t. injection of [D-Ala^2]deltorphin II (A) and DPDPE (B). Naltriben (NTB, open squares) and 7-benzylidene naltrexone (BNTX, closed squares) were cumulatively injected i.t. every 20 min starting 20 min after i.t. injection of the δ-opioid agonist. The tail-flick response was measured 10 min after each i.t. antagonist injection. The number in parentheses denotes the number of rats and the vertical line indicates S.E.M. $^*P < 0.05$, $^{**}P < 0.01$, compared with rats injected with i.t. saline.

blocked the δ_1- and δ_2-opioid receptors, respectively, the effects of naltriben and 7-benzylidene naltrexone on the inhibition of the tail-flick response induced by i.t. administered [D-Ala^2]deltorphin II and DPDPE were studied. [D-Ala^2]deltorphin II at a dose of 20 μg (25.6 nmol) or DPDPE at a dose of 20 μg (31.0 nmol) produced marked inhibition of the tail-flick response. The antinociception induced by [D-Ala^2]deltorphin II was dose-dependently blocked by naltriben (0.2–20 nmol) but not by 7-benzylidene naltrexone (6.4 and 21 nmol). 7-Benzylidene naltrexone even at a high dose (64 nmol) caused only a small degree of reversal of [D-Ala^2]deltorphin II-induced inhibition (Fig. 3A). Both naltriben and 7-benzylidene naltrexone given i.t. were found to reverse dose-dependently the DPDPE-induced inhibition. 7-Benzylidene naltrexone, however, was slightly more potent and effective than naltriben in antagonizing DPDPE-induced tail-flick inhibition (Fig. 3B).

3.3. Effects of CTOP or nor-binaltorphimine given i.t. on the inhibition of the tail-flick response induced by DAMGO or U50,488H given i.t.

To ascertain if the doses of CTOP and nor-binaltorphimine used for i.t. injection were selective in blocking the μ- and κ-opioid receptors, respectively, the effects of i.t. CTOP and nor-binaltorphimine on the inhibition of the tail-flick response induced by i.t. DAMGO or U50,488H were studied. DAMGO at a dose of 120 pmol given i.t. produced a marked inhibition of the tail-flick response. The antinociception induced by DAMGO was selectively blocked dose-dependently by i.t. administered CTOP (0.19–0.9 nmol) but not nor-binaltorphimine (13.6–27.2 nmol). At a higher dose (95.3 nmol), i.t. administered nor-binaltorphimine blocked slightly (< 20% reversal) the inhibition induced by i.t. administered DAMGO (Fig. 4A). On the other hand, the inhibition of the tail-flick

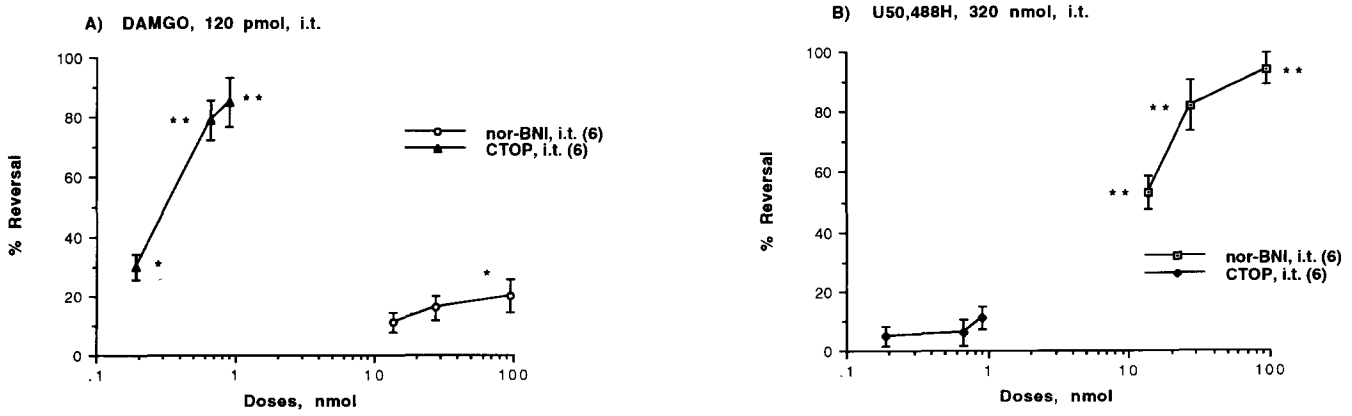

Fig. 4. Effects of i.t. injection of various doses of nor-binaltorphimine and CTOP on the inhibition of the tail-flick response induced by i.t. injection of U50,488H (A) and DAMGO (B). Nor-binaltorphimine (nor-BNI, open circles) and CTOP (closed triangles) were cumulatively injected i.t. every 20 min starting 20 min after i.t. injection of the opioid agonists. The tail-flick response was measured 10 min after each i.t. antagonist injection. The number in parentheses denotes the number of rats and the vertical line indicates S.E.M. $^*P < 0.05$, $^{**}P < 0.01$, compared with rats injected with i.t. saline.

response induced by i.t. administered U50,488H was blocked dose-dependently by i.t. administered nor-binaltorphimine (13.6–95.3 nmol), but not CTOP (0.19–0.9 nmol) (Fig. 4B). Although there was a small loss of selectivity at the highest dose of i.t. administered nor-binaltorphimine, the results indicated that CTOP and nor-binaltorphimine at the doses used in the present studies selectively blocked the μ- and κ-opioid receptors, respectively.

4. Discussion

We have previously demonstrated that the antinociception induced by β-endorphin given supraspinally is mediated by the stimulation of ε-opioid receptors which subsequently induces the release of [Met^5]enkephalin and stimulation of opioid receptors in the spinal cord in mice and rats (Tseng and Fujimoto, 1984, 1986; Tseng et al., 1985, 1986; Suh and Tseng, 1990; Xu et al., 1992). The opioid receptors in the spinal cord involved in i.c.v. administered β-endorphin-induced antinociception have been identified to be δ_2-, but not δ_1-, μ-, or κ-opioid receptors in mice (Suh and Tseng, 1990; Tseng et al., 1993; Xu et al., 1992). Previous studies performed in anesthetized rats using micro-intracerebral injection techniques have indicated that the brain sites sensitive to β-endorphin for the production of antinociception are localized in several nuclei (Tseng et al., 1980, 1990; Tseng and Wang, 1992). In the brainstem area, the sites sensitive to β-endorphin for the production of antinociception were found to be in the nucleus raphe obscurus and nucleus raphe pallidus (Tseng et al., 1990). Nucleus raphe obscurus was therefore chosen as the β-endorphin injection site for the study. Microinjection of β-endorphin into nucleus raphe obscurus selectively activates the descending pain control system for the production of the tail-flick inhibition which involves the release of [Met^5]enkephalin acting on opioid receptors. Intrathecal injection of naloxone, but not methysergide or yohimbine blocks the inhibition of the tail-flick response induced by β-endorphin from nucleus raphe obscurus in the pentobarbital-anesthetized rats, indicating that opioid receptors, but not 5-HT or α_2-adrenoceptors in the spinal cord are involved in β-endorphin-induced antinociception (Tseng and Collins, 1991). The purpose of the present study was to identify the type of opioid receptor involved in the β-endorphin-induced antinociception in the rat using a selective δ_1-opioid receptor antagonist, 7-benzylidene naltrexone, a δ_2-opioid receptor antagonist, naltriben, CTOP, a selective μ-opioid receptor antagonist, and nor-binaltorphimine, a κ-opioid receptor antagonist. The results of the present study in the rat are consistent with previous experiments in the mouse (Tseng et al., 1993), indicating that δ_2- but not δ_1-, μ- or κ-opioid receptors in the spinal cord are involved in supraspinally administered β-endorphin-induced antinociception. This conclusion is supported by the findings that naltriben, but not 7-benzylidene naltrexone, the respective δ_2- and δ_1-opioid receptor antagonists, effectively blocked the inhibition of the tail-flick response induced by β-endorphin given into nucleus raphe obscurus. CTOP and nor-binaltorphimine, at doses sufficient to selectively block μ- and κ-opioid receptors, did not affect the inhibition of the tail-flick response induced by β-endorphin. The present results performed in rats are consistent with the results obtained in mice (Tseng et al., 1993; Xu et al., 1992).

The control experiments were also performed to verify that δ_2- and δ_1-opioid receptors in the spinal cord were selectively blocked by selective receptor antagonists at doses used to study the attenuation of β-endorphin-induced antinociception. [D-Ala^2]deltorphin II is a δ_2-opioid receptor agonist (Mattia et al., 1991). Intrathecal injection of [D-Ala^2]deltorphin II (20 μg) given i.t. selectively stimulates δ_2-, but not δ_1-opioid receptors. This is verified in the present finding that the inhibition of the tail-flick response induced by [D-Ala^2]deltorphin II given i.t. was blocked by i.t. naltriben, but not 7-benzylidene naltrexone. DPDPE has been reported to stimulate δ_1-opioid receptors. This is evidenced by the finding that the inhibition of the tail-flick response induced by i.t. administered DPDPE was blocked by 7-benzylidene naltrexone, a δ_1-opioid receptor antagonist. However, 7-benzylidene naltrexone, although less potent than naltriben, also blocked the inhibition induced by DPDPE, indicating that DPDPE not only stimulates δ_1-opioid receptor but it also has efficacy on δ_2-opioid receptors. The lack of absolute selectivity of DPDPE on stimulation of subtypes of δ-opioid receptors has been reported by Vanderah et al. (1994). However, our results differ from the reports by Stewart and Hammond (1993, 1994), who demonstrated in rats that naltriben given i.t. did not antagonize i.t. DPDPE-induced antinociception. The reason for the discrepancy is not known. Naltriben may not be a selective δ_2-receptor antagonist. They also reported that naltriben blocked DAMGO-induced antinociception.

In conclusion, the present results indicate that δ_2-, but not δ_1-, μ- or κ-opioid receptor is involved in the inhibition of the tail-flick response induced by β-endorphin given into nucleus raphe obscurus in pentobarbital-anesthetized rats.

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