Pharmacology Biochemistry & Behavior, Vol. 38, pp. 185–190. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Delta Opioid Antagonist, Naltrindole, Selectively Blocks Analgesia Induced by DPDPE But Not DAGO or Morphine

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Received 16 May 1990

CALCAGNETTI, D. J. AND S. G. HOLTZMAN. *Delta opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine.* PHARMACOL BIOCHEM BEHAV **38**(1) 185–190, 1991.—Initial reports suggest that naltrindole hydrochloride (NTI), a recently developed opioid, acts as a selective delta (δ) antagonist in vivo. Three experiments were conducted in rats to test NTI for its ability to dose-dependently and selectively block the analgesia produced by a δ-selective opioid agonist without affecting analgesia produced by mu (μ) receptor opioid agonists. Intracerebroventricular (ICV) administration of the δ-selective agonist, DPDPE (30 μg/rat), and the μ-selective agonist, DAGO (0.3 μg/rat), increased paw-lick latency (2-fold relative to baseline) in the hot-plate assay. NTI (0.01–1.0 μg/rat, ICV) dose-dependently attenuated DPDPE-induced analgesia (1.0 μg reduced paw-lick latency to baseline), but failed to affect DAGO-induced analgesia at any dose tested. A third experiment determined whether the ICV administration of NTI (1.0 μg/rat) would attenuate restraint stress-induced potentiation of morphine analgesia as indexed by the tail-flick assay. Rats that underwent 5 days of 1 h restraint stress and nonstressed rats were injected subcutaneously with morphine (1.0–8.0 mg/kg). The magnitude (>2-fold) and duration of morphine-induced analgesia in restrained rats were significantly potentiated compared to nonstressed rats. NTI (1 μg, ICV) failed to affect the magnitude and duration of morphine-induced analgesia regardless of restraint treatment. Thus, NTI failed to attenuate the analgesia produced by DAGO or morphine (in two assays of antinociception), whereas NTI (0.01–1.0 μg, ICV) antagonized dose-dependently DPDPE-induced analgesia. These results support the view that NTI is a selective δ-receptor antagonist in vivo.

Opioid antagonist Intracerebroventricular Delta and mu opioid receptors Naltrindole DPDPE DAGO Morphine Restraint Stress Hot-plate Analgesia Tail-flick

CHARACTERIZATION of the functional roles of delta (δ) opioid receptors in vivo has proceeded at a much slower rate than the characterization of mu (μ) and kappa (κ) opioid receptors. This situation is due, at least in part, to the lack of highly selective, nonneurotoxic and cost-effective δ receptor antagonists that are capable of penetrating the blood-brain barrier. Recently, it has been suggested that the presently available δ antagonist, ICI-174864 (8), is less than ideal for in vivo testing because it lacks the aforementioned qualities (7,23).

Portoghese, Takemori and colleagues have been instrumental in designing selective opioid ligands based upon the "message-address" concept (28). Their approach has led to the synthesis and development of naltrindole hydrochloride (17-cyclopropylmethyl-6,7-dehydro-4,5-epoxy-3,14-dihydroxy-6,7,2′,3′-indolomorphinan), a nonpeptide, δ-receptor antagonist (28). In vitro evidence from their laboratory demonstrated that naltrindole (NTI, Ke = 0.1–0.3 nM range) potently antagonized δ-selective agonists, but not μ- or κ-selective agonists in guinea pig ileum, mouse vas deferens and rat vas deferens smooth muscle preparations (27). Furthermore, in mice, the subcutaneous administration of NTI (20 mg/kg) selectively antagonized the analgesia produced by a δ-receptor preferring agonist, (D-Ser^2, Leu^5, Thr^6) enkephalin (DSLET), but not analgesia produced by the κ agonist U50488H (31) or morphine (27). These data indicate that NTI can penetrate the blood-brain barrier.

Evidence from two reports is consistent with the initial findings that NTI selectively antagonize the effects of δ agonists and suggests that NTI also antagonizes analgesia produced by endogenous (presumably enkephalin-mediated) mechanism(s) that may involve δ receptors. When young rats (45–50 days old) are made to swim for 3 min, a form of stress-induced analgesia is produced that can be assessed using the hot water (55°C) tail-flick as an endpoint (21,22). This swim-stress-induced analgesia has been reported to involve δ-receptors based on the finding that it blocked by ICI-174864 (10.0 mg/kg) (21). NTI (1.0 mg/kg) was also attenuated swim-stress-induced analgesia in a dose-related manner in rats and did so at doses that did not alter baseline pain sensitivity (22). Moreover, the intrathecal (IT) administration of NTI (0.01–1.0 μg) in rats blocked dose-dependently the analgesia produced by the IT administration of the highly selective δ-re-

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ceptor agonist, (D-Pen^2, D-Pen^5) enkephalin, (DPDPE), but had no effect on morphine-induced (1 μg, IT) analgesia as indexed by the radiant heat tail-flick assay (11). NTI (1.0 μg) completely blocked DPDPE-induced analgesia within 30 min. Nonspecific effects of NTI (IT) were not reported, even at a dose of 30 μg (11).

The activation of either spinally or supraspinally located δ-receptors, by administration of DPDPE, produces analgesia (11,19). However, in the rat, observable analgesia is dependent upon the route of administration and the antinociceptive assay employed. For example, the intracerebroventricular (ICV) administration of DPDPE produces observable analgesia in the formalin test (5) and hot-plate assay (3); however, the ICV administration of DPDPE (30–100 μg/rat) fails to produce analgesia in the tail-flick assay (4,16). Thus it appears that the hot-plate assay is more suitable than the tail-flick assay for assessing the role δ-receptors in analgesia resulting from the ICV administration of DPDPE.

The objectives of the present experiments were two-fold: 1) to demonstrate further the selectivity of NTI (upon ICV administration) by testing its ability to antagonize analgesia produced by DPDPE and by the μ-selective agonist, (D-Ala^2, N-Me-Phe^4, Gly^5-ol) enkephalin (DAGO) (15) in the hot-plate assay and 2) to explore the possible contribution of δ-receptors in restraint stress-induced potentiation of morphine analgesia. The magnitude and duration of DPDPE- and morphine-induced analgesia are potentiated to about the same degree (1.5–2-fold) in restraint-stressed rats (4,6). Thus the contribution of δ-receptors to restraint-induced potentiation of opioid analgesia remains unclear. In mice morphine has been reported to produce analgesia by activation of δ-receptors (17, 20, 30), which may be part of the hypothesized "μ-δ receptor complex" [see (18) for review]. We therefore sought to employ NTI to determine whether δ-receptors were necessary for and/or contributed to morphine analgesia and the expression of restraint-induced potentiation of morphine analgesia.

METHOD

Subjects

Adult male rats (weighing 260–300 g upon arrival) of Sprague-Dawley descent (SASCO/King, Omaha, NE) served as subjects. Subjects were maintained in a colony room (temperature ~22–24°C) and individually housed in hanging stainless steel cages where they had ad lib access to food (Purina rodent lab chow No. 5001) and tap water. Their body weights ranged from 320–465 g over the time of testing. Testing took place in a room separate from the colony and was conducted during the latter half of the light cycle of the animals, between 1300–1600 h (lights on from 0700–1900 h).

Surgery

All rats were anesthetized with 100 mg/kg of ketamine hydrochloride. Under aseptic conditions, a stainless steel outer cannula guide (22 gauge, Plastics One, Roanoke, VA) was stereotaxically (Kopf) implanted into the right lateral ventricle with the skull leveled between lambda and bregma landmarks (coordinates used were 0.5 mm posterior to bregma, 1.5 mm lateral to midline, and 3.2 mm ventral to the surface of the cortex) (26).

Drugs and Injection

Naltrindole hydrochloride (purchased from Research Biochemicals Inc., Natick, MA), DPDPE and DAGO (purchased from Bachem Inc., Torrance, CA) were all dissolved in distilled, sterile water. Sterile, distilled water also served as the vehicle (VEH) control injection. The peptides were prepared fresh on the day of testing. Morphine sulfate (Penick Corp., Newark, NJ) was dissolved in 0.9% saline which also served as the control vehicle. All doses are expressed as the free base.

Prior to drug testing, cannula patency was verified behaviorally by measuring water intake after ICV injection of angiotensin II (10 ng/5 μl), a potent dipsogen (1,2). Data from animals that failed to drink at least 5 ml of water in 10 min after angiotensin II injection were excluded from testing.

Intraventricular injections were performed by backloading the drug into a 28-gauge internal cannula (Plastics One) via a 18 cm length of PE-20 tubing (Intramedic, No. 7406). The internal cannula tip was ground to a point and extended 0.5 mm beyond the guide cannula. A 25 μl Hamilton syringe mounted in a hand-held repeating dispenser was used to deliver a 5 μl injection volume at a rate of 1 μl/5 s. The inner cannula was allowed to remain in place for at least 10 s after the drug injection to allow for complete drug delivery and equalization of pressure. During infusion each subject was gently held by hand. After every injection, the internal cannula was checked for possible occlusion. Visual inspection for positive flow assured drug delivery throughout the injection procedures.

General Procedure

Subjects were allowed to recover for 10–21 days following surgery prior to testing for cannula patency with angiotensin II. During recovery, all subjects underwent periodic handling, habituation to transport, and habituation to the testing room (at least 4 days). After recovery from surgery, two groups of naive rats served as subjects. Group 1 underwent 3 test days [2 tests with DPDPE (including a replication) followed by a final test with DAGO]. Drug treatment and testing was conducted once a week over three consecutive weeks to minimize the development of tolerance and allow adequate drug clearance. Additionally, subjects were randomized among treatment groups after each experiment. Rats in Group 2 underwent testing with morphine twice a week.

DPDPE and DAGO: Hot-Plate Analgesic Testing

Analgesic testing was conducted on a hot-plate (Technilab Inst., Pequannock, NJ) set at 55°C. The surface of the hot-plate measured 26.5 × 28 cm and was surrounded by Plexiglas walls 30 cm high. Subjects were randomly assigned to one of four treatment groups and underwent 3 predrug baseline trials on the hot-plate spaced 5–10 min apart. Each rat was placed on the hot-plate and the latency to lick a rear paw was recorded with a stopwatch. If this behavior was not observed within 30 s the subject was removed from the hot-plate, given a score of 30 for its paw-lick latency and returned to its home cage. At no time did we observe jumping by rats exposed to the hot-plate and therefore jumping was not measured as an endpoint. The average of the last two trials served as the baseline predrug paw-lick latency.

Ten min after 3 baseline trials on the hot-plate, the drug injection schedule started with a single ICV injection of VEH or NTI (0.01–1.0 μg/rat). After 10 min, all subjects received their second ICV injection consisting of agonist (DPDPE 30 μg/rat). Ten min following the injection of agonist (thus 20 min after NTI), they were placed on the hot-plate for 3 trials (at 10, 20 and 40 min after agonist injection). Thus dose- and time-response curves were generated.

The doses of NTI pretreatment interval were chosen on the basis of published reports (11,22). The same subjects were used in Experiments 1 and 2. The order of weekly testing was: Experiment 1 and its replication using DPDPE, and Experiment 2 wherein an equianalgesic dose (4) of DAGO (0.3 μg) was substituted for DPDPE as the agonist. This standard design conformed to a 2×2 factorial design with 4 levels of dose and 3 postdrug testing trials with repeated measures on trials.

Restraint Stress and Tail-Flick Analgesic Testing

In Experiment 3, naive subjects were assigned to one of two groups according to treatment conditions: 1) restraint stressed and 2) nonstressed. Stressed rats were habituated to 1 h of restraint at 1300 h for 5 consecutive days. Restraint was accomplished by immobilizing each subject in a well-ventilated Plexiglas cylinder measuring 5–6 cm in diameter. The cylinder was sealed with a No. 12 rubber stopper (to which a wedge-shaped slice had been removed) such that the tail was left exposed and freely mobile. Day 6 served as the start of the drug regimen and testing. Drug doses were randomly administered over the 4 test days. Stressed rats were weighed and fitted into their cylinders and remained there as specified during testing. All subjects underwent 3 predrug tail-flick baseline trials conducted at 10–15 min intervals. Scores from the third trial served as the baseline measure for each subject.

Upon the conclusion of baseline trials, each subject received an ICV injection of NTI (VEH or 1.0 μg/rat) followed immediately by a subcutaneous (SC) injection of saline or one of 3 doses of morphine (1.0, 2.0, 4.0 and 8.0 mg/kg), depending upon restraint treatment. Stressed rats received a 1.0 mg/kg dose of morphine instead of the 8.0 mg/kg dose (given to nonstressed rats) because doses greater than 4.0 mg/kg often result in fatalities due to respiratory depression. Stressed subjects were removed for approximately 2 min, given their ICV injection and returned to the cylinder. Nonstressed rats were merely injected ICV and returned to their home cage. Tail-flick latencies were recorded 15, 30, 45, 60, 90 and 120 min after morphine injection. This experiment conformed to a design with 2 levels of restraint treatment (stressed and nonstressed), 2 levels of antagonist treatment (NTI 1 μg or VEH) and 4 levels of dose (morphine) with repeated measures on the time of testing after morphine injection.

We employed the tail-flick assay described by D'Amour and Smith (10), with modifications (14). Briefly, nonstressed subjects were loosely wrapped in a towel for testing, whereas stressed subjects remained in their restraint cylinder allowing for each tail to be positioned along a "v"-shaped copper groove housing a light sensitive sensor. A beam of radiant heat (from a 20-V, high amperage 100-watt bulb situated 18 cm above the tail) was aimed at the dorsal portion of each subjects' tail (about 5 cm from the tip). A device automatically recorded (in 10ths of a s) the latency between the onset of the beam and the tail-flick, at which point the beam was terminated. The maximum duration of each test was set at 6.0 s to minimize tissue damage. The stimulus intensity was set so that baseline tail-flick latencies were approximately 2.0 s. Subjects failing to respond within 3.0 s were excluded from testing.

Data Treatment and Statistical Analysis

Statistical analyses were performed on the dose-response curves by analysis of variance (ANOVA) and, where appropriate, were followed by Newman-Keuls' test (29). The time-course of tail-flick latency was expressed as the mean percent maximum possible effect (% MPE) according to the following formula:

$$\% \text{ MPE} = \frac{\text{drug latency} - \text{predrug latency}}{\text{6 sec (cut-off time)} - \text{predrug latency}} \times 100.$$

Dose-effect curves for the tail-flick assay were derived by computing the area under the corresponding 15–120-min time-course-% MPE curves; areas were calculated using the trapezoidal rule (29). The level of statistical significance was set at $p<0.05$. The effective dose that produced 50% of the maximum response (ED_{50}) was calculated by simple linear regression of the area of analgesia values per each subject and averaged.

RESULTS

Analgesic Effects of DPDPE and DAGO

ANOVA of the predrug baseline paw-lick latencies failed to reveal significant between group differences for DPDPE-induced analgesia and its replication, $F(7,55)=1.8$, $p=0.1$. Given that the baseline scores of the DPDPE plus NTI experiment and its replication also did not differ, these results were pooled for further statistical consideration and are collectively referred to as Experiment 1.

Figure 1 (top panel) depicts the time-course of analgesia (mean and SEM) of rats pretreated (10 min) with one of four doses of NTI (VEH, 0.01, 0.1 and 1.0 μg/rat, n = 15–16 per dose) followed by an ICV injection of agonist (DPDPE 30 μg/rat) over three trials on the hot-plate (10, 20 and 40 min after ICV administration of DPDPE).

Two-factor ANOVA of postdrug paw-lick latencies yielded a significant main effect for dose, $F(3,59)=7.3$, $p<0.003$, as well as a significant main effect for time of testing, $F(3,59)=28.2$, $p<0.001$. The dose by trial interaction was also significant, $F(9,177)=6.4$, $p<0.001$. Subsequent time-course analyses (Newman-Keuls test) revealed that the paw-lick latency scores from both VEH and 0.01 μg groups were significantly ($p<0.05$) different from baseline at 10 and 20 min after DPDPE. Paw-lick latencies of the VEH-treated group remained significantly different from baseline even at the 40-min trial. Newman-Keuls analyses of latencies from rats receiving the 0.1 and 1.0 μg doses of NTI failed to reveal significant differences from baseline at any time of testing.

These results demonstrate that the ICV administration of DPDPE (30 μg) reliably elevated response latency (2-fold above baseline) for at least 20 min in rats receiving VEH and 0.01 μg dose of NTI. Rats receiving the 0.1 and 1.0 μg doses of NTI displayed paw-lick response latencies similar to that of baseline, thus demonstrating that NTI blocked dose-dependently DPDPE-induced analgesia.

In Experiment 2, DAGO and NTI (0.01–1.0 μg) were tested using the same method as in Experiment 1 (see Fig. 1, bottom panel). ANOVA of baseline paw-lick scores failed to reveal reliable differences for dose groups, $F(3,28)=1.1$, $p=0.34$. ANOVA of postdrug scores failed to reveal significant differences between antagonist dose groups, $F(3,28)=1.0$, $p=0.39$, however, a significant main effect was found for the time of testing, $F(3,28)=36.4$, $p<0.001$. The interaction was not significant, $F(9,84)=0.59$, $p=0.8$.

Newman-Keuls analyses of time-course revealed that all treatment groups were significantly different ($p<0.05$) from baseline at the 10- and 20-min testing trials but failed to differ from baseline at the 40-min trial. These results demonstrate that DAGO, like DPDPE, reliably elevated response latency (approximately

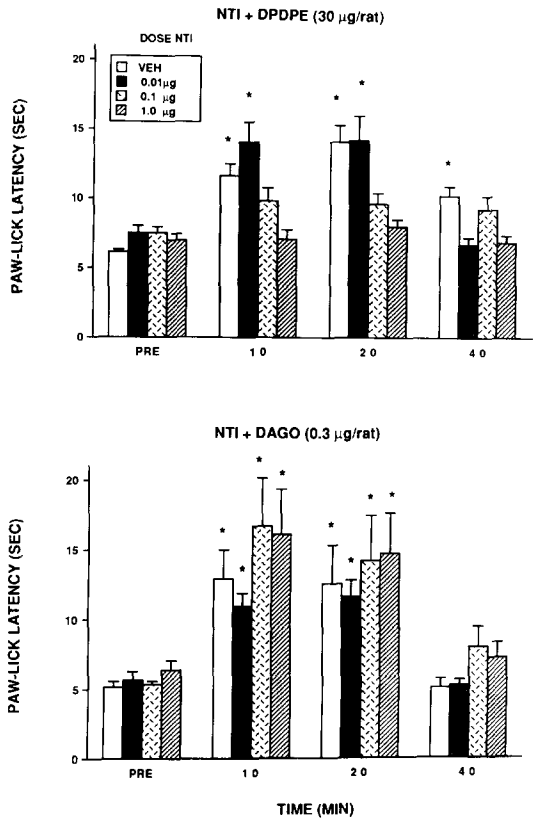

FIG. 1. Depicts the time-course of analgesia in rats pretreated (10 min) with one of four ICV administered doses of naltrindole (VEH, 0.01, 0.1 and 1.0 μg/rat) followed by a second ICV injection of with 30 μg DPDPE (top panel; n = 15–16 per dose) or 0.3 μg DAGO (bottom panel; n = 8 per dose). Subjects were tested on a hot-plate 10, 20 and 40 min after injection of DPDPE or DAGO. Each bar represents the mean (± SEM) paw-lick latency in rats. "VEH" refers to the control vehicle for naltrindole. An asterisk indicates significant difference ($p<0.05$) between predrug baseline (PRE) and postagonist testing trials (Newman-Keuls' test).

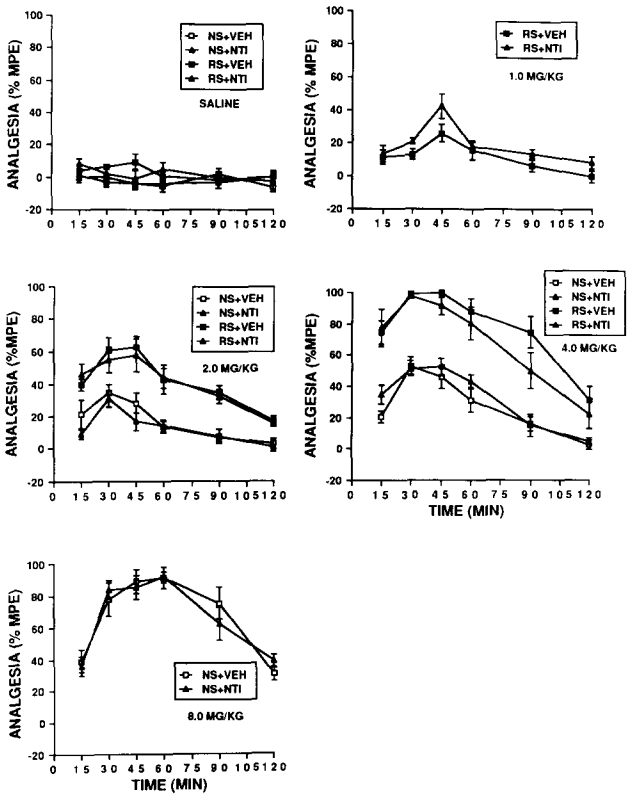

FIG 2. Illustrates the time-course effects of naltrindole (NTI; VEH or 1.0 μg/rat, ICV) plus saline or morphine (1.0, 2.0, 4.0 and 8.0 mg/kg, SC) injections in nonstressed (NS) and restraine stressed (RS) subjects. The mean (± SEM) tail-lick latency in rats (n = 7–9 per point) is presented as the % maximum possible effect (% MPE). "VEH" refers to the control vehicle.

2–3 times > baseline) for 20 min. However, unlike results with DPDPE, NTI failed to block DAGO-induced analgesia at any dose tested.

Restraint Stress Potentiation of Morphine Analgesia

One-factor ANOVA of predrug baseline tail-flick latencies (s) between restraint stressed and nonstressed groups indicated a significant main effect, $F(1,125) = 32.4 < 3.1$, $p<0.001$. These results show that the mean tail-flick latency of restrained rats was significantly higher than nonstressed rats [restraint mean and standard error of the mean (SEM) = 2.3 ± 0.26, nonstressed mean and SEM = 2.1 ± 0.29] even though the difference between group means was only 0.2 s. However, ANOVA of latencies within either treatment group failed to reveal significant differences; restraint stressed groups, $F(7,54) = 1.7$, $p = 0.13$; nonstressed groups, $F(7,57) = 1.3$, $p = 0.28$.

Two-factor ANOVA of postdrug tail-flick latencies (transformed to area in order to generate dose-effects curves) were performed on stressed and nonstressed rats that received antagonist (NTI or VEH) plus saline. ANOVAs failed to reveal a significant main effects for restraint treatment groups, $F(1,29) = 3.4$, $p = 0.07$, and antagonist treatment, $F(1,29) = 0.047$, $p = 0.83$. These results confirm two important findings: 1) the latencies between restraint treatment groups, which were significantly different in the predrug baseline measurements, failed to remain significantly different in postdrug saline testing, and 2) antagonist treatment (NTI 1.0 μg or VEH) did not affect pain sensitivity.

Three-factor ANOVA of antagonist treatment by the doses of morphine common to both stressed and nonstressed groups revealed significant main effects for restraint treatment, $F(1,84) = 168.4$, $p<0.001$, and doses of morphine, $F(2,84) = 242.4$, $p<0.001$. Only the restraint by morphine dose treatment interaction was significant, $F35.3 = p<0.001$. The main effect for antagonist treatment was not significant, $F(1,84) = 1.1$, $p = 0.3$. These results indicate that NTI failed to affect either the morphine-induced analgesia in nonstressed rats or the restraint stress-induced potentiation of morphine analgesia.

Rats were injected with NTI (VEH or 1.0 μg/rat) and saline or one of 3 doses of morphine (1.0, 2.0, 4.0 and 8.0 mg/kg, n = 7–9 per point), depending upon restraint treatment. Figure 2 illustrates the time-course of analgesia in stressed and nonstressed rats (expressed as mean % MPE and SEM) using the tail-flick assay.

The magnitude of peak morphine analgesia was increased by

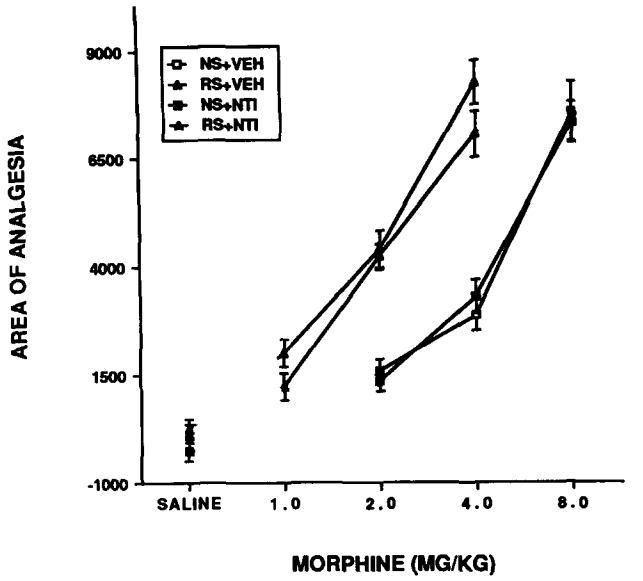

FIG 3. Shows naltrindole (NTI) plus morphine dose-response data presented as the area of analgesia (%MPE-min). The nonstressed (NS) and restraint stressed (RS) subjects (n = 7–9) had received an ICV injection of NTI immediately followed by an injection of saline or morphine (1.0–8.0 mg/kg). "VEH" refers to the NTI control vehicle. Areas were derived from data shown in Fig. 2.

restraint. For example, the analgesic effect of 4.0 mg/kg of morphine in nonstressed rats was increased 3-fold in stressed rats at the 60-min point. The duration of action of morphine analgesia was also significantly increased (about 2-fold) in stressed rats compared to nonstressed rats. For example, the % MPE of morphine (4.0 mg/kg) at 60 min was about 35% in nonstressed rats, whereas in stressed rats, the % MPE at this dose remained above 80%. Figure 3 shows dose-response curves generated from area of analgesia under the time-course curves (data presented in Fig. 2).

These dose-response curves and the ED_{50}s for morphine (Table 1) indicate that the analgesic potency of morphine in restrained rats was approximately twice that in nonstressed rats regardless of whether the rats were treated with ICV vehicle or with 1.0 μg of NTI.

TABLE 1

ED_{50} (95% CONFIDENCE LIMITS)* IN mg/kg FOR MORPHINE-INDUCED ANALGESIA IN NONSTRESSED (2.0–8.0 mg/kg) AND RESTRAINT STRESSED (1.0–4.0 mg/kg) RATS THAT RECEIVED NALTRINDOLE (NTI, 1 μg/rat, ICV) OR ITS VEHICLE (VEH)

Nonstressed + VEH	5.0(2.6–16)
Nonstressed + NTI	5.0(3.0–11.1)
Restraint Stressed + VEH	2.1(1.7–3.1)
Restraint Stressed + NTI	2.3(1.7–4.0)

*ED_{50} was determined by regression analysis based on 7–9 independent observations per dose.

DISCUSSION

The ICV administration of DPDPE or DAGO significantly increased paw-lick latency for at least 20 min. These results replicate previous findings (13) and findings from our laboratory (3) wherein we report full dose- and time-response curves for ICV administration of DPDPE (3–30 μg) and DAGO (0.03–0.3 μg). The lowest dose of NTI (0.01 μg) failed to attenuate DPDPE-induced analgesia, whereas the 0.1 and 1.0 μg doses blocked the increase in paw-lick latency, demonstrating the dose-dependent antagonism of DPDPE-induced analgesia. NTI failed to antagonize DAGO-induced analgesia at every dose tested. Thus with doses as high as 1 μg, NTI acted selectively to antagonize DPDPE- but not DAGO-induced analgesia.

DPDPE has been considered one of the most selective agonists for δ-over μ-receptors, based primarily on the strength of binding data (25). However, the in vivo selectivity of DPDPE has sometimes been questioned. For example, Cowan and colleagues have offered in vivo evidence that DPDPE also produces μ-receptor-like effects in rats (9). Other evidence that DPDPE results in μ-receptor-like effects comes from the demonstration that DPDPE hyperpolarized a subset of DAGO-sensitive guinea pig hypothalamic arcuate neurons, resulting in increased potassium conductance (24). Therefore, we cannot exclude the possibility that under some conditions DPDPE and μ-receptor agonists produce analgesia via a similar cellular mechanism. The differential effects of NTI demonstrate that in the present study DPDPE produced analgesia via a receptor mechanism different from the one mediating the analgesic effect of DAGO.

In Experiment 3, we administered NTI (1.0 μg, ICV) to restraint stressed and nonstressed rats. Following morphine administration, these rats were tested for pain sensitivity using the tail-flick assay. Morphine dose-dependently increased the latency to tail-flick. The magnitude and duration of morphine analgesia in restrained rats were significantly potentiated in comparison to nonstressed rats. These findings are consistent with our previous findings (1, 4, 6, 12).

Response latencies in the groups of rats receiving the morphine vehicle (saline) plus NTI VEH or 1.0 μg NTI did not significantly differ from each other. These results demonstrate that NTI fails to alter baseline pain sensitivity and is consistent with the findings of previously mentioned reports (11,22). NTI failed to affect either morphine analgesia (in nonstressed rats) or the restraint stress-induced potentiation of morphine analgesia. The fact that ICV administered NTI did not attenuate either morphine-induced analgesia or restraint stress-induced potentiation of morphine analgesia provides no support for the view that these analgesic effects of morphine are mediated in part by δ-receptors. However, we cannot exclude the possibility that NTI may attenuate morphine-induced analgesia after IT administration. To our knowledge, there is no evidence to support the hypothesis that antagonism of supraspinally located δ-receptors will alter the ability of spinally located δ-receptors to contribute to morphine-induced analgesia.

In conclusion, our results demonstrate that NTI acts as a selective antagonist of DPDPE but not DAGO-induced analgesia. Moreover, the ICV administration of NTI failed to affect morphine-induced analgesia and restraint stress potentiation of morphine analgesia using the tail-flick assay and at a dose of NTI (1.0 μg) which selectively antagonized DPDPE-induced analgesia. The usefulness of NTI as a tool in the exploration of δ-receptor functions awaits additional characterization in behavioral tests known to involve opioid mechanisms.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institute on Drug Abuse (No. DA00541) and by a Research Scientist Award (No. DA00008).

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