Pharmacology Biochemistry and Behavior, Vol. 60, No. 4, pp. 899–907, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00074-4

Relative Involvement of Spinal Opioid Receptors in Physical Dependence on Intrathecal Butorphanol and Morphine

W. WONGCHANAPAI,* B. K. TSANG,† Z. HE† AND I. K. HO*

*Department of Pharmacology and Toxicology, †Department of Anesthesiology, University of Mississippi Medical Center, Jackson, MS 39216

Received 17 October 1997; Revised 5 February 1998; Accepted 19 February 1998

WONGCHANAPAI, W., B. K. TSANG, Z. HE AND I. K. HO. *Relative involvement of spinal opioid receptors in physical dependence on intrathecal butorphanol and morphine.* PHARMACOL BIOCHEM BEHAV. **60**(4) 899–907, 1998.— The present study was carried out to investigate the relative involvement of spinal opioid receptors in the development of physical dependence on intrathecal (IT) butorphanol in comparison with IT morphine. Dependence was induced by continuous IT infusion of butorphanol (52 nmol/h) and morphine (26 nmol/h) for 4 days in male Sprague–Dawley rats. Naloxone, CTOP, naltrindole, and nor-binaltorphimine (nor-BNI) were administered IT to precipitate behavioral signs of withdrawal. Administration of IT naloxone produced a significantly greater increase in the profile of withdrawal signs in IT morphine dependence than that in IT butorphanol dependence. An IT nor-BNI challenge elicits behavioral signs of withdrawal only in rats dependent on IT butorphanol, but not in rats dependent on IT morphine. CTOP administered IT precipitated withdrawal signs in IT morphine dependence that were greater than that in IT butorphanol dependence. An IT treatment with naltrindole produced equivalent signs of withdrawal in both IT butorphanol- and morphine-dependent rats. These results suggest that continuous IT butorphanol results in the development of less physical dependence than that of IT morphine. Spinal κ- rather than δ- and μ-opioid receptors play a major role in the development of IT butorphanol dependence, whereas spinal μ-opioid receptors play a more important role than δ-opioid receptors in IT morphine dependence. © 1998 Elsevier Science Inc.

Intrathecal Butorphanol Morphine Physical dependence

SPINAL administration of opioids in humans and animals has been found to produce intense, selective, and prolonged analgesia (3,37,42). Unfortunately, this treatment can cause undesirable side effects in both human and nonhuman subjects. Of all potential complications, the risk of respiratory depression generates the most clinical concern. In contrast to μ-opioid receptors, activity at the κ-opioid receptors may not be associated with significant respiratory depression (1,27). Mixed agonist-antagonist analgesics (including butorphanol) have been suggested to offer the potential benefit of greater receptor site selectivity, while diminishing the incidence of adverse sequelae (15). Furthermore, spinal butorphanol has also been reported to cause neither pruritis nor urinary retention (26).

Intrathecal (IT) administration of butorphanol and morphine produces an antinociceptive effect through opioid receptors in a dose-dependent manner that is reversed by naloxone (36,38,44). However, the antinociceptive effects of IT butorphanol and IT morphine are mediated through μ-, δ-, and κ-opioid receptors in different relative orders. IT butorphanol exerts analgesic effects in the dorsal horn of the spinal cord primarily through the κ-opioid receptor, whereas IT morphine preferentially acts on the μ-opioid receptor (39,41). However, chronic IT administration of some opioids leads to the development of tolerance and physical dependence (40). Previous studies have demonstrated that the physical dependence on IT opioid may develop primarily in the spinal cord (5,22,29). Withdrawal signs in morphine dependence could be precipitated by naloxone injected IT to morphine-implanted animals (6) and naloxone given intraperitoneally (IP) to IT morphine-dependent rats (31). Recently, the development of physical dependence on IT infused morphine after an IT naloxone challenge was also demonstrated (5). Most studies

Requests for reprints should be addressed to Dr. I. K. Ho, Professor and Chairman, Department of Pharmacology & Toxicology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216.

of spinal opioid dependence have focused on morphine, while the development of IT butorphanol dependence has not been determined. Furthermore, because the actions of the two analgesics at spinal opioid receptors are dissimilar, the relative participation of these receptors in the development of dependence on the analgesics should be different. Therefore, the present study was designed to determine the relative involvement of μ-, δ-, and κ-opioid receptors in IT opioid-dependent animals. Naloxone, CTOP, naltrindole, and nor-binaltorphimine (nor-BNI) were injected IT to precipitate withdrawal signs in animals rendered dependent on IT butorphanol or morphine.

METHOD

Animals and Chemicals

For a week prior to the experiment, male Sprague–Dawley rats (Harlan–Sprague–Dawley Inc. Indianapolis, IN), weighing 250–300 g, were kept in a room with an ambient temperature of 21 ± 2°C and 12 L:12 D cycle with free access to food and water. Butorphanol was a generous gift of the Bristol-Myers Corporation (Syracuse, NY). Morphine, CTOP, naltrindole, and nor-BNI were purchased from Research Biochemicals International (Natick, MA). Naloxone was obtained from Sigma Chemical Company (St. Louis, MO).

Surgical Procedures

Rats were implanted with polyurethane microspinal catheters (35) (inner diameter 0.12 mm, outer diameter 0.35 mm, Preferred Medical Products, a division of Ballard Medical Products, Lewiston, NY) under general anesthesia with 1–3% halothane and 100% oxygen. The surgical technique was carried out according to modification of the method described previously (43). Briefly, a sterile incision was made 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 externalized percutaneously on the top of the skull and connected with a 4-cm piece of Tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL) that was then plugged by a 28 gauge stylet. The incision was then closed with sutures. The external tubing was shielded by a plastic protector sutured at the top of the head and dental acrylic (Lang Dental, Wheeling, IL) was applied to strengthen the protector. The animals were given 30,000 units of procaine penicillin G subcutaneously (SC) to prevent infection. Kept individually, animals were allowed to recover for 1 week before implantation of osmotic minipumps. Animals with any neurological deficit were excluded from the study.

Induction of IT Butorphanol and Morphine Dependence

Animals were rendered dependent on butorphanol and morphine by IT infusion of butorphanol tartrate (52 nmol/μl/h) or morphine hydrochloride (26 nmol/μl/h) for 4 days via osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA). The control rats received an IT infusion of normal saline (1 μl/h) for the same period of time. Both the infusion period and the dose paradigm had been determined to be optimal from preliminary experiments. Under halothane anesthesia, each animal was implanted SC with a minipump between the scapulae. Before being connected to the minipump, the IT catheter with the Tygon connector was filled with 7 μl of butorphanol, morphine, or saline to eliminate the dead space. Butorphanol, morphine, or saline vehicle solutions were passed through a 0.2-μm sterile Acrodisk filter (Gelman Sciences, Ann Arbor, MI) before being introduced into the pumps. The minipumps were primed overnight at room temperature in normal saline so that the nominal flow rate (1 μl/h) was obtained.

Measurement of Behavioral Sings During IT Butorphanol and Morphine Withdrawal

After implantation, each rat lived in his own polycarbonate cage (48 cm long; 27 cm wide; 20 cm high) in the animal room and was moved to the laboratory 4 h before withdrawal testing. The IT infusion was terminated by cutting the connector; the remaining drug solution inside the catheter was then flushed out with 10 μl of normal saline. The butorphanol-, morphine-, and saline-infused rats were randomly assigned to four groups of eight animals per group. Three hours later, each rat was injected IT with naloxone, CTOP, NTI, or nor-BNI (48 nmol/10 μl) followed by a 10 μl saline flush to clear the catheter. This dose, selected for different compounds, was based on previous studies in this laboratory (9,11,17,34). All drugs were dissolved in sterile physiological saline and were administered IT by a hand-driven syringe pump. To diminish disturbance of resting activity and environment, the injection procedures were performed while rats were still in their cages but with the grid tops removed. This was reflected in the very low incidence of spontaneous horizontal and vertical exploration during control observation. Withdrawal signs (wet shakes, teeth chattering, rearing, locomotion, stretching, scratching, digging, grooming, penis licking, forepaw tremor, vocalization, and ptosis) were then observed for 30 min. A simple scoring sytsem (one point for each sign, and summation of the scores for each of the signs to give a composite score) was used to compare responses between the treatment groups. Withdrawal signs were counted quantally as the number of animals exhibiting each sign at any time during the 30-min observation period. In addition, the total number of withdrawal signs was recorded to assess the severity of abstinence signs precipitated by IT opioid antagonists (17,19). For instance, locomotion (movement or walking episodes in circle), rearing (standing up on the hindlegs), or wet shake (shaking of the entire trunk) was assessed quantitatively by recording the frequency of the sign.

Statistics

Quantal (all or none) data from the behavioral studies on experimental groups and saline control were compared using the chi-square test. The comparability of the three treatment groups for the intensity (quantity) of withdrawal signs (mean ± SEM) was analyzed using one-way ANOVA to compare all groups together. Post hoc pairwise comparisons were made using Student–Newman–Keuls (SNK) test. A difference was considered significant at $p < 0.05$.

RESULTS

Withdrawal Signs Precipitated by IT Naloxone

IT naloxone precipitated withdrawal signs in both the IT butorphanol-infused group and the IT morphine-infused group are presented in Table 1. The composite withdrawal score was significantly greater in the IT butorphanol-dependent group ($\chi^2 = 12.46$, $p < 0.01$) and in the IT morphine-dependent group ($\chi^2 = 34.03$, $p < 0.01$), than in the IT saline-infused group. Similarly, the score in the morphine group was

TABLE 1

WITHDRAWAL SIGNS ELICITED BY IT INJECTION OF NALOXONE, CTOP, NATRINDOLE, OR NOR-BNI IN SALINE-INFUSED RATS, AND BUTORPHANOL- OR MORPHINE-DEPENDENT RATS

	Naloxone (48 nmol)			CTOP (48 nmol)		
Sign	Saline	Butorphanol	Morphine	Saline	Butorphanol	Morphine
Wet shakes	ND	5/8*	5/8*	1/8	5/8	7/8*
Teeth chattering	ND	3/8	7/8†	ND	2/8	5/8*
Rearing	1/8	2/8	1/8	5/8	2/8	5/8
Locomotion	1/8	4/8	8/8†	ND	4/8	7/8†
Stretching	1/8	2/8	4/8	ND	ND	4/8
Scratching	ND	1/8	4/8	2/8	4/8	3/8
Digging	2/8	2/8	5/8	ND	2/8	1/8
Grooming	1/8	ND	ND	3/8	3/8	5/8
Penis licking	ND	1/8	1/8	ND	ND	1/8
Forepaw tremor	ND	1/8	3/8	3/8	5/8	5/8
Vocolization	ND	1/8	3/8	ND	ND	5/8*
Ptosis	ND	3/8	4/8	ND	1/8	4/8
Composite score	6/96	25/96†	45/96†‡	14/96	28/96*	52/96†‡
	Naltrindole (48 nmol)			nor-BNI (48 nmol)		
Sign	Saline	Butorphanol	Morphine	Saline	Butorphanol	Morphine
Wet shakes	1/8	2/8	3/8	1/8	6/8*	4/8
Teeth chattering	ND	1/8	2/8	ND	1/8	1/8
Rearing	2/8	2/8	3/8	2/8	4/8	2/8
Locomotion	ND	5/8*	5/8*	1/8	8/8†	4/8
Stretching	ND	1/8	4/8	ND	6/8†	ND
Scratching	ND	ND	1/8	2/8	3/8	3/8
Digging	2/8	5/8	3/8	1/8	3/8	ND
Grooming	1/8	1/8	ND	1/8	3/8	ND
Penis licking	ND	ND	1/8	ND	2/8	1/8
Forepaw tremor	ND	ND	2/8	1/8	3/8	1/8
Vocalization	ND	ND	1/8	ND	1/8	1/8
Ptosis	ND	ND	2/8	ND	4/8	ND
Composite score	6/96	17/96*	27/96†	9/96	44/96†‡	17/96

The values given for each sign denote the number of rats showing positive signs over the total number of rats tested. ND = nondetected.

*$p < 0.05$, †$p < 0.01$ vs control; ‡$p < 0.01$ butorphanol vs. morphine.

greater than that in the IT butorphanol-dependent group ($\chi^2 = 5.87$, $p < 0.05$). The IT injection of naloxone in IT morphine-dependent animals significantly induced wet shakes, teeth chattering, and locomotion compared with the saline-infused group. In the IT butorphanol-dependent group, only wet shakes was significantly precipitated by IT naloxone.

The intensity of abstinence signs, indicated as the severity of a particular withdrawal sign, is presented in Fig. 1. The occurrence of wet shakes, teeth chattering, locomotion, scratching, and ptosis was significantly increased in the morphine group after IT naloxone, while an increase in the number of wet shakes was observed in the butorphanol group precipitated by IT naloxone. In addition, locomotion in IT morphine-dependent rats that received IT naloxone was much more severe than that of the IT butorphanol-dependent rats.

Withdrawal Signs Precipitated by IT CTOP

In rats receiving 48 nmol of IT CTOP, the composite withdrawal score was higher in IT morphine-dependent animals than in both saline-infused rats ($\chi^2 = 23.48$, $p < 0.01$) and IT butorphanol-dependent rats ($\chi^2 = 6.92$, $p < 0.01$) (Table 1). An IT injection with CTOP also precipitated significant abstinence signs in the butorphanol group ($\chi^2 = 4.69$, $p < 0.05$). CTOP, given IT, significantly induced wet shakes, teeth chattering, locomotion, and vocalization in morphine-dependent rats. Figure 2 demonstrates that the severity of wet shakes, teeth chattering, locomotion, stretching, and ptosis was significantly increased in the morphine group when compared to both the saline and the butorphanol groups. In contrast, no particular withdrawal sign observed in IT butorphanol-dependent rats receiving IT CTOP was significantly severe when compared to the saline group.

Withdrawal Signs Precipitated by IT Naltrindole

After IT naltrindole injection, the composite withdrawal score in both IT butorphanol-dependent group and IT morphine-dependent group was significantly higher than that of control group ($\chi^2 = 4.94$, $p < 0.05$; $\chi^2 = 14.64$, $p < 0.01$, respectively) (Table 1). In addition, only locomotion was signifi-

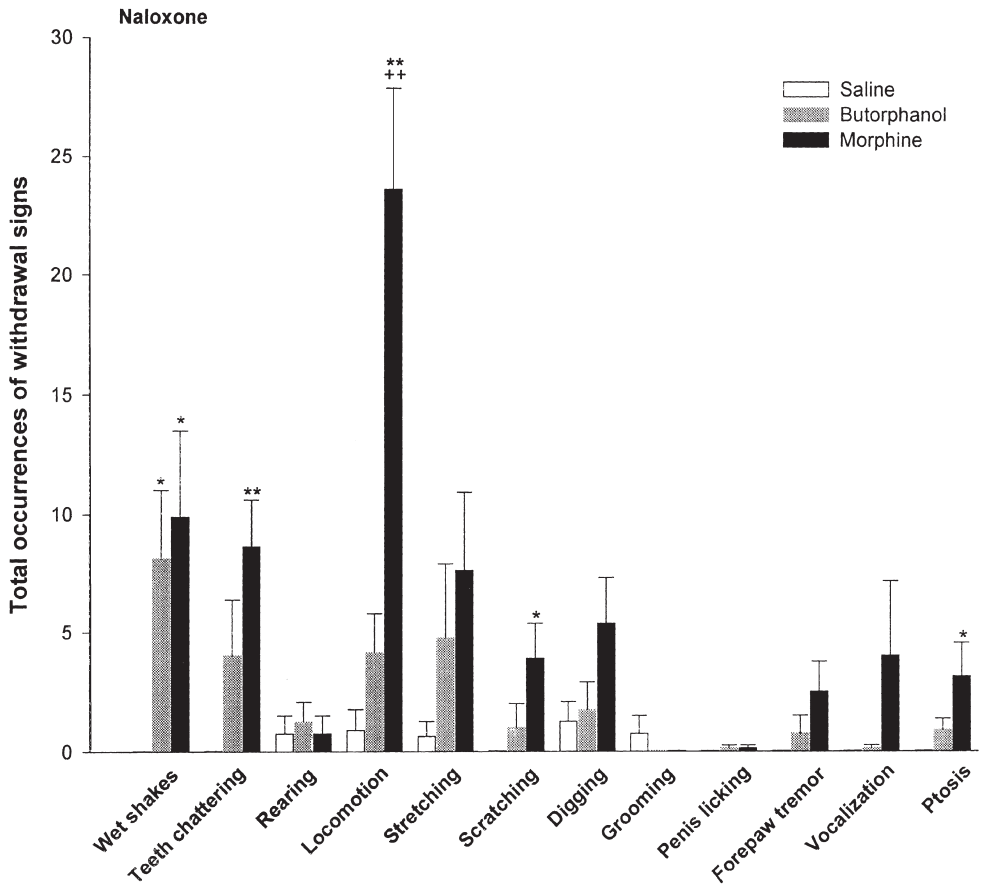

FIG. 1. Withdrawal signs (total occurrences) elicited by IT injection of the nonselective opioid receptor antagonist, naloxone, (48 nmol/10 μl) in animals given continuous IT infusion of saline (1 μl/h), butorphanol (52 nmol/μl/h), or morphine (26 nmol/μl/h) for 4 days. Data are mean ± SEM of eight animals in each group. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group; $^{++}p < 0.01$ significantly different between butorphanol- and morphine-dependent groups.

cantly increased in both dependent groups. As shown in Fig. 3, IT naltrindole administration produced a high intensity of locomotion only in both the IT butorphanol- and the IT morphine-infused rats.

Withdrawal Signs Precipitated by IT nor-BNI

Behavioral evidence of withdrawal was observed only in IT butorphanol-dependent rats (Table 1). The composite withdrawal score was significantly greater in the butorphanol-dependent group than in either the saline-treated group ($\chi^2 = 26.69$, $p < 0.01$) or morphine-dependent group ($\chi^2 = 15.15$, $p < 0.01$). Administration of IT nor-BNI did not precipitate abstinence signs in the IT morphine-infused group. Wet shakes, locomotion, and stretching were significantly increased in the IT butorphanol-dependent animals that received 48 nmol of IT nor-BNI. In addition, the severity of wet shakes, locomotion, stretching, and ptosis was markedly observed in IT butorphanol-dependent rats (Fig. 4). The intensity of rearing, stretching, and ptosis in the butorphanol group was significantly increased when compared to the morphine group.

DISCUSSION

The present results indicate that continuous IT infusion of both butorphanol and morphine results in the development of physical dependence. An IT administration of opioid antagonists was shown to precipitate withdrawal in the IT butorphanol-dependent and morphine-dependent animals. It is possible that supraspinal level might be involved in the development of dependence by migration and/or redistribution of the drugs following an IT injection and continuous IT infusion. However, several studies have demonstrated that the distribution of an IT-infused or injected dye is almost entirely localized in the spinal lumbar region (21,23). Therefore, the withdrawal signs precipitated by IT opioid antagonists in this study are most likely from the primary site of drug action in the spinal cord.

The phenomenon of withdrawal in rats elicited from different routes of naloxone administration has been determined. In morphine-dependent animals, behavioral signs of withdrawal elicited by intracerebroventricular (ICV), IT, and intraarterial (IA) routes of naloxone injection have been demonstrated to be qualitatively and quantitatively similar (22). In butorphanol-dependent rats, behavioral signs of withdrawal were observed following ICV and IP naloxone chal-

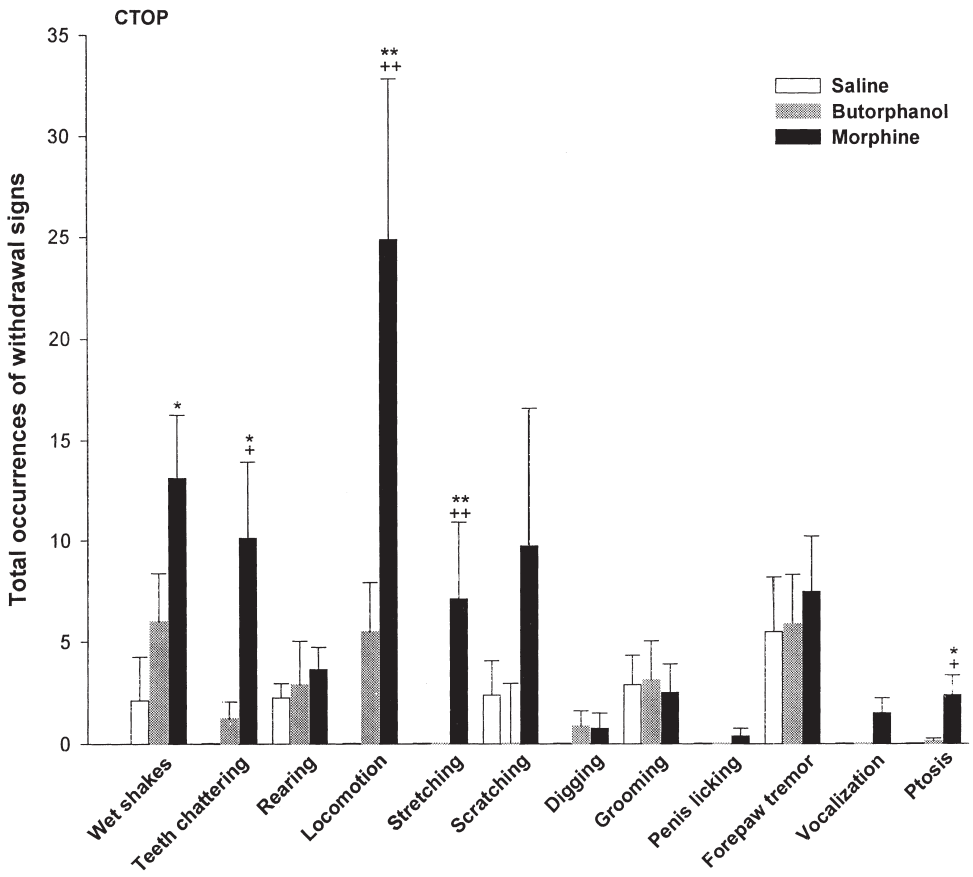

FIG. 2. Withdrawal signs (total occurrences) elicited by IT injection of the μ-opioid receptor antagonist, CTOP (48 nmol/10 μl), in animals given continuous IT infusion of saline (1 μl/h), butorphanol (52 nmol/μl/h), or morphine (26 nmol/μl/h) for four days. Data are mean ± SEM of eight animals in each group. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group; $^{+}p < 0.05$, $^{++}p < 0.01$ significantly different between butorphanol- and morphine-dependent groups.

lenge (9,34). Although administration of IT naloxone to butorphanol-dependent animals in the present study also significantly precipitated withdrawal signs, it is likely that behavioral signs of withdrawal elicited by IT naloxone administration were less than those observed with the ICV and IA naloxone challenge.

In a previous study from this laboratory, no differences in the severity or incidence of withdrawal signs were detected between rats chronically infused (ICV) with an equimolar concentration (52 nmol/h) of either butorphanol or morphine when withdrawal was precipitated by SC injection of naloxone (14). However, in the present study, precipitation of withdrawal by IT naloxone elicited a significantly greater incidence and severity of withdrawal in rats treated with 26 nmol/h of IT morphine than those receiving 52 nmol/h of IT butorphanol. Thus, it seems likely that morphine has a higher potency in the induction of dependence at the spinal level than butorphanol. This occurrence may be due to the fact that the μ-opioid receptor appears to mediate the majority of withdrawal signs in opioid dependence (4,7), that the distribution of opioid receptors in the spinal cord is relatively different (from highest to lowest) μ- >> δ- > κ-opioid receptors (20,30), and/or that the opioid receptor activities between butorphanol and morphine are different. Morphine is known to stimulate primarily μ- and possibly δ-opioid receptors (2,12), while butorphanol activates μ-, and δ-, as well as κ-opioid receptors (13). Therefore, the involvement of spinal opioid receptors in the development of dependence on IT butorphanol should be different from morphine.

The use of selective opioid antagonists in this study has shown the relative participation of different opioid receptors in the mediation of IT butorphanol and morphine dependence. CTOP, a cyclic somatostatin octapeptide, is a potent ligand with a 1000-fold selectivity for μ- over δ- and κ-opioid receptors in opioid receptor binding assays (28). Moreover, the ability of CTOP to antagonize chronic effects of morphine in vivo has been reported as well (12). The involvement of μ-opioid receptor in morphine dependence at both spinal and supraspinal levels is well established (6,24,29,31). An earlier report from this laboratory found that the μ-opioid receptor is also involved in the development of dependence on ICV butorphanol (11,25). Although our data are consistent with the participation of μ-opioid receptor in both butorphanol- and morphine-dependent animals, the activity of IT butorphanol at μ-opioid receptor is much less than that of IT morphine.

Involvement of δ-opioid receptor in the development of dependence on butorphanol and morphine is less well established. However, administration of naltrindole, a selective

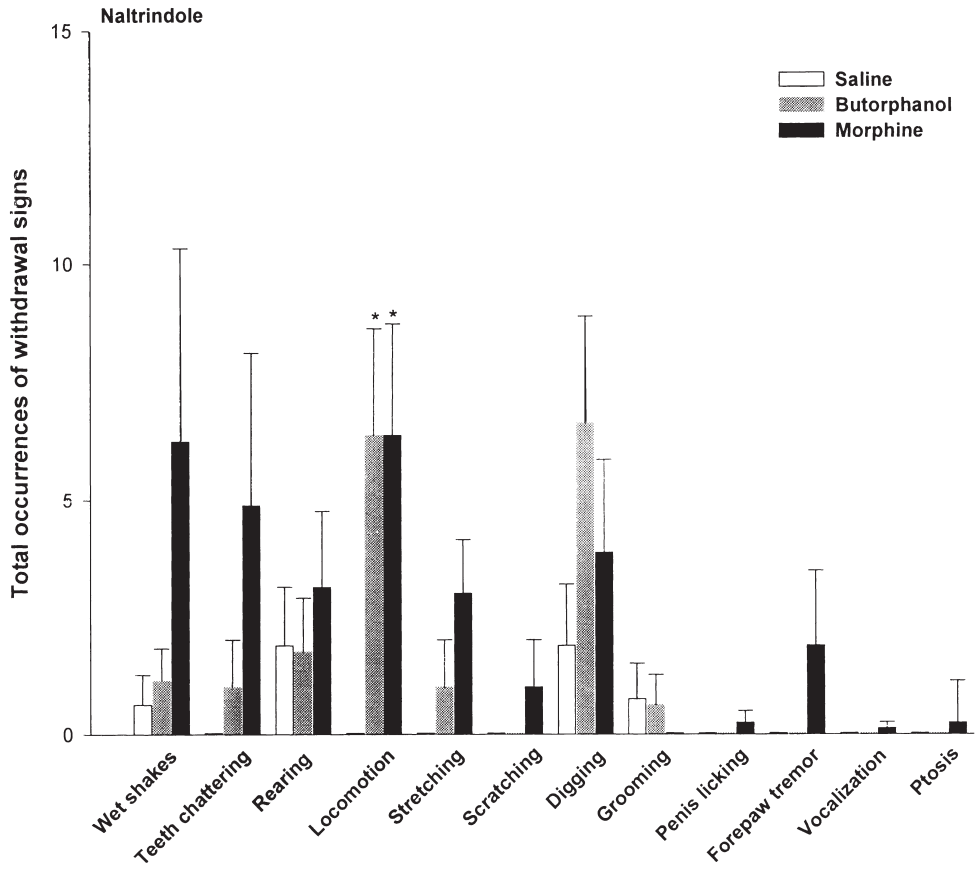

FIG. 3. Withdrawal signs (total occurrences) elicited by IT injection of the selective δ-opioid receptor antagonist, naltrindole (48 nmol/10 μl), in animals given continuous IT infusion of saline (1 μl/h), butorphanol (52 nmol/μl/h), or morphine (26 nmol/μl/h) for 4 days. Data are mean ± SEM of eight animals in each group. *$p < 0.05$ significantly different from control group.

δ-opioid receptor antagonist, has been demonstrated to precipitate withdrawal in both ICV butorphanol- and morphine-dependent rats (11,17). Furthermore, pretreatment with naltrindole was observed to prevent the development of butorphanol and morphine dependence (2,16). Recently, Suzuki et al. (32) demonstrated that pretreatment with highly selective δ-opioid receptor subtype antagonists, naltriban and naltrindole 5′-isothiocyanate for δ_2 and 7-benzylidenenaltrexone for δ_1 during chronic treatment with morphine, significantly suppressed naloxone precipitated withdrawal signs in morphine-dependent mice. The findings in this study also provide additional evidence in the role of δ-opioid receptor in the development of butorphanol and morphine dependence at the spinal level.

Nor-BNI, a long-acting κ-opioid receptor antagonist, was shown to have a 100-fold selectivity for the κ- over μ-opioid receptor when tested against U-50,448H (a selective κ-opioid receptor agonist) and morphine in the mouse writhing test (33). In addition, nor-BNI has a 160-fold selectivity for κ-, compared to μ-, and δ-opioid receptors in radioligand binding assays (33). Previous studies in this laboratory have demonstrated that ICV nor-BNI challenge can precipitate withdrawal in butorphanol-dependent rats (19), and pretreatment with ICV nor-BNI also blocks behavioral signs of naloxone-precipitated withdrawal in butorphanol-dependent rats (18). In the present study, IT nor-BNI administration can precipitate withdrawal from butorphanol, but not from morphine dependence. These results provide additional evidence at the spinal level to support the earlier conclusion from a previous study that κ-opioid receptor mediates behavioral signs of withdrawal in butorphanol-, but not morphine-dependent rats (10).

Although naloxone exerts relatively nonselective antagonistic effects with high affinity at μ-opioid receptor, its action in adequate doses is also effective to antagonize δ- and κ-opioid receptors as well (8). The relatively high dose (48 nmol) of naloxone used in this study was chosen to determine withdrawal signs that mediated through all three types of opioid receptors. Because of additive effects of these opioid receptors, the intensity of withdrawal signs elicited by naloxone was not lower than those of CTOP. Contrast to the present results, Gulya et al. (12) reported previously that CTOP administered ICV was 10–400 times more potent than naloxone in antagonizing morphine-induced analgesia and in producing withdrawal signs in ICV morphine-dependent animals. This may be due to difference in experimental designs. The previous study observed only withdrawal hypothermia and body weight loss by ICV injection, while the present study measured other withdrawal signs, such as locomotion, wet shakes,

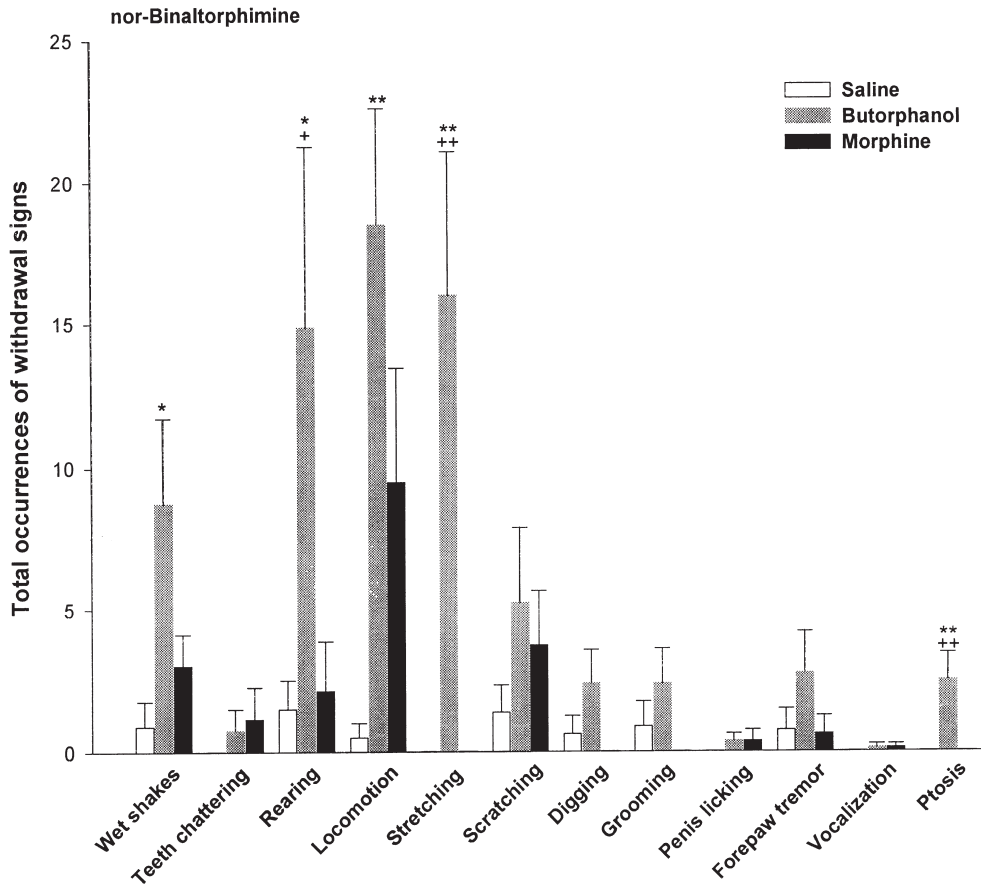

FIG. 4. Withdrawal signs (total occurrences) elicited by IT injection of the selective κ-opioid receptor antagonist, nor-binaltorphimine (nor-BNI) (48 nmol/10 μl), in animals given continuous IT infusion of saline (1 μl/h), butorphanol (52 nmol/μl/h), or morphine (26 nmol/μl/h) for 4 days. Data are mean ± SEM of eight animals in each group. $*p < 0.05$, $**p < 0.01$ vs. control group; $^{+}p < 0.05$, $^{++}p < 0.01$ significantly different between butorphanol- and morphine-dependent groups.

stretching, rearing, etc., via IT administration. For the κ antagonist, nor-BNI binds to both high-affinity κ_1-opioid receptor sites (45) and U-69,593 insensitive, low-affinity κ_2-opioid receptor sites. It has been reported that κ_1-opioid receptor sites predominate in rats' spinal cord (27). Therefore, IT butorphanol-dependent animals challenged with IT nor-BNI would exhibit more behavioral signs of withdrawal than those challenged with IT naloxone.

Of all spinal withdrawal signs, our observation indicates that locomotion is the most common sign in opioid-dependent animals precipitated by IT opioid antagonists. Thus, μ-, δ-, and κ-opioid receptors in the spinal cord are involved in this behavior. Wet shakes have been reported to be mediated by both spinal and supraspinal μ-opioid receptors (29). However, κ-opioid receptor in the brain has also been indicated to be involved in this behavioral sign (18,19). Present results suggest a role of κ-opioid receptors at spinal level in this behavior as well. Supraspinal μ- and κ-opioid receptors have been demonstrated to be involved in teeth chattering (19,34). Nevertheless, our findings indicate that spinal μ-, but not κ-opioid receptors, may also mediate this withdrawal sign. Furthermore, present observation also provides additional evidence suggesting that spinal μ- and κ-opioid receptors are possibly involved in stretching and ptosis, while only μ-opioid receptors are associated with vocalization. Surprisingly, one of the most characteristic signs of opioid withdrawal sign, namely stereotyped jumping or escape attempts, was not observed in this study probably due to the bigger area of container. This absence of jumping has also been reported previously in ICV morphine-dependent animals precipitated withdrawal by ICV injection of CTOP (12). However, the participation of spinal opioid receptors in behavioral signs of opioid withdrawal need to be further investigated.

While the selected doses of compounds are very important, full dose–response data of each agonist and antagonist would be ideal to complete pharmacological study. The dose of antagonists used in the present study was selected based on our previous studies that demonstrated the effective doses of these compounds to discriminate the withdrawal patterns in ICV opioid-dependent rats (9,11,17,34). Although they are highly selective opioid receptor antagonists, all of these agents may have interaction with additional opioid receptors. Therefore, the present results may not completely exclude the possible nonselective actions of these antagonists. However, these results may, at least, give credence to the importance of the spinal cord in development of dependence on opioids and

the relative contributions of different types of opioid receptors to the dependence development.

In summary, the present results demonstrate that continuous IT infusion of butorphanol and morphine leads to the development of dependence at the spinal level. However, the profile of withdrawal signs elicited by IT naloxone in IT morphine-dependent animals was higher than that in IT butorphanol-dependent animals. Spinal κ-opioid receptors are the major opioid receptors mediating IT butorphanol dependence, while spinal δ-, and, at least partly, μ-opioid receptors have a minor role in IT butorphanol dependence. On the other hand, spinal μ- and, partially, δ-opioid receptors play a major role in the development of IT morphine dependence.

ACKNOWLEDGEMENTS

This work was supported by Grant DA 05828 from the National Institute on Drug Abuse.

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