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# Differential Involvement of Opioid Receptors in Intrathecal Butorphanol-Induced Analgesia: Compared to Morphine

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WONGCHANAPAI, W., B. K. TSANG, Z. HE AND I. K. HO. *Differential involvement of opioid receptors in intrathecal butorphanol induced analgesia: Compared to morphine.* PHARMACOL BIOCHEM BEHAV **59**(3) 723–727, 1998.—The present experiments were performed to investigate the differential involvement of the opioid receptor subtypes in the antinociception of intrathecal (IT) butorphanol compared to IT morphine. A single dose (26 nmol) of IT nor-binaltorphimine (nor-BNI), β-funaltrexamine (β-FNA), and naltrindole (NTI) demonstrated a significant attenuation in the overall antinociception of IT butorphanol (52 nmol) or IT morphine (26 nmol). However, IT butorphanol elicits thermal antinociceptive effect through $\kappa > \delta \geqslant \mu$, whereas morphine acts on $\mu > \delta >> \kappa$. These results indicate that the antinociceptive effect of both IT butorphanol and IT morphine are mediated through μ, δ, and κ opioid receptors in different relative orders. © 1998 Elsevier Science Inc.

Antinociceptive Butorphanol Intrathecal Morphine

BUTORPHANOL tartrate (17-cyclobutylmethyl-3,14-dihydroxymorphinan), Stadol®, is a potent mixed agonist-antagonist opioid analgesic that belongs to the group of opioids known as morphinans (11). Butorphanol, administered parenterally in pre- and postoperative situations, exerts an analgesic action with a potency about five to seven times greater than that of morphine (2,8), the prototypical opioid analgesic. Butorphanol produces its analgesic action by interacting with κ, as well as μ and δ opioid receptors (4), whereas morphine acts primarily through the μ, and δ opioid receptors (1,28). However, in vitro binding studies have suggested that morphine has affinity not only for μ, and δ but also for κ opioid receptors (16,26). The analgesic effects of opioid agonists, especially morphine, injected directly into the lumbar spinal subarachnoid space, have been extensively investigated (23,30). Previously, we have shown the dose-dependent effect of the antinociceptive response of intrathecally injected butorphanol on thermal stimuli, which is reversed by naloxone (25). However, the relative involvement of the opioid-receptor subtypes in mediating analgesia of IT butorphanol remains unknown.

Recently, nor-binaltorphimine (nor-BNI), β-funaltrexamine (β-FNA), and naltrindole (NTI) have been demonstrated as the selective antagonists at κ, μ, and δ opioid receptors, respectively (3,6,19). For κ antagonism, intracerebroventricularly (ICV) injected nor-BNI has been reported to have a duration of antagonistic actions ranging from 10 min to 56 days in the tail-flick test in mice (5,19). However, we have observed that pretreatment with nor-BNI at 12 h prior to IT opioid injection was generally more effective in blocking the thermal antinociception of IT butorphanol than those pretreatment at 10 min prior. In the present study, these antagonists were, therefore, used to investigate the involvement of different opioid receptor subtypes in the antinociception of IT butorphanol, compared to morphine.

## METHOD

### *Animals and Chemicals*

Male Sprague–Dawley rats (Harlan Sprague–Dawley Inc. Indianapolis, IN), weighing 250–300 g, were kept in a room

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with an ambient temperature of 21 ± 2°C and 12 L:12 D cycle with free access to food and water, for a week prior to the experiment. Butorphanol was a generous gift of the Bristol-Myers Corporation (Syracuse, NY). Morphine was purchased from Research Biochemicals International (Natick, MA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

*Surgical Procedures*

Rats were implanted with polyurethane microspinal catheters (22) (inner diameter 0.12 mm, outer diameter 0.35 mm, Tecoflex, Preferred Medical Products, Ontario, Canada) under general anesthesia with 1–3% halothane and 100% oxygen. The surgical technique was carried out according to modification of the method described previously (30). Briefly, an incision was made sterilely along the occipital ridge and the nuchal muscles were reflected. The atlanto-occipital membrane was then identified and incised through the midline. The microspinal catheter was inserted into the subarachnoid space and gently advanced 8.5 cm caudally to the lumbar enlargement. The proximal end of the catheter was then externalized percutaneously on the top of the skull, flushed with 10 μl of normal saline, and plugged with a 28 gauge stylet. The incision was then closed with sutures. The animals were given 30,000 units of procaine penicillin G subcutaneously to prevent infection. Kept individually, animals were allowed to recover for 1 week before drug administration. Animals with any neurological deficit were excluded from the study.

*Drug Administration*

To assess the effects of nor-BNI on the antinociceptive responses of butorphanol, the animals were given IT saline or nor-BNI (10.4, 26, or 52 nmol/10 μl/rat) at 10 min and nor-BNI (26 nmol) at 12 h before the injection with an equieffective dose of IT butorphanol (52 nmol). To determine the involvement of opioid receptors in the antinociceptive responses of butorphanol compared to morphine, animals were pretreated intrathecally with saline, nor-BNI, β-FNA, or NTI (26 nmol) at 12 h before antinociceptive testing with IT butorphanol (52 nmol) or IT morphine (26 nmol). The doses selected for different compounds tested were based on preliminary experiments. The antinociceptive responses were evaluated every 15 min for up to 120 min in each rat. Six to eight animals were used per group. All animals were only used once for antinociceptive testing.

All drugs, except β-FNA, were dissolved in sterile physiological saline and were administered IT by a hand-driven syringe pump. β-FNA was dissolved in sterile water. Agents were prepared such that each dose was delivered in a volume of 10 μl followed by a 10-μl saline flush to clear the catheter.

*Analgesic Tests*

Antinociception was measured by tail-flick latency defined as the time from the onset of radiant heat to tail withdrawal. A cutoff time of 10 s was set to prevent thermal injury of the tail. Before IT administration, any animal having a reaction time greater than 3 SD from the mean reaction time of all prescreened animals were excluded from the study. Antinociception was determined by percentage of maximal possible analgesic effect (%MPE), calculated by the formula: %MPE = (test latency baseline latency)/(cutoff time − baseline latency) × 100. The overall antinociceptive response was determined by area under the curve (AUC) (21). AUC was calculated by a trapezoidal approximation of the MPE curve and expressed as percent maximum AUC (100%) when all animals in a group reached the cutoff point of 10 s.

*Statistics*

Data (mean ± SEM) were analyzed using one-way ANOVA to compare all groups together. Post hoc pairwise comparisons were made using Student–Neuman–Keuls (SNK) test. Significance was set at $p < 0.05$.

## RESULTS

The antinociceptive effects of IT butorphanol after IT pretreatment at different times with different doses of nor-BNI are illustrated in Fig. 1, and there were significantly depended on dose, $F(4, 30) = 12.423$, $p < 0.001$, and time, $F(3, 23) = 17.230$, $p < 0.001$. Intrathecal pretreatment at 10 min with nor-BNI (10.4 and 26 nmol) failed to antagonize the antinociceptive effects of butorphanol in the tail-flick test. However, the highest dose of IT nor-BNI (52 nmol, −10 min) in the present studies significantly antagonized the antinociceptive responses to equieffective dose of butorphanol (52 nmol) ($q = 5.384$, $p < 0.01$). IT nor-BNI exhibited a long-term action of opioid receptor antagonism rather than a short-term effect, as a single pretreatment at 12 h (but not 10 min) was able to block the antinociception produced by IT butorphanol ($q = 7.150$, $p < 0.01$).

The effects of opioid antagonist pretreatment on the antinociceptive actions of IT butorphanol and morphine are shown in Figs. 1 and 2, respectively. A statistic analysis re-

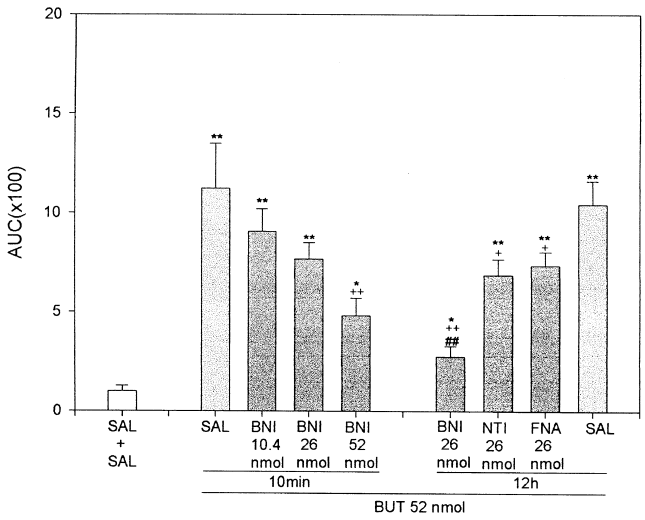


FIG. 1. Effect of pretreatment with nor-BNI, NTI, and β-FNA on the overall antinociceptive activity of IT butorphanol in the tail-flick test. Animals were pretreated with IT nor-BNI (10.4, 26, or 52 nmol) 10 min and IT nor-BNI, NTI, or β-FNA (26 nmol) 12 h before single dose of IT butorphanol (52 nmol). The tail-flick assay was performed on each rat at 15-min intervals for 120 min. The overall antinociception calculated from areas under the curve of the maximal possible effect (MPE) time curves. Data are mean ± SEM of six to eight animals in each group. *$p < 0.05$; **$p < 0.01$ vs. control. +$p < 0.05$; ++$p < 0.01$ vs. pretreated-saline group. ##$p < 0.01$ indicates a significant difference between the time of pretreatment.

vealed that selective opioid antagonist pretreatment significantly reduced the antinociceptive effects of IT butorphanol, $F(4, 30) = 24.703$, $p < 0.001$, and IT morphine, $F(4, 33) = 37.117$, $p < 0.001$. Intrathecal nor-BNI, β-FNA, or NTI (26 nmol, −12 h) alone had no effect on antinociception in this study. Pretreatment with IT nor-BNI, β-FNA, and NTI resulted in a significant attenuation in the overall antinociception of IT butorphanol (52 nmol) ($q = 9.623$ $p < 0.01$, $q = 3.861$, $p < 0.05$, and $q = 4.149$ $p < 0.05$, respectively) (Fig. 1). Similarly, IT nor-BNI, β-FNA, and NTI pretreatment also markedly antagonized the analgesic responses of IT morphine (26 nmol) ($q = 3.841$, $p < 0.05$, $q = 13.003$, $p < 0.01$, and $q = 7.908$, $p < 0.01$, respectively) (Fig. 2).

It was not possible to determine the antinociceptive responses of IT morphine at 52 nmol because administration of this dose produced rigidity of the tail, invaliding the tail-flick test. Although IT administration of 26 nmol of butorphanol did demonstrate the antinociceptive effect measured by tail-flick test, the antinociceptive response was not high enough to be differentiated by selective antagonists. However, IT morphine, 26 nmol, produced an overall antinociception significantly greater than that of IT butorphanol 52 nmol, $t(14) = 4.01$, $p < 0.001$. In comparison, data obtained on % analgesia of both butorphanol and morphine were shown in Table 1. The antinociception produced by IT butorphanol after IT nor-BNI pretreatment was lower than that of morphine. In other words, pretreatment with nor-BNI reduced the overall analgesia of butorphanol more than that of morphine. In contrast, β-FNA and NTI decreased the overall antinociception of butorphanol less than that of morphine. The order (from highest to lowest) of % analgesia of IT butorphanol and morphine produced by the different opioid antagonists was: butorphanol: $\kappa > \delta \geq \mu$ and morphine: $\mu > \delta >> \kappa$.

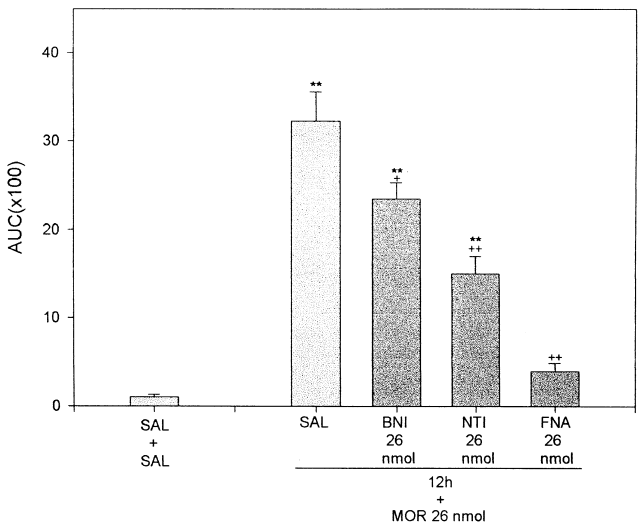


FIG. 2. Effect of pretreatment with IT nor-BNI, NTI, and β-FNA on the antinociceptive response to IT morphine. Animals were pretreated with IT not-BNI, NTI, or β-FNA (26 nmol) 12 h before antinociceptive testing with a single dose of IT morphine (26 nmol). The tail-flick test was performed at 15-min intervals for 120 min. Areas under the curve calculated from the maximal possible effect (MPE) time curves. Data are mean ± SEM of 6 to 10 animals in each group. $^{**}p < 0.01$ vs. control. $+p < 0.05$; $++p < 0.01$ vs. pretreated-saline group.

## DISCUSSION

The intrathecal injection of opiates, especially morphine, has been demonstrated to produce a dose-dependent antinociception, and this effect is readily antagonized by IT naloxone (23,29). Previously, we have shown that IT butorphanol also produced an antinociceptive effect in the tail-flick test in a dose-dependent and naloxone reversal manner (25). In the present study, IT butorphanol was less efffective in thermal analgesia than IT morphine. The different potency in thermal analgesia between two opioids may result from the nature of the opioid receptors acted upon by the drugs to produce the effect and the major action of the drugs on different receptors.

It has been emphasized that there are multiple classes of opioid receptors in the central nervous system. Biochemical and pharmacological evidence has been reported to support the presence of multiple subtypes for opioid receptors including μ, δ, and κ receptors in the substantia gelatinosa, a region of the dorsal horn in the spinal cord where most afferent pain fibers terminate (7,14,24). In thermal nociceptive tests, μ and δ agonists have been shown to have potent analgesic effect (27), whereas κ agonists do produce antinociception with their activity dependent on the intensity of the nociceptive stimulus (9,13). It has been suggested that the relative activity ordering of the opioid receptors on thermal antinociception is $\mu > \delta > \kappa$ (28). Becaused morphine acts mainly on μ receptor, the thermal antinociceptive efficacy of morphine is, therefore, greater than that of butorphanol. However, butorphanol administered subcutaneously has been demonstrated to have greater potent analgesic effect on visceral pain than morphine in mice (15).

Selective opioid antagonists are pharmacological probes that allow the analysis of the individual opioid receptor subtype mediating the effects of exogenous opioids. For the κ opioid binding site, nor-BNI has been reported to be a potent and highly selective antagonist based on in vitro binding studies (19) and in vivo studies when administered as a single dose even at 100 nmol (17). The duration of antagonistic action of nor-BNI has been reported to produce a remarkably long-lasting (10 min to 56 days) blockade of κ opioid receptor after a single ICV injection (5). In this study, IT pretreatment at 10 min with nor-BNI (10.4 and 26 but not 52 nmol) was found to be unable to exhibit antagonistic action. However, IT nor-BNI was found to have high antagonistic activity in long-term action (12 h) rather than in short-term effect (10 min). This appearance may be related to the increase in antagonistic activity and potency of this compound at the κ opioid receptor with time.

It has been reported that pretreatment at 12 h with ICV β-FNA (18.8 nmol) antagonized analgesic effects of a single dose of ICV morphine (6) and treatment with β-FNA (12–48 nmol)

TABLE 1

PERCENT ANALGESIA IN TAIL-FLICK ASSAY INDUCED BY IT BUTORPHANOL AND MORPHINE AFTER PRETREATMENT WITH NOR-BNI, NTI, OR β-FNA*

| Pretreatment | BUT (52 nmol) | MOR (26 nmol) |
|---|---|---|
| Saline | 100 | 100 |
| nor-BNI (26 nmol) | 26.08 | 72.80 |
| NTI (26 nmol) | 65.77 | 46.56 |
| β-FNA (26 nmol) | 70.37 | 12.14 |

* Data derived from Figs. 1 and 2 as calculated by the formula: % analgesia = 100 × antagonist pretreatment/saline pretreatment.

significantly diminished naloxone-induced withdrawal signs in butorphanol-dependent rats (12). Additionally, IT NTI at concentrations as high as 66 nmol, has also been demonstrated to produce a selective antagonism for the δ selective agonist, cyclic[D-penicillamine$^2$-D-penicillamine$^5$]enkephalin (DPDPE) (3). Although different intervals for pretreatment have been used (6,10,17), the results from the present study suggest that antagonistic actions at 12 h after treatment with IT nor-BNI, β-FNA, and NTI were effective for comparison among the antagonists with equal molar concentration.

In the present study, pretreatment with IT nor-BNI, β-FNA, and NTI significantly antagonized butorphanol- as well as morphine-induced analgesia. These results suggest that IT butorphanol and morphine produced antinociceptive effect through μ, δ, and κ opioid receptors. However, IT butorphanol-induced analgesia is mediated mainly through opioid receptors by the relative order of $\kappa > \delta \geqslant \mu$. This result corresponds to the affinity of butorphanol on κ, δ, and μ receptors in binding studies (4). In contrast, it is likely that morphine elicits a thermal antinociceptive effect in the relative order of $\mu > \delta >> \kappa$ receptors. Although the present results cannot exclude the possible nonselective actions of these antagonists, these results may at least partly substantiate and provide additional evidence in support of the earlier in vitro characterization of morphine as an agonist that has high affinity for μ and δ receptors and has some binding activity on the κ, receptor (16,26). In addition, previous studies have reported the role of κ, receptor on the action of morphine. The κ-selective antagonist, binaltorphimine, has been demonstrated to inhibit morphine-induced analgesia in β-FNA–treated mice (20). Pretreatment with the κ antagonist has been shown to potentiate the development of tolerance to morphine analgesia and aggravate the naloxone-precipitated body weight loss in morphine-dependent mice and rats (18).

In summary, this study reveals that IT butorphanol and IT morphine exert their antinociceptive effects by acting at μ, δ, and κ opioid receptors, but with different orders of receptor contributions. Butorphanol produces analgesic effects through $\kappa > \delta \geqslant \mu$ receptors, whereas morphine possibly acts on $\mu > \delta >> \kappa$ receptors. However, further studies will be required to clarify the differential involvement of spinal opioid receptors in the antinociceptive effects of IT butorphanol and IT morphine.

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