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Spinal Delta Opioid Receptor Subtype Activity of 6-monoacetylmorphine in Swiss Webster Mice

JODIE J. RADY,*[1] DANIEL BAEMMERT,* A. E. TAKEMORI,†
P. S. PORTOGHESE‡ AND JAMES M. FUJIMOTO*

*Research Service and Department of Pharmacology, VA Medical Center and Medical College of Wisconsin, Milwaukee, WI
†Medical School and ‡College of Pharmacy, University of Minnesota, Minneapolis, MN

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RADY, J. J., D. BAEMMERT, A. E. TAKEMORI, P. S. PORTOGHESE AND J. M. FUJIMOTO. *Spinal delta opioid receptor subtype activity of 6-monoacetylmorphine in Swiss Webster mice.* PHARMACOL BIOCHEM BEHAV **56**(2) 243–249, 1997.—Heroin and 6-monoacetylmorphine (6MAM) given intracerebroventricularly in Swiss Webster mice, act on supraspinal delta (δ) opioid receptors to produce antinociception in the tail flick test. More specifically, this action of heroin involves δ_1 and 6MAM involves δ_2 opioid receptors. Even though 6MAM given intrathecally (IT) in Swiss Webster mice also activates δ receptors to produce antinociception, the subtype of δ receptor in the spinal cord is not known. The present study addressed this question. First, in order to confirm the subtype selectivity of the δ opioid receptor antagonists in the spinal cord, 7-benzylidenenaltrexone (BNTX, a selective δ_1 receptor antagonist) and naltriben (a selective δ_2 receptor antagonist) were administered IT against the prototypic δ_1 and δ_2 peptide agonists [D-Pen^{2,5}]enkephalin (DPDPE) and [D-Ser^2,Leu^5]enkephalin-Thr (DSLET), respectively. DPDPE-induced antinociception was inhibited by BNTX, but not naltriben. The opposite selectivity occurred for DSLET; naltriben, but not BNTX, administered IT inhibited IT DSLET-induced antinociception. Therefore, the antagonists differentiated between spinal δ_1 and δ_2 opioid receptor subtype agonist actions. This differentiation was further demonstrated by administration of the antagonists IT against the antinociceptive action of β-endorphin given intracerebroventricularly. The antinociceptive action of β-endorphin is due to spinal release of met-enkephalin which results in spinal δ_2 receptor activation. This antinociception was reduced by IT naltriben, but not BNTX, administration. The antagonists were then administered against IT 6MAM-induced antinociception. Neither BNTX nor naltriben given alone, each at twice the usual dose, altered IT 6MAM-induced antinociception. When the antagonists were administered together, each at the usual dose, the antinociceptive action of 6MAM was inhibited. Thus, even though a differentiation between spinal δ_1 and δ_2 opioid receptor activity can be obtained with naltriben and BNTX, blockade of the individual δ receptor subtypes does not appear to alter IT 6MAM antinociception. Therefore, these results suggest that 6MAM, given IT, is acting on a δ opioid receptor but this receptor in the spinal cord appears to be different from the δ_2 receptor on which 6MAM acts in the brain. **Copyright © 1997 Elsevier Science Inc.**

Spinal delta opioid receptor subtype 6-monoacetylmorphine Antinociception Naltriben 7-benzylidenenaltrexone DPDPE DSLET

HEROIN is converted to 6-monoacetylmorphine (6MAM) and subsequently to morphine (42–45). The major pharmacological activity of heroin is ascribed to the 6MAM and morphine (10,38,39,42–45) which act on the mu (μ) opioid receptors (10). Antinociception produced in the tail flick test by these μ agonists in the brain is mediated through descending spinal noradrenergic and serotonergic systems (1,19,22,24,46). An unusual situation arises in Swiss Webster mice where heroin and 6MAM given systemically or intracerebroventricularly (ICV) activate supraspinal delta (δ) opioid receptors and not μ receptors even though morphine acts on μ receptors (19, 22). The δ receptor response in the brain to heroin and 6MAM is further differentiated as to δ opioid receptor subtype activity. Administration of the δ_1 and δ_2 receptor antagonists,

[1] Correspondence should be addressed to: James Fujimoto, Ph.D., VA Medical Center, Research Service-151, Milwaukee, WI 53295; Fax: (414)382-5374.

7-benzylidenenaltrexone (BNTX) and naltriben, respectively, demonstrates that heroin acts on supraspinal δ_1 receptors while 6MAM acts on supraspinal δ_2 receptors (23). Correspondingly, supraspinal heroin-induced antinociception, like that of [D-Pen^{2,5}]enkephalin (DPDPE, a δ_1 receptor agonist), is mediated by activation of spinal $GABA_A$ and $GABA_B$ receptors (8, 20). The action of 6MAM like that of [D-Ser^2,Leu^5]enkephalin-Thr (DSLET, a δ_2 receptor agonist), is mediated by spinal $GABA_A$ but not $GABA_B$ receptors (21). Therefore, not only can heroin and 6MAM action be differentiated from morphine action in Swiss Webster mice, but heroin and 6MAM action can be differentiated from each other as well.

Differentiation between heroin and 6MAM antinociception also occurs after intrathecal (IT) administration in Swiss Webster mice. Heroin given IT acts on spinal μ opioid receptors to produce antinociception, an action inhibited by naloxone (22). In contrast, 6MAM given IT produces antinociception through activation of spinal δ opioid receptors, an action inhibited by naltrindole (a nonspecific δ receptor antagonist), but not naloxone (a μ receptor anatagonist) or norbinaltorphimine (a κ receptor antagonist) (19). From the fact that 6MAM acts on δ_2 receptors in the brain, it would follow that the δ agonist action of 6MAM in the spinal cord would also be on δ_2 receptors. Taking this extrapolation as the working hypothesis, the present study examined the δ receptor subtype involved in the spinal action of 6MAM in Swiss Webster mice.

Subtypes for the δ opioid receptor have been described for the spinal cord (13,25,27) as well as the brain (12,14,17,26, 27). One approach to determination of δ receptor subtypes is based on differential inhibition of δ agonist-induced antinociception by relatively selective δ receptor antagonists. Thus, the agonist action of DPDPE is inhibited by BNTX, a δ_1 opioid receptor selective antagonist, and that of DSLET is inhibited by naltriben, a δ_2 opioid receptor selective antagonist (12,17,18,23,27,32). As a further example, in the spinal cord of mice, naltriben and BNTX have been administered IT to examine the spinal δ receptor subtype involved in the antinociceptive action of β-endorphin given ICV. ICV β-endorphin produces antinociception through a spinal opioid (more specifically δ opioid) mechanism, in ICR mice (24,29,33,35). Further studies demonstrate that met-enkephalin is released into the spinal perfusates of rats after ICV β-endorphin administration (36). Also, in ICR mice a met-enkephalin antibody given IT attenuates ICV β-endorphin-induced antinociception (37). The antinociceptive action of met-enkephalin (in the presence of an enkephalinase inhibitor) in Swiss Webster mice is inhibited by IT naltriben, but not BNTX, indicating δ_2 receptor involvement (30). Furthermore, naltriben administration IT also inhibits the antinociceptive response to ICV β-endorphin while BNTX has no effect in both ICR and Swiss Webster mice (30,35). Therefore, exogenously administered and endogenously released met-enkephalin act on δ_2 receptors. Because the working hypothesis in the present study is that IT 6MAM acts on spinal δ_2 receptors, ICV β-endorphin was also used to activate spinal δ_2 receptors for comparison.

In the present study, naltriben and BNTX were administered IT to determine which δ receptor subtype was involved in the antinociceptive action of IT 6MAM. The δ subtype selectivity of the antagonists (BNTX and naltriben) was characterized using DPDPE and DSLET as the selective agonists (17,32). Therefore, the antagonists were administered with DPDPE and DSLET to confirm the selectivity of these antagonists. As previously published (17,32), BNTX inhibited DPDPE-induced antinociception and naltriben attenuated DSLET-induced antinociception. In contrast to these results, neither antagonist altered the δ receptor mediated antinociceptive action of IT 6MAM. However, administration of both antagonists together inhibited 6MAM-induced antinociception. These results suggest that the spinal δ receptor involved in IT 6MAM-induced antinociception is not the δ_1 receptor involved in IT DPDPE action; nor does it appear to be the same as the δ_2 receptor involved in IT DSLET- or ICV β-endorphin-induced antinociception.

METHODS

Animals and Antinociception

Male Swiss Webster mice weighing 25–40 g (Hilltop Lab Animals, Scottdale, PA) were used for all experiments. Each mouse was only used once. All studies were performed in compliance with the Institutional Animal Care and Use Committee (Animal Studies Subcommittee).

The radiant heat tail flick test (4) was used to measure antinociception. Control tail flick latencies were 2–4 sec and a 10 sec cutoff time was used to prevent tail damage. The change in tail flick latencies were converted to percent maximum possible effect (% MPE) according to the following formula (5):

$$\% \text{ MPE} = \frac{(\text{post drug latency} - \text{pre drug latency})100}{(10 - \text{pre drug latency})}.$$

Significant differences for control data, cross-selectivity of the antagonists for μ and κ receptor action, and effect of the 1 pmol and 25 pmol doses of BNTX and naltriben, respectively, on 6MAM antinociception were determined using analysis of variance (ANOVA) followed by Newman-Keuls' test with $P < 0.05$ indicating significance (28).

Dose-Response Relationships

To construct dose-response curves, opioid agonists were administered IT (except for β-endorphin which was given ICV) at varying doses, to groups of 8–10 mice, along with IT saline or opioid receptor antagonists. The IT injections of 5 μl were made by the method of Hylden and Wilcox (9). The antagonists were given IT 10 min before the tail flick test. 6MAM and DSLET were given IT 5 min and DPDPE 15 min before the tail flick test. β-endorphin was given ICV in a 4 μl volume 10 min before the tail flick test (7) under light halothane anesthesia. The route and time of administration are given with each experiment and were obtained from previous publications (17,19,23,27,30). The mean % MPE (± SEM) was calculated and was plotted (on a probit scale) against the agonist dose (on a log scale) on semilog-probit paper. The method of Litchfield and Wilcoxon (as described by Dewey et al. (5)) was used to analyze these data. Briefly, straight lines were fitted to the data by eye, the χ^2 goodness of fit test determined that the line fit the data, the slopes and ED_{50} values (95% confidence interval) were determined. If the slopes were not significantly different (indicating that the lines were parallel) then the ED_{50} values were compared for significant differences using the potency ratio.

Drug Sources

The antagonists (BNTX and naltriben) were synthesized as previously described (17,18). 6MAM (free base) was obtained from the National Institute on Drug Abuse (Rockville, MD). Peptides were purchased from commercial sources: β-endorphin and DSLET (Peninsula Laboratories, Belmont, CA),

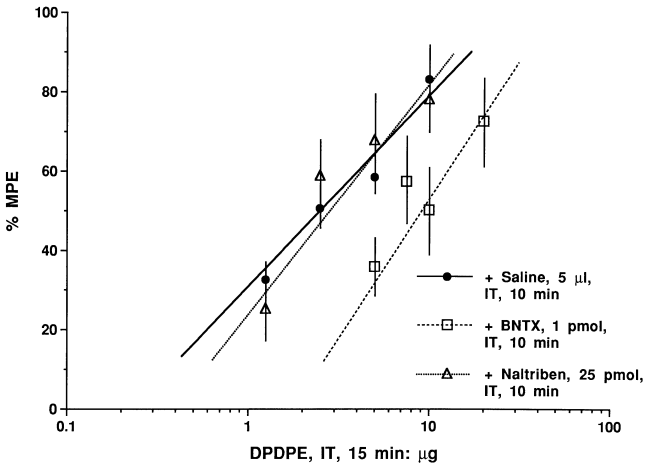

FIG. 1. Dose response curves for DPDPE, given IT 15 min before the tail flick test, in the presence of saline or the antagonists. In the presence of IT saline (5 μl, 10 min before the tail flick test) DPDPE produced dose dependent antinociception (●). This antinociceptive effect was decreased by administration of BNTX (1 pmol, IT 10 min before the tail flick test) as indicated by a rightward shift in the dose response curve (□). Administration of naltriben (25 pmol, IT 10 min before the tail flick test) did not produce a shift in the dose response curve for DPDPE antinociception (△). In this and subsequent figures each point and vertical line represents the mean % MPE and SEM, respectively, for a group of 8 to 10 mice given the agonist at the plotted dose.

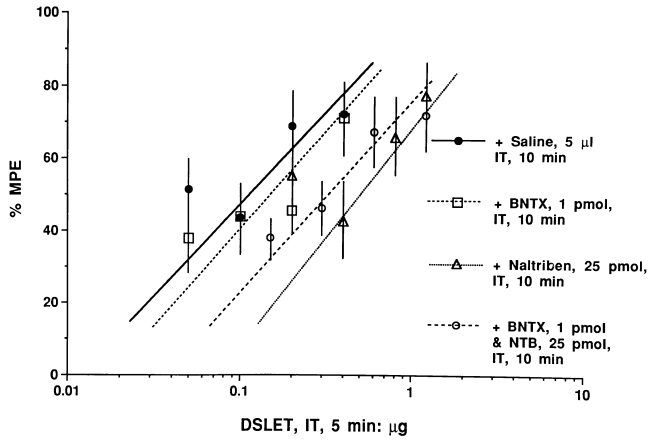

FIG. 2. Dose response curves for DSLET, given IT 5 min before the tail flick test, in the presence of saline or the antagonists. Administration of DSLET with saline (5 μl, 10 min before the tail flick test) produced a dose dependent antinociceptive response (●). Unlike for DPDPE, administration of IT BNTX (1 pmol, 10 min before the tail flick test) did not shift the DSLET dose response curve (□). Naltriben administration IT at 25 pmol 10 min before the tail flick test, on the other hand, produced a rightward shift of the dose response curve for DSLET (△). A further shift did not occur when the antagonists (naltriben and BNTX) were administered together (at the usual doses) against DSLET (○).

DPDPE (Sigma, St. Louis, MO), [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]-enkephalin, DAMGO (BACHEM, Torrance, CA) and trans-(+-3,4-dichloro-*N*-Methyl-*N*-(2-(1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide methanesulfonate, U50,488H (UpJohn, Kalamazoo, MI). Peptides were dissolved in 0.1% Triton-X-100 solution in 0.9% saline. The antagonists and 6MAM were dissolved in 0.9% saline. For 6MAM, a few drops of 0.1M hydrochloric acid and slight heating was needed to aid in dissolving the free base.

RESULTS

Confirmation of the δ Receptor Subtype Selectivity of BNTX and Naltriben

Dose response curves for DPDPE, given IT 15 min before the tail flick test, with saline or antagonists, given IT 10 min before the tail flick test, are presented in Fig. 1. IT DPDPE given along with IT saline produced dose dependent antinociception (●). Administration of BNTX (1 pmol) attenuated the antinociceptive response to DPDPE (□). The dose response curve for DPDPE shifted to the right in a parallel manner about fourfold; the ED_{50} value (95% confidence limit) changed from 2.5 (1.2–5.3) μg to 8.9 (5.0–15.8) μg. As expected, the dose response curve for DPDPE was not altered by naltriben (25 pmol) administration (△); the ED_{50} value was 2.9 (1.6–5.4) μg.

IT naltriben and BNTX also differentially inhibited IT DSLET-induced antinociception (Fig. 2). Administration of DSLET, IT at 5 min along with saline at 10 min before the tail flick test resulted in a dose dependent antinociceptive response (●); the ED_{50} value was 0.11 (0.06–0.23) μg. When BNTX (1 pmol) was administered IT 10 min before the tail flick test, no shift in the DSLET dose response curve occurred; the ED_{50} value was 0.15 (0.08–0.29) μg (□). On the other hand, the ED_{50} value for DSLET in the presence of naltriben (25 pmol, 10 min before the tail flick test) was 0.50 (0.29–0.87) μg which represented a fourfold, parallel rightward shift in the DSLET dose response curve (△). This study went one step further than previous studies. Another dose response study was performed for DSLET in the presence of the antagonists given together, that is both naltriben and BNTX were administered at the same time (○). The ED_{50} value for DSLET in this paradigm was 0.32 (0.16–0.62) μg. This represents a threefold, rightward parallel shift compared to the DSLET + saline curve indicating that the combination of antagonists still resulted in antagonism of DSLET antinociception. However, this dose response line was not significantly different from the curve for DSLET when naltriben was given alone. Thus, the combination of the antagonists did not produce greater antagonism than the naltriben alone.

The results in Table 1 demonstrate that BNTX and naltriben given alone or in combination did not alter the antinociceptive activity of the μ and κ receptor selective agents DAMGO and U50,488H. This along with the above data demonstrates that the antagonists have selectivity for the δ, but not μ or κ, opioid receptors. Administration of saline, saline with Triton X-100 or the combination of the antagonists resulted in a mean %MPE (SEM) of 2.0 (2.1), 1.1 (3.2) and 13.7 (6.6), respectively. No siginificant differences were found between these groups using ANOVA; $P > 0.05$.

Spinal δ Receptor Subtype for IT 6MAM-Induced Antinociception

Administration of 1 pmol of BNTX or 25 pmol of naltriben IT did not alter the antinociceptive action of 6MAM. The mean %MPE (SEM) for 6MAM given in the presence of saline was 85.7 (6.0). When the BNTX or the naltriben was given with the 6MAM, the mean %MPE was 64.7 (10.6) and

TABLE 1

LACK OF EFFECT OF BNTX AND NALTRIBEN, GIVEN IT, ON THE ANTINOCICEPTIVE ACTION OF IT DAMGO AND U50,488H

Antagonist Treatment	% MPE (SEM)
Agonist: DAMGO, 10 ng, IT, 15 min	
+Saline, 5 μl, IT, 10 min	78.9 (7.6)
+BNTX, 2 pmol, IT, 10 min	69.7 (7.4)
+Naltriben, 50 pmol, IT, 10 min	80.8 (6.6)
+BNTX, 1 pmol + Naltriben, 25 pmol, IT, 10 min	73.2 (6.0)
Agonist: U50, 488H, 30 μg, IT, 10′	
None	83.5 (7.7)
+BNTX, 2 pmol, IT, 10 min	79.0 (6.0)
+Naltriben, 50 pmol, IT, 10 min	72.0 (7.2)
+BNTX, 1 pmol + Naltriben, 25 pmol, IT, 10 min	77.7 (6.8)

66.7 (8.3), respectively. No significant differences were found between these data using ANOVA; $P > 0.05$. Next, the doses of the antagonists were doubled to rule out the possibility of lack of antagonism due to the antagonist dose being too low.

Dose response curves for 6MAM given in the presence of saline or the antagonists are given in Fig. 3. IT administration of 6MAM at 5 min along with IT saline treatment at 10 min before the tail flick test produced a dose dependent antinociceptive response (●); the ED_{50} value was 0.79 (0.36–1.7) μg. IT administration of BNTX (2 pmol; □) or naltriben (50 pmol; △) IT, 10 min before the tail flick test, did not alter the 6MAM dose response curve; the ED_{50} values were 0.63 (0.29–1.4) μg and 0.95 (0.40–2.2) μg, respectively. The antinociceptive action of IT 6MAM, unlike that of the other agonists, was not inhibited by either antagonist given alone. Yet, it was shown in a previous publication (19) that naltrindole, a subtype non-selective δ receptor antagonist, inhibits 6MAM action. Because naltrindole inhibits both δ_1 and δ_2 receptors, BNTX and naltriben were administered together IT in the next experiment. Together, the antagonists, given at one half the dose of each given alone, produced a parallel rightward shift in the 6MAM dose response curve (○). The ED_{50} value was changed to 4.0 (2.6–6.0) μg which represented a fivefold shift in the dose response curve.

Spinal δ Receptor Subtype Activity for ICV β-endorphin-Induced Antinociception

The dose response curves for ICV β-endorphin given with IT saline or the antagonists are given in Fig. 4. Administration of β-endorphin ICV at 10 min along with IT saline at 10 min before the tail flick test produced dose dependent antinocicep-

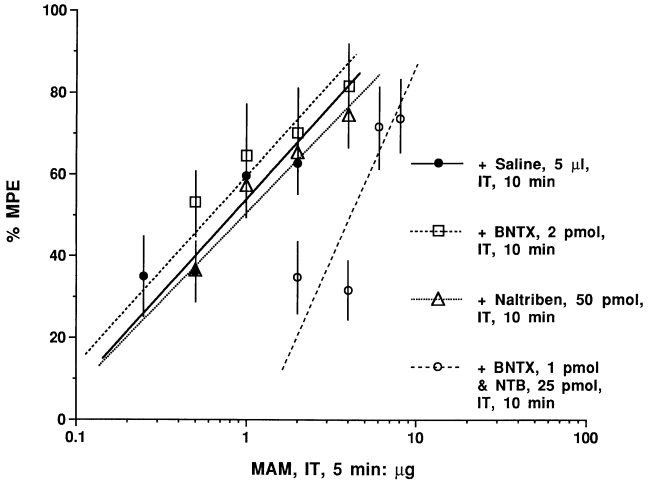

FIG. 3. Dose response curves for 6MAM, given IT 5 min before the tail flick test, in the presence of saline or the antagonists. Like the other agonists, 6MAM administration with saline (5 μl, 10 min before the tail flick test) resulted in dose dependent antinociception (●). However, unlike the other agonists, no shift in the 6MAM dose response curve was observed when BNTX (2 pmol) or naltriben (50 pmol) were administered 10 min before the tail flick test (□ and △, respectively). Coadministration of BNTX and naltriben (NTB) at doses of 1 pmol and 25 pmol, respectively, given 10 min before the tail flick test produced a rightward shift of the 6MAM dose response curve (○).

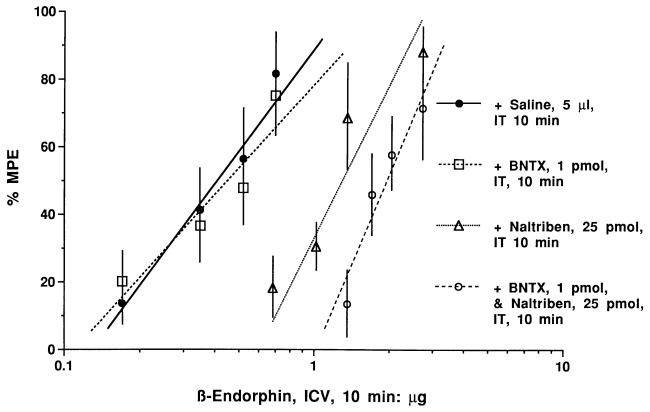

FIG. 4. Dose response curves for β-endorphin, given ICV 10 min before the tail flick test, in the presence of saline or the antagonists given IT. Administration of β-endorphin ICV produced antinociception in a dose dependent manner when saline was given IT, 10 min before the tail flick test (●). As with DSLET, administration of BNTX (1 pmol) IT, 10 min before the tail flick test, did not produce a shift of the β-endorphin dose response curve (□). Naltriben (25 pmol) given IT, 10 min before the tail flick test, caused a rightward shift of the dose response curve for β-endorphin (△). Also depicted is the dose response curve for ICV β-endorphin when naltriben (NTB) and BNTX were coadministered IT (○). Coadministration of the antagonists resulted in a further shift of the β-endorphin dose response curve.

tion (●). This antinociceptive effect was inhibited by administration of naltriben IT (25 pmol, 10 min before the tail flick test), as indicated by the threefold, parallel rightward shift in the β-endorphin dose response curve (△). The ED_{50} value shifted from 0.42 (0.27–0.63) μg to 1.3 (0.9–1.8) μg. Administration of BNTX (1 pmol, 10 min before the tail flick test) IT produced no alteration in the dose response curve for β-endorphin (□); the ED_{50} value was 0.44 (0.28–0.63) μg. These results suggest that the antinociception produced by ICV β-endorphin involved spinal δ_2 receptor activation, as previously reported (30,35). A further experiment examined the effect of administration of the two antagonists together on ICV β-endorphin-induced antinociception (○). In this experiment, the dose response curve for β-endorphin was shifted in a parallel manner to the right even further than with the naltriben alone; the ED_{50} value was 2.1 (1.7–2.6) μg. This represents a fivefold and twofold shift in the β-endorphin dose response curves compared to that in the presence of saline and naltriben, respectively.

DISCUSSION

Both heroin and 6MAM (a metabolite of heroin) produce antinociception through activation of supraspinal δ receptors after ICV administration in Swiss Webster mice (19,22). However, heroin acts on supraspinal δ_1 receptors while 6MAM acts on supraspinal δ_2 receptors (20,21,23). Receptor differences for heroin and 6MAM antinociception also occur in the spinal cord (19,22). Heroin antinociception is inhibited by blockade of μ receptors and 6MAM antinociception is attenuated by blockade of δ receptors. Because 6MAM produces antinociception through activation of supraspinal and spinal δ receptors it might be anticipated that the specific subtype to be the same at both sites.

The results from the present study for the activity of the antagonists against DPDPE and DSLET confirmed the reported selectivities for naltriben and BNTX (17,23,27,32). BNTX administration antagonized the antinociception produced by DPDPE, but not DSLET, and naltriben administration attenuated the antinociceptive activity of DSLET without altering that of DPDPE. Furthermore, ICV β-endorphin antinociception was inhibited by IT administration of naltriben, but not BNTX. This result confirms the involvement of spinal δ_2 receptors that has been reported for ICV β-endorphin-induced antinociception (30,35). These results demonstrate that BNTX and naltriben can be used to differentiate between the activity of the δ receptor subtypes. Furthermore, administration of the antagonists did not alter μ (DAMGO) or κ (U50,488H) agonist- induced antinociception (Table 1) confirming selectivity of the antagonists for the δ receptor (17,32). Therefore, the antagonists were administered against IT 6MAM to determine which δ receptor subtype was involved in spinal 6MAM antinociception in Swiss Webster mice.

Despite the ability of the antagonists to inhhibit the prototypic δ subtype agonists (DPDPE and DSLET), they had no effect on 6MAM-induced antinociception even when the doses of the antagonists were doubled. That the antagonists had no effect was somewhat puzzling because these antagonists were able to differentiate the δ_2 action of ICV 6MAM from the δ_1 action of ICV heroin (23) as well as the δ subtype activity of DPDPE and DSLET in the spinal cord (17,27,32 and in the experiments for this study). Also, as mentioned above, one would expect the δ receptor activity of 6MAM in the brain and spinal cord to involve the same δ subtype. However, leu- and met-enkephalin act on δ_1 receptors in the brain of mice but act on δ_2 receptors in the spinal cord (30). Therefore, it is possible that the subtype of a receptor that an agonist acts on may be different between the brain and the spinal cord. A further example of a difference between brain and spinal receptor subtype activity is seen for morphine with μ receptors. In CXBK and CD-1 mice, morphine interacts with μ receptors in the brain but μ_2 receptors in the spinal cord (15). However, in the case of 6MAM neither of the known δ subtypes appear to be involved selectively in the spinal antinociceptive action.

Consistent with δ receptor involvement, the administration of BNTX and naltriben together reduced the antinociceptive activity of 6MAM. This suggests that both of the receptors needed to be inhibited in order to decrease 6MAM antinociception. Coadministration of the antagonists also resulted in a greater shift in the ICV β-endorphin dose response curve than occurred for naltriben given alone. These results could suggest the possibility of some type of coupling or cooperativity occurring between the two receptor subtypes. However, β-endorphin-induced antinociception was antagonized by administration of naltriben alone which suggests more of a potentiative effect of BNTX on naltriben activity. Potentiation of other receptor-induced activity by possible cooperativity between δ receptor subtypes has been demonstrated. DPDPE, a δ_1 receptor agonist, potentiates the antinociceptive action of morphine through a δ_2 receptor mediated effect (11,16). A further note here is that combined antagonist treatment against DSLET did not yield similar results. Coadministration of the BNTX did not produce greater antagonism than the naltriben alone. It must be noted that DSLET is reported to have some activity at the μ receptor (3,6). Therefore, blocking the δ receptor may shift the action of DSLET to μ receptor interaction. From examination of Fig. 3, it appears that the slope of the 6MAM dose-response curve in the presence of the two antagonists together is slightly (but not significantly) steeper than the others. This suggests that the effect of lower doses of 6MAM were antagonized to a greater extent than the higher doses. This could be due to the higher doses recruiting another receptor type. 6MAM is known to interact with spinal μ receptors in ICR mice (19). Regardless of the mechanism for the slight, but insignificant, change in slope, administration of the combined antagonists does significantly antagonize the antinociceptive activity of 6MAM, but not DSLET.

Another possibility is that there is a distinct difference between the δ receptors in the brain and those in the spinal cord. Studies with antisense oligodeoxynucleotides to the δ receptor suggest differences between the two sites. The antisense differentiates between δ_1 and δ_2 activity in the brain; δ_2 receptor-induced antinociception is reduced by the antisense oligodeoxynucleotide while δ_1 activity is not (2). However, in the spinal cord the activities of both subtypes are affected by the antisense oligodeoxynucleotide (2,34). Preliminary studies performed in this laboratory confirm that the activities of both δ receptor agonists in the spinal cord are inhibited by an antisense oligodeoxynucleotide to the δ receptor (unpublished data). This same antisense oligodeoxynucleotide, like the subtype specific antagonists, has no effect on IT 6MAM antinociception (unpublished data). Therefore, it may be more difficult to differentiate δ_1 and δ_2 receptor activity in the spinal cord. This latter explanation seems somewhat unlikely because the spinal subtype activity involved in IT DPDPE, IT DSLET and ICV β-endorphin have been clearly demonstrated in the past (27,30,32,35) and in the present study.

Another possibility is that there is a difference between the activity of peptide agonists and non peptide agonists. Studies

in the literature provide support for such a difference. In the guinea pig ileum β-funaltrexamine treatment differentiates between peptide and non peptide μ receptor agonists (31,41). Treatment of a neuroblastoma-glioma hybrid cell line (NG108-15) with opioid agonists alters the binding of peptide, but not opiate alkaloid, agonists to δ receptors (40). Therefore, it is possible that lack of antagonism of 6MAM antinociception by BNTX and naltriben given separately is due to 6MAM being a non peptide agonist. However, in the present study ICV β-endorphin antinociception caused by release of spinal met-enkephalin (a peptide) was inhibited by naltriben alone and it was antagonized further when BNTX was given with naltriben. Therefore, it is unlikely that a difference between peptide and non peptide agonist action is responsible for the 6MAM results.

Regardless of the mechanism, the results demonstrate that IT 6MAM antinociception does not involve the same spinal δ receptors as does IT DPDPE and IT DSLET. The antinociceptive actions of all of these agents as well as ICV β-endorphin are inhibited by the nonspecific δ receptor antagonist naltrindole given IT. Until now the δ agonists have fallen into two subtypes, δ_1 and δ_2, that could be distinguished by BNTX and naltriben, respectively. Even though the antagonism of ICV β-endorphin-induced antinociception is greater when the antagonists are combined, antagonism occurred with naltriben given alone. Thus, 6MAM appears to be different from β-endorphin as well. 6MAM action does not seem to fit with either of the δ receptor subtypes. Further studies are needed to clarify the differences and to determine the exact mechanism involved in the action of the antagonists given together.

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