Modulation of Nociception by Microinjection of Delta-1 and Delta-2 Opioid Receptor Ligands in the Ventromedial Medulla of the Rat

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ABSTRACT
In this study, we characterized the role of delta-1 and delta-2 opioid receptors in the ventromedial medulla (VMM) in the modulation of thermal nociception. Male Sprague-Dawley rats were prepared with an intracerebral guide cannula aimed at the nucleus raphe magnus or nucleus reticularis gigantocellularis. Microinjection of the delta-1 opioid receptor agonist [D-Pen\(^2\),D-Pen\(^5\)]enkephalin (DPDPE) or the delta-2 opioid receptor agonist [D-Ala\(^2\),Glu\(^4\)]deltorphin (DELT) in the VMM increased response latency in the radiant heat tail-flick test with respective ED\(_{50}\) values (95% CL) of 0.66 (0.07–1.5) nmol and 0.1 (0.03–0.21) nmol. In the 55°C hot-plate test, DELT produced a modest, transient increase in response latency and DPDPE was ineffective. The antinociception produced by DPDPE was antagonized by microinjection at the same site of 1.5 pmol of the delta-1 opioid receptor antagonist 7-benzylidenenaltrexone (BNTX) but not by 0.15 nmol of the delta-2 opioid receptor antagonist naltriben (NTB). Conversely, the antinociception produced by DELT was antagonized by microinjection at the same site of 0.15 nmol of NTB but not by 1.5 pmol of BNTX. These doses of BNTX or NTB alone did not alter either tail-flick or hot-plate latency when microinjected in the VMM. However, at 10-fold higher doses, BNTX lost its selectivity for delta-1 opioid receptor, and NTB by itself increased tail-flick and hot-plate latencies. These results collectively implicate both delta-1 and delta-2 opioid receptors in the VMM in the modulation of nociception. They also indicate that the antinociceptive effects of DPDPE and DELT can be distinguished by the specific supraspinal sites at which delta-1 opioid receptor agonists act to produce antinociception. With the recent development of antibodies to the delta opioid receptor, the neuroanatomic distribution of these receptors can be visualized with greater resolution than previously afforded by in vitro autoradiography. These studies have localized delta opioid receptors to fibers and varicosities in the NRM and NGCP\(_{\alpha}\) (Arvidsson et al., 1995a; Kalyuzhny et al., 1996), two nuclei in the VMM that are implicated in the bulbospinal modulation of nociceptive transmission (Gebhart, 1982; Hammond, 1986; Jones, 1992). Although several investigators have reported that microinjection of delta opioid receptor agonists in the

Two subtypes of the delta opioid receptor, termed delta-1 and delta-2, have been proposed on the basis of considerable pharmacological, behavioral and biochemical evidence (Hammond, 1993; Porreca and Burks, 1993; Zaki et al., 1996). The role of these receptor subtypes in the production of antinociception at the level of the spinal cord has been extensively studied in the mouse (Heyman et al., 1987; Mattia et al., 1992; Sofuoglu et al., 1991; Takemori and Portoghese, 1993), as well as the rat (Hammond et al., 1995; Malmberg and Yaksh, 1992; Stewart and Hammond, 1993, 1994). In contrast, comparatively little is known about the role of supraspinal delta opioid receptors in the production of antinociception, the specific sites at which delta opioid receptor agonists act or the relative involvement of the two different subtypes of delta opioid receptor in the supraspinal modulation of nociception. Most studies of supraspinal delta opioid receptors in the mouse (Jiang et al., 1991; Mattia et al., 1991; Roerig and Fujimoto, 1989; Sofuoglu et al., 1991) and the rat (Adams et al., 1993; Miaskowski et al., 1991; Negri et al., 1991) administered the agonists intracerebroventricularly. Unfortunately, such studies provide little information about the specific supraspinal sites at which delta opioid receptor agonists act to produce antinociception. With the recent development of antibodies to the delta opioid receptor, the neuroanatomic distribution of these receptors can be visualized with greater resolution than previously afforded by in vitro autoradiography. These studies have localized delta opioid receptors to fibers and varicosities in the NRM and NGCP\(_{\alpha}\) (Arvidsson et al., 1995a; Kalyuzhny et al., 1996), two nuclei in the VMM that are implicated in the bulbospinal modulation of nociceptive transmission (Gebhart, 1982; Hammond, 1986; Jones, 1992). Although several investigators have reported that microinjection of delta opioid receptor agonists in the

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ABBREVIATIONS: VMM, ventromedial medulla; NRM, nucleus raphe magnus; NGCP\(_{\alpha}\), nucleus reticularis gigantocellularis pars \(\alpha\); DPDPE, [D-Pen\(^2\),D-Pen\(^5\)]enkephalin; DELT, [D-Ala\(^2\),Glu\(^4\)]deltorphin; BNTX, 7-benzylidenenaltrexone; NTB, naltriben; CL, confidence limits; GABA, \(\gamma\)aminobutyric acid; HBC, 2-hydroxypropyl-\(\beta\)-cyclodextrin.
VMM produces antinociception (Jensen and Yaksh, 1986; Ossipov et al., 1995; Rossi et al., 1994), the role of the different subtypes of δ opioid receptor in the production of antinociception is not well understood. Also, despite the high concentration of enkephalin (Zamir et al., 1985), the relatively high densities of enkephalineric terminals (Finley et al., 1981; Khachaturian et al., 1983) and existence of opioid receptors in the VMM (Arvidsson et al., 1995a, 1995b; Mansour et al., 1988, 1994), it is not known whether neurons in this region are tonically modulated by enkephalinergic inputs. The present study was therefore undertaken to (1) characterize the antinociception produced by microinjection of the prototypic δ-1 opioid receptor agonist DPDP or the δ-2 opioid receptor agonist DELT into the NRM and NGCp using the radiant heat tail-flick and 55°C hot-plate tests in the rat and (2) determine the pharmacological specificity of this antinociception by challenging these agonists with the δ-1 opioid receptor antagonist BNTX and the δ-2 opioid receptor antagonist NTB. A final aim of this study was to determine whether neurons of the NRM and NGCp receive a tonic inhibitory enkephalinergic input mediated by δ opioid receptors by assessing the effects of the antagonists themselves on nociceptive threshold. Preliminary reports of this work have been published in abstract form (Thorat and Hammond, 1996, 1997).

Methods and Materials

These experiments were approved by the Institutional Animal Care and Use Committee of the University of Chicago. All procedures were conducted in accordance with the “Guide for Care and Use of Laboratory Animals” as published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Animals. Male Sprague-Dawley rats (Sasco, Madison, WI) weighing 300 to 350g were housed three per cage and maintained on a 12-hr light/dark cycle with lights on at 6:00 a.m. Animals had free access to food and water. Rats were used only once in this study.

Surgical procedures. After induction of anesthesia with a mixture of ketamine hydrochloride (85 mg/kg i.p.) and xylazine (13 mg/kg i.p.), rats were stereotaxically prepared with a stainless steel guide cannula (26-gauge; Plastics One, Roanoke, VA) aimed either at NRM (interaural coordinates: AP, −1.6 to −2.5 mm; ML, 0.0 mm; DV, 4.0 to 5.0 mm) or NGCp (interaural coordinates: AP, −1.6 to −2.5 mm; ML, +0.7 mm; DV, 4.0 to 5.0 mm). The incisor bar was fixed at −2.5 mm. The guide cannula was secured to the skull with stainless steel screws and dental acrylic cement. A 33-gauge stainless steel stylette was placed in each guide cannula to maintain its patency. After surgery, the rats were housed individually and allowed at least 7 days to recover from surgery.

Behavioral procedures. Nociceptive threshold was assessed by using the radiant heat tail-flick and 55°C hot-plate tests. Briefly, in the tail-flick test, a high-intensity light beam was focused on the blackened tail of the rat (ITT, Woodlawn Hills, CA). The tail-flick reflex latency was measured to the nearest tenth of a sec. Two successive determinations of the response latency were made at different sites on the distal two thirds of the tail, and the average was recorded as the tail-flick latency. In the hot-plate test, the rat was placed on a covered copper hot-plate maintained at 55°C and the time between placement of the rat on the hot-plate and the occurrence of either a hind paw lick or a jump off the surface was recorded as the hot-plate latency. Rats with base-line tail-flick latencies of >5.2 sec or base-line hot-plate latencies of >15 sec were eliminated from the study. Mean base-line latencies for 20 treatment groups ranged from 3.5 to 4.5 sec in the tail-flick test and from 8.1 to 11.4 sec in the hot-plate test. Motor function was evaluated using the inclined plane test (Rivlin and Tator, 1977), which determined the largest angle at which the rat was able to maintain its position on the inclined plane. Mean base-line inclined plane angle varied from 40º to 50º. The tail-flick, inclined plane and hot-plate tests were conducted in succession in that order. All experiments were done between 10:00 a.m. and 4:00 p.m.

Experimental design. The first series of experiments determined the time course and dose dependence of the effects of DPDP or DELT on nociceptive threshold. After determination of base-line response latencies and motor competency, rats were microinjected with either DPDP (0.2–8.8 nmol), DELT (0.03–3.0 nmol) or their respective vehicle. Tail-flick and hot-plate latencies were re-determined 15, 30, 45 and 60 min later. Rats that did not respond within 14 sec on the tail-flick test or 40 sec on the hot-plate test after drug administration were removed to prevent tissue damage and assigned the cutoff latency. Dose-response lines for DPDP or DELT were constructed using the individual tail-flick and hot-plate latencies obtained at the time of peak effect (45 and 30 min, respectively). The ED50 was defined as the dose that produced 50% of the maximum possible increase in tail-flick or hot-plate latency (i.e., to 7 sec in the tail-flick test and to 25 sec in the hot-plate test). Fieller’s theorem was used to determine the 95% CI (Finney, 1964). A two-way analysis of variance for repeated measures was used to compare the effects of the δ opioid receptor agonists to those of their respective vehicle. The Newman-Keuls test was used for post hoc comparisons among the individual mean values.

Experiments in the second series determined the pharmacological specificity of the antinociception produced by microinjection of DPDP or DELT in the VMM. After determination of base-line tail-flick and hot-plate latencies and motor competency, rats were microinjected with either vehicle (45% w/v HBC), 1.5 to 45 pmol of BNTX or 0.15 to 1.5 nmol of NTB. Five min later, 5.3 nmol of DPDP or 1.2 nmol of DELT was microinjected at the same site and the tail-flick and hot-plate latencies were re-determined 15, 30, 45 and 60 min later. The effect of DPDP or DELT in BNTX- or NTB-treated rats was compared with its effect in vehicle-pretreated rats by two-way analysis of variance for repeated measures. Post hoc comparisons of individual mean values were made by Newman-Keuls test.

The final series of experiments examined whether microinjection of the δ opioid receptor antagonists by themselves affected nociceptive threshold. After determination of base-line responses in the tail-flick, hot-plate and inclined angle test, either vehicle (45% w/v HBC), 45 pmol of BNTX or 0.15–1.5 nmol of NTB was microinjected into the VMM. Tail-flick and hot-plate latencies were re-determined 15, 30, 45 and 60 min later. Two-way analyses of variance for repeated measures were used to compare the effect of the antagonists to that of vehicle. Comparisons of mean values for individual treatment groups were made by Newman-Keuls test.

Histology. At the end of the experiment, each rat was killed by inhalation of CO2. The brain was removed and fixed by immersion in 10% formalin containing 30% sucrose. Twenty-five-μm transverse sections were cut through the region traversed by the guide cannula using a cryostat microtome, collected on gelatinized slides and stained with Cresyl violet. The location of the injection sites was verified by two individuals without reference to the behavioral data and plotted on appropriate coronal sections from an atlas of the rat brainstem modified from that provided by Neurographics (Kanata, Ontario, Canada).

Drugs and microinjections. All drug and vehicle solutions were microinjected over a period of 60 sec in a volume of 0.4 μl using a 33-gauge injection cannula that protruded an additional 3 mm beyond the guide cannula. Delivery of the drug solution was monitored by following movement of a bubble in the calibrated tubing that connected the injector to a syringe mounted on a microinfusion pump. The drugs and their vehicle solutions were freshly prepared. Naltrexen (lot no. XX1-146.3; molecular weight, 465.5) was a gift from G. D. Searle & Co. (Skokie, IL). BNTX hydrochloride (lot no. WY-III-
69B; molecular weight, 466.0) was obtained from Research Biochemicals (Natick, MA) through the Research Technology Branch of the National Institute of Drug Abuse. Naltriben and BNTX were dissolved in 45% (w/v) HBC (lot no. UCD-695A; Research Biochemicals). DELT (Sigma Chemical, St. Louis, MO; lot no. 44H08641; molecular weight, 782.9) was dissolved in either saline for the dose-response studies or 45% (w/v) HBC for the antagonism studies. DPDPE (Sigma Chemical; lot no. 85H58551; molecular weight, 654.8, or Research Biochemicals; lot no. FRY-296F, molecular weight, 647.8) was dissolved in distilled water for the dose-response studies or saline for the antagonism studies.

Results

Distribution of microinjection sites in the VMM. Histological analysis revealed that the microinjection sites were distributed throughout the rostrocaudal extent of the NRM and NGCpα. The very large number of rats and different treatment groups in this study precluded presentation of all the injection sites for each treatment group. Therefore, as there were no major differences in the distribution of microinjection sites among the various treatment groups, only the distribution of microinjection sites for the 1.2-nmol dose of DELT is presented (fig. 1). A number of sites located dorsal to the NGCpα and ventral to the dorsal border of the facial nucleus, as well as a few sites in the most rostral aspect of the nucleus reticularis paragigantocellularis lateralis, were included within the NGCpα for purposes of analysis. Microinjection sites located outside the NRM and NGCpα included sites in the dorsal, lateral or caudal aspects of the nucleus reticularis gigantocellularis, as well as the facial nucleus, the medial longitudinal fasciculus, the pyramids, inferior olive or nucleus raphe obscurus.

Effects of DPDPE in the VMM. Microinjection of 0.2 to 8.8 nmol of DPDPE in either the NRM or NGCpα increased tail-flick latency in a dose-dependent manner, with the peak effect occurring between 30 and 45 min (fig. 2A). There was no difference between these nuclei in either the magnitude or the onset to effect of DPDPE (P > .27 for each treatment group). For example, microinjection of 8.8 nmol of DPDPE at seven sites in the NRM increased tail-flick latency to 6.4 ± 1.2, 9.0 ± 0.9 and 10.5 ± 1.1 sec at 15, 30 and 45 min, respectively. This same dose in the NGCpα increased tail-flick latency to 5.8 ± 1.0, 8.2 ± 1.3 and 9.3 ± 1.1 sec at 15, 30 and 45 min, respectively. Therefore, the sites were grouped together as the VMM for subsequent analysis. Linear regression analysis estimated the ED50 (95% CL) of DPDPE in the tail-flick test to be 0.66 (0.07–1.5) nmol (fig. 3A). Doses of DPDPE of >8.8 nmol could not be tested due to its limited solubility.

No dose of DPDPE significantly increased hot-plate latency beyond that produced by the vehicle, distilled water, which itself transiently increased response latency compared with base-line in this test (fig. 2B). Although hot-plate latencies in rats that received 0.2 nmol of DPDPE were consistently less than in the vehicle-treated group, this effect is likely to be an artifact of the shorter base-line latencies of this particular treatment group. No adverse motor effects or decrement in the angle maintained on the inclined plane test occurred after microinjection of doses of DPDPE as high as 8.8 nmol.

Microinjection of either 2.93 or 8.8 nmol of DPDPE at three sites in the dorsal aspect of the nucleus reticularis gigantocellularis increased tail-flick latency to the same extent and

![Fig. 1. Representative rostrocaudal distribution of the sites in the VMM at which 1.2 nmol DELT was microinjected in the rat. Numbers refer to distance caudal to the interaural line. • Sites in the NRM and NGCpα. ○ Sites outside these two nuclei. 4th vent, 4th ventricle; 7, facial motor nucleus; 7g, genu of the seventh cranial nerve; 7t, tract of the seventh cranial nerve; dcn, dorsal cochlear nucleus; icp, inferior cerebellar peduncle; mlf, medial longitudinal fasciculus; ngc, nucleus reticularis gigantocellularis; P, pyramid; tr, trapezoid body; V, spinal trigeminal tract.](image1)

![Fig. 2. Time course of the change in response latencies in the (A) tail-flick and (B) hot-plate tests after microinjection of either distilled water (△) or 0.2 (○), 1.0 (●), 2.9 (●) or 8.8 (■) nmol of the delta-1 opioid receptor agonist DPDPE in the VMM. Symbols represent the mean ± S.E.M. of determinations in 12 to 17 rats. *, P < .05; ***, P < .01 compared with vehicle at the corresponding time point.](image2)
with the same onset as did microinjection of these doses in the NRM and NGCp. In contrast, microinjection of these doses at sites in the facial nucleus, caudal nucleus reticularis paragigantocellularis lateralis or the pyramids was without effect.

**Effects of DELT in the VMM.** Microinjection of 0.03 to 3.0 nmol of DELT in either the NRM or NGCp produced a dose-dependent increase in tail-flick latency, with the peak effect occurring within 30 min (fig. 4A). These sites were grouped together as the VMM for subsequent analysis because they did not differ with respect to either the magnitude or onset to effect of DELT (P > .2 for all treatment groups). For example, tail-flick latency determined 15, 30 and 45 min after microinjection of 1.2 nmol of DELT at seven sites in the NRM was 6.3 ± 0.9, 8.6 ± 1.2 and 8.9 ± 1.1 sec, respectively. Tail-flick latency determined 15, 30 and 45 min after microinjection of this same dose at seven sites in the NGCp was 5.8 ± 0.7, 9.2 ± 0.8 and 9.5 ± 0.8 sec, respectively. The ED$_{50}$ (95% CL) of DELT in the tail-flick test was 0.1 (0.03–0.21) nmol (fig. 3A). Microinjection of either 1.2 or 3.0 nmol of DELT at five sites in the nucleus reticularis gigantocellularis increased tail-flick latency with the same onset and to the same extent as did microinjection of these doses in the NRM and NGCp. Doses of DELT of >3.0 nmol could not be tested due to limited solubility.

Microinjection of DELT also produced a significant, although short-lived, increase in hot-plate latency (fig. 4B). However, this increase was of modest magnitude and was not dose dependent (fig. 3B). No adverse motor effects or decrement in the angle maintained on the inclined plane test occurred after microinjection of doses of DELT as high as 3.0 nmol.

**Effects of BNTX or NTB in the VMM.** Figure 5 illustrates the time course of the change in response latencies produced by microinjection of BNTX or NTB in the VMM. Neither tail-flick nor hot-plate latencies were altered after microinjection of 45 pmol of BNTX in the VMM. Similarly, microinjection of 0.15 nmol of NTB in the VMM did not alter either tail-flick or hot-plate latencies compared with vehicle. However, tail-flick and hot-plate latencies were significantly increased after microinjection of a 10-fold higher dose of NTB, 1.5 nmol, in the VMM (fig. 5, A and B). No consistent effects were noted on the inclined plane test after microinjection of BNTX or NTB (data not shown).

**Effects of BNTX or NTB on the antinociception produced by microinjection of DPDPE or DELT in the VMM.** Microinjection of the delta-1 opioid receptor antagonist BNTX in the VMM selectively antagonized the antinociception produced by microinjection of DPDPE at the same sites. However, the selectivity of this antagonism was dependent on the dose of BNTX. Pretreatment with 1.5 pmol of BNTX significantly attenuated the increase in tail-flick latency produced by microinjection of 5.3 nmol of DPDPE (fig. 6A) and produced a only marginal and transient antagonism of the increase in tail-flick latency produced by microinjection of DELT (fig. 6B). Increasing the dose of BNTX by 10-fold to 15 pmol completely antagonized the antinociceptive effect of DPDPE (fig. 6A). However, pretreatment with this higher dose of BNTX also antagonized the antinociceptive effect of
Delt to a greater extent and for a longer duration (fig. 6B). Microinjection of 45 pmol of BNTX did not produce any greater antagonism of the antinociceptive effect of DELT than did the 15-pmol dose (data not shown).

Pretreatment with the delta-2 opioid receptor antagonist NTB selectively antagonized the antinociception produced by microinjection of DELT but not DPDPE in the VMM. Microinjection of 0.15 nmol of NTB nearly completely antagonized the increase in tail-flick latency produced by subsequent microinjection of 1.2 nmol of DELT at the same sites (fig. 6B). This same dose of NTB did not antagonize the increase in tail-flick latency produced by 5.3 nmol of DPDPE, with the exception of the 60-min time point (fig. 6A). A higher dose of NTB could not be tested because it increased tail-flick and hot-plate latencies by itself (see above).

Similar findings were made in the hot-plate test. In rats that were pretreated 5 min earlier with the HBC vehicle, microinjection of 1.2 nmol of DELT increased tail-flick latency to 19.6 ± 4.2 sec. This increase was completely prevented in rats that were pretreated with 0.15 nmol of NTB (12.1 ± 1.4 sec). Neither 1.5 nor 15 pmol of BNTX antagonized the increase in hot-plate latency produced by DELT (19.8 ± 3.4 and 22.3 ± 3.4 sec, respectively); however, the 15-pmol dose of BNTX did significantly prolong the antinociception through 60 min (24.5 ± 3.4 sec at 45 min; 21.2 ± 3.2 sec at 60 min) (P < .02). Microinjection of 5.3 nmol of DPDPE did not significantly increase hot-plate latency when microinjected in rats that were pretreated 5 min earlier with either the HBC vehicle, NTB or BNTX (data not shown).

Influence of solvent on drug efficacy. In the antagonism study, 1.2 nmol of DELT increased tail-flick latency to a significantly greater extent than in the dose-response analysis (compare figs. 4A and 6B). This difference in effect could arise from (1) pretreatment with the HBC vehicle or (2) the use of HBC, rather than saline, as the solvent for DELT in the antagonism study. Additional animals were tested to examine this discrepancy. Microinjection of 1.2 nmol of DELT dissolved in saline in five rats that were pretreated 5 min earlier with HBC increased tail-flick latency to 8.8 ± 1.6 sec. These values were not different from the 8.9 ± 0.7 sec latency determined for this dose of DELT in the dose-response study in which it was also dissolved in saline (P > .8; fig. 3A). When 1.2 nmol of DELT was dissolved in HBC and injected by itself, it increased tail-flick latency to 11.6 ± 1.1 sec (n = 7). These values were significantly greater than the effect of this dose of DELT dissolved in saline (P < .05) but did not differ from the increase produced by microinjection of this formulation in rats that were pretreated with HBC (12.3 ± 0.7 sec). A similar enhancement occurred in the hot-plate test, in which response latency was increased to a greater extent and for a longer duration when DELT was dissolved in HBC compared with saline (data not shown). The enhanced efficacy of DELT in this set of experiments is

Fig. 5. Time course of the change in response latencies in the (A) tail-flick and (B) hot-plate tests after microinjection of either 45% (w/v) HBC ( ), 45 pmol of the delta-1 opioid receptor antagonist BNTX ( ) or 0.15 ( ) or 1.5 (A) nmol of the delta-2 opioid receptor antagonist NTB in the VMM. Symbols represent the mean ± S.E.M. of determinations in 12 to 17 rats. *, P < .05; **, P < .01 compared with HBC at the corresponding time point.

Fig. 6. Time course of the antagonism of the increase in tail-flick latency produced by microinjection of (A) 5.3 nmol of DPDPE or (B) 1.2 nmol of DELT in the VMM of rats pretreated 5 min earlier by microinjection of either HBC ( ), 0.15 nmol of the delta-2 opioid receptor antagonist NTB ( ) or 1.5 pmol (A) or 15 pmol ( ) of the delta-1 opioid receptor antagonist BNTX. Symbols represent the mean ± S.E.M. of determinations in 7 to 13 rats. *, P < .05; **, P < .01 compared with the rats pretreated with HBC at the corresponding time point.
therefore most likely due to the use of HBC as the solvent for DELT, and not to prior microinjection of HBC. The antinoceptive potency of intrathecally administered morphine, [d-Ala², d-Leu⁵]enkephalin and other peptidic analogs of enkephalin is similarly enhanced when HBC is used as the vehicle (Jang et al., 1992; Yaksh et al., 1991).

**Discussion**

**Medullary delta-1 opioid receptors modulate nociception.** The first finding of this study was that microinjection of DPDPE, a delta-1 opioid receptor agonist, in the NRM and NGCp increased tail-flick latency in a dose-dependent manner. This increase in tail-flick latency was antagonized by microinjection of as little as 1.5 pmol of the delta-1 opioid receptor antagonist NTB at the same site. However, it was not attenuated by pretreatment with 0.15 nmol of the delta-2 opioid receptor antagonist BNTX. The selective antagonism of the antinoceptive effect of DPDPE by BNTX but not NTB is strong evidence that the increase in tail-flick latency is specific and is mediated by a delta-1 opioid receptor. The finding that microinjection of DPDPE in the VMM increased tail-flick latency is contrary to previous reports that doses as high as 40 nmol of DPDPE were ineffective (Ossipov et al., 1995; Rossi et al., 1994). However, this study differs from previous reports in two important respects. First, DPDPE was administered in a much smaller volume in the present study (0.4 compared with 1.0 µl). Second, comparison of base-line tail-flick latencies suggests that the thermal stimulus in this study was less intense than that used by Rossi et al. (1994). Studies with intrathecally administered mu opioid receptor agonists indicate that antinoceptive potency is inversely related to the intensity of the noxious stimulus (Saeki and Yaksh, 1993). Even with the moderate intensity stimulus used in this study, the highest dose of DPDPE that could be tested did not increase tail-flick latency beyond 10 sec. Therefore, the antinoceptive effect of DPDPE may not have been observed in previous studies simply because the thermal stimulus was too intense.

Doses as high as 8.8 nmol of DPDPE were ineffective in the 55°C hot-plate test. This lack of effect could be attributed to insufficient dose because higher doses could not be tested due to limited solubility. However, Ossipov et al. (1995) also did not observe an effect of 40 nmol of DPDPE administered in a larger volume in the 55°C hot-plate test. Like the tail-flick test, the thermal stimulus in the hot-plate test may have been too intense for detection of the antinoceptive effect of DPDPE.

**Medullary delta-2 opioid receptors modulate nociception.** Another finding of this study was that microinjection of DELT, a delta-2 opioid receptor agonist, in the NRM and NGCp also produced a dose-dependent increase in tail-flick latency, as well as a modest increase in hot-plate latency. The increase in tail-flick and hot-plate latency was antagonized by microinjection of 0.15 nmol of NTB at the same site but was not attenuated by 1.5 pmol of BNTX. The selective antagonism of the antinoceptive effect of DELT by NTB, but not BNTX, is strong evidence that the increase in tail-flick latency is specific and is mediated by a delta-2 opioid receptor. These observations are consistent with previous reports that microinjection of DELT in the VMM increases response latency in the radiant heat and warm-water tail-flick tests, as well as the hot-plate test (Ossipov et al., 1995; Rossi et al., 1994), and that the antinoceptive effect of DELT is antagonized by the nonequilibrium delta-2 opioid receptor antagonist [d-Ala²,Cys³]deltorphin but not the delta-1 opioid receptor antagonist [d-Ala²,Leu⁵,Cys⁶]enkephalin (Ossipov et al., 1995). The doses of DELT that increased tail-flick latency in this study were 10-fold lower than those used in prior reports. The greater potency of DELT is likely due to our use of a less intense stimulus in the tail-flick test. Consistent with this interpretation, microinjection of 20 nmol of DELT in the VMM increased response latency to 47% of the maximum possible effect in the 52°C warm water tail-flick test but was inactive in the 55°C warm water tail-flick test (Ossipov et al., 1995).

Neurons of the VMM are not regulated by a tonically active enkephalinergic input. This study is one of two to systematically examine whether neurons of the VMM receive a tonically active inhibitory enkephalinergic input and the first to specifically assess the contribution of delta opioid receptors. Prior studies in which opioid receptor antagonists were microinjected into the VMM or periaqueductal gray assessed the ability of these agents to reverse the antinoception produced by opioid receptor agonists but did not assess the effects of the antagonists by themselves. Neither BNTX nor NTB altered response latencies in the tail-flick or hot-plate test when microinjected in the VMM at doses that selectively antagonized the antinoceptive effects of DPDPE and DELT. Similarly, microinjection of naltrexone or the selective mu opioid receptor antagonist Cys²,Tyr³,Orn⁵,Pen⁷-amide in the VMM also did not alter tail-flick latency (Roychowdhury and Fields, 1996). These findings suggest that neurons of the NRM and NGCp are not subject to a tonically active, inhibitory enkephalinergic input mediated by delta-1, delta-2 or mu opioid receptors. In this respect, enkephalinergic inputs to the NRM and NGCp differ from GABAergic and noradrenergic inputs to this region, which are tonically active (Drower and Hammond, 1988; Hammond et al., 1980; Heinricher et al., 1991).

**Mechanisms by which delta-1 and delta-2 opioid receptors may produce antinociception.** Immunochemical studies indicate that delta opioid receptor immunoreactivity in the NRM and NGCp is localized to fibers and varicosities and that immunoreactive soma are not present (Arvidsson et al., 1995a; Kalyuzhny et al., 1996). In fact, half of all spinally projecting neurons in the NRM are apposed by varicosities immunoreactive for the delta opioid receptor (Kalyuzhny et al., 1996). These observations suggest that delta opioid receptors are predominantly situated presynaptically in the NRM and NGCp. Because no somal staining for the delta opioid receptor was observed in this region, DELT or DPDPE probably do not produce antinociception by inhibiting the pain-facilitatory pathways that also originate in this region (Gebhart, 1993; Zhuo and Gebhart, 1990, 1991). Rather, microinjection of DELT or DPDPE in the VMM may produce antinociception by presynaptically inhibiting inhibitory inputs to these neurons. Candidate inhibitory inputs include those mediated by GABA, norepinephrine or enkephalin. Definition of the mechanism of action will require additional immunochemical studies of the relationship of delta opioid receptors to immunocytochemically defined inhibitory inputs.
puts in this region, as well as neurochemical studies of the effects of delta opioid receptor agonists on the release of GABA, norepinephrine and enkephalin.

**Selectivity of BNTX and NTB as antagonists of the delta-1 and delta-2 opioid receptor.** Microinjection of 1.5 pmol of BNTX selectively antagonized the antinociception produced by DPDPDE without affecting that produced by DEDLT. Conversely, microinjection of 0.15 nmol of NTB almost completely blocked the antinociception produced by DEDLT without attenuating the antinociception produced by microinjection of DPDPDE. These findings are consistent with the characteristics of the delta-1 and delta-2 subtypes of the delta opioid receptor (Hammond, 1983; Zaki et al., 1996). However, the selectivity of BNTX and NTB is maintained in a limited dose range and the selection of dose requires careful attention. For example, a 10-fold increase in the dose of BNTX to 15 pmol resulted in a complete antagonism of the effects of DPDPDE but also caused a significant, prolonged antagonism of the effects of DEDLT.

**Summary.** The present results provide strong evidence that activation of delta-1, as well as delta-2, opioid receptors in the NRM and NGCpr results in antinociception. The differential antagonism of the antinociceptive effects of DPDPDE and DEDLT by the subtype-selective antagonists BNTX and NTB complements earlier studies of spinal delta opioid receptors and supports the existence of delta-1 and delta-2 opioid receptors at supraspinal, as well as spinal loci. These findings implicate both subtypes of the delta opioid receptor in the supraspinal modulation of nociception. However, neurons of the VMM that modulate nociception do not appear to receive a tonically active, inhibitory enkephalinergic input mediated by either delta-1 or delta-2 opioid receptors.

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**References**


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