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Opioid Antagonists and Butorphanol Dependence

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JAW, S. P., B. HOSKINS AND I. K. HO. *Opioid antagonists and butorphanol dependence.* PHARMACOL BIOCHEM BEHAV **44**(3) 497–500, 1993. – Butorphanol has been known to act on μ-, δ-, and κ-opioid receptors. μ- and possibly δ-receptors are thought to mediate morphine dependence. Relative to morphine, butorphanol has a higher affinity for μ- and δ-receptors. In the present study, β-funaltrexamine (β-FNA) and naltrindole (NTI) (nonequilibrium μ- and δ-antagonist, respectively) were used to precipitate withdrawal in butorphanol-dependent rats. It was found that β-FNA (12, 24, 48, and 100 nM) did not elicit significant withdrawal behaviors, while NTI caused teeth-chattering (100 nM), wet shakes (100 nM), forepaw tremors (24 nM), yawning (48 and 100 nM), ejaculation (24 nM), and urination (100 nM). The present results indicate that δ-opioid receptors may be involved in mediating butorphanol dependence, while the involvement of μ-opioid receptors needs to be further investigated.

Butorphanol dependence β-Funaltrexamine Naltrindole μ- and δ-opioid receptors Precipitated withdrawal

NONMEDICAL uses of butorphanol, a synthetic opioid agonist/antagonist, have been reported (2,5). Butorphanol dependence has been documented in humans as well as in animals (8,17). Upon withdrawal, patients were presented with rhinnorhea, gastric discomfort, vomiting, dysphoria, emotional lability, and irritability. μ- and possibly δ-opioid receptors are thought to mediate morphine dependence (1). However, the relative involvement of opioid receptor subtypes in mediating butorphanol dependence remains an enigma. It is known that butorphanol can act on μ-, δ-, κ-, and possibly σ-opioid receptors (7). In comparison to morphine, butorphanol has higher affinities for μ- and δ-opioid receptors (7). And yet, relative to morphine, butorphanol is considered a partial agonist at μ- (16) and possibly δ-opioid receptors. In the present study, β-funaltrexamine (18,23) and naltrindole (19) (the nonequilibrium μ- and δ-opioid antagonists, respectively) were used to precipitate withdrawal in butorphanol-dependent rats. The study was designed to investigate the involvement of μ- and δ-opioid receptors in butorphanol withdrawal, which in turn provides us an insight into the involvement of μ- and δ-opioid receptors in the development of butorphanol dependence.

METHOD

Animals

Seven- to 8-week-old male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 251–300 g, were used. They were kept in a room with an ambient temperature of 21 ± 2°C and a 12 L : 12 D cycle for 1 week before surgery.

Surgical Procedures

Rats were anesthetized with Equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4 \cdot 7H_2O$, 0.972 g pentobarbital Na, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to make the final volume of 100 ml), 0.3 ml/100 g body weight, IP and then placed in a stereotaxic instrument.

An indwelling stainless steel guide cannula (26 ga, 10 mm long) was implanted into the right lateral cerebral ventricle (AP −0.5, LAT +1.3, and DV −4.5) (15). Lang's jet acrylic was applied to the surface of the skull, and a protective cap was placed around the cannula. After the acrylic had hardened, the animal was removed from the stereotaxic frame. A stylet (32-ga stainless steel tubing) was placed into the guide cannula to remain patent. The presence of cerebrospinal fluid (CSF) in the guide cannula was examined to assure proper placement. After surgery, rats were given 300,000 U procaine penicillin G (Pfizerpen-AS, Pfizer Corp., New York, NY) SC and allowed at least 1 week to recover before commencing the infusion of butorphanol (a generous gift from Bristol-Myers Corp., Evansville, IN).

Induction of Butorphanol Dependence

Animals were infused with butorphanol tartrate (26 nM/h) for 3 days via osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA). This infusion period and dose paradigm were determined to be optimal from preliminary experiments. Under ether anesthesia, animals were implanted SC with minipumps between the scapulae. A 4-cm piece of Tygon tubing

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(0.38 mm inner diameter, Cole-Palmer, Chicago, IL) was applied to connect the minipump to a piece of "L"-shaped stainless steel injector tubing (32 ga, 30 mm long) with the length of the guide cannula. Butorphanol tartrate solution was passed through a 0.2-mm Acrodisk filter (Gelman Scientific, Ann Arbor, MI) before it was introduced into the pumps. The delivery apparatus was assembled under sterile conditions. Minipumps were primed overnight at room temperature in normal saline so that optimal flow rates (1 μl/h) were obtained.

Measurement of Abstinence Signs

After 3 days of ICV infusion of butorphanol, rats were weighed. Three hours after termination of butorphanol infusion, these butorphanol-infused rats were randomly divided into three groups and then individually placed into 1-gal mayonnaise jars. A piece of filter paper was placed on the bottom of the jar to aid visualization of withdrawal-associated urination and diarrhea. After a 10-min acclimation period, rats were challenged, respectively, with β-funaltrexamine [Research Biochemicals, Inc. (RBI), Natick, MA], naltrindole (RBI) (12 nM/5 μl/rat; 24 nM/5 μl/rat; 48 nM/5 μl/rat; 100 nM/5 μl/rat for both drugs; at least six animals were used for each dose), or saline (5 μl/rat; 12 animals used) ICV. All drugs were dissolved in saline. Precipitated withdrawal signs were observed for 30 min on a quantal basis (i.e., number of animals exhibiting more than two episodes of teeth-chattering, rearing, wet shakes, forepaw tremors, and ejaculation; and number of animals showing a single episode of escape behavior, yawning, ptosis, salivation, diarrhea, and urination). Body weight and rectal temperature were measured before and 1 h after administration of opioid antagonists. In addition, the total number of withdrawal signs (teeth-chattering, wet shakes, forepaw tremors, and ejaculation) was recorded for a 30-min period to assess the severity of precipitated withdrawal by μ- or δ-antagonists. The treatments animals received were blind to the independent observers. Examinations of brains after injections of methylene blue dyes through cannulae revealed that the drug solutions were infused directly into the right cerebral lateral ventricle.

Statistics

Quantal (all or none) data from the behavioral studies on the experimental groups and saline controls were compared using the χ^2 test. The two-tailed Student's paired *t*-test (mean ± SEM) was used to compare total occurrence data between experimental groups and saline controls, as well as changes in body weight and rectal temperature of animals before and after challenge with the antagonists. A p value < 0.05 was considered significant.

RESULTS

β-Funaltrexamine-Precipitated Butorphanol Withdrawal

No behavioral signs were detected in butorphanol-dependent rats after receiving ICV β-FNA except at 12 nM, at which one of six animals exhibited ejaculation and urination. In contrast to butorphanol-dependent animals receiving ICV saline, ICV β-FNA-treated rats showed a suppression of overall activities throughout the dose range tested.

Both β-FNA and saline exerted a hyperthermic effect in butorphanol-dependent rats. No significant difference was found in the loss of body weight between β-FNA- and saline-treated dependent animals.

In terms of total occurrence of each withdrawal sign, no significant differences were observed for dependent animals receiving β-FNA and saline.

Naltrindole-Precipitated Butorphanol Withdrawal

In rats receiving 100 nM naltrindole ICV all butorphanol-dependent animals showed teeth-chattering and wet shakes (Table 1). After receiving 24 nM naltrindole ICV, a significantly higher percentage of dependent rats exhibited forepaw tremors ($p < 0.025$) when compared with ICV saline controls (Table 1). Yawning was significantly increased in animals that received 48 nM ($p < 0.05$) and 100 nM ($p < 0.01$) of ICV naltrindole (Table 1). At 24 nM, ICV naltrindole elicited ejaculation in a significantly higher number of dependent animals compared with those receiving ICV saline ($p < 0.025$). Urination was detected in a significantly higher percentage of

TABLE 1

WITHDRAWAL SIGNS PRECIPITATED BY ICV-ADMINISTERED NALTRINDOLE (AN IRREVERSIBLE δ-ANTAGONIST) IN BUTORPHANOL-DEPENDENT RATS

	naltrindole (nM/5 μl/rat)				
	0 (n = 12)	12 (n = 13)	24 (n = 13)	48 (n = 10)	100 (n = 6)
Teeth-chattering	1/12	n.d.	3/13	4/10	6/6*
Wet shakes	2/12	n.d.	7/13	5/10	6/6*
Forepaw tremors	1/12	n.d.	7/13†	2/10	2/6
Yawning	n.d.	n.d.	2/13	3/10‡	3/6§
Ptosis	1/12	2/13	1/13	n.d.	2/6
Ejaculation	2/12	1/13	8/13†	3/10	2/6
Urination	2/12	5/13	5/13	n.d.	4/6‡
Diarrhea	n.d.	n.d.	n.d.	n.d.	n.d.
Hyperthermia¶	0.8 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	0.6 ± 0.2
Weight loss (%)	1.8	1.6	1.3	1.4	1.3

n.d., none detected.

*$p < 0.01$, †$p < 0.05$, ‡$p < 0.025$, §$p < 0.01$ (values are significantly higher than the control values as determined by the χ^2 test).

¶Numbers in °C.

TABLE 2

WITHDRAWAL SIGNS (TOTAL OCCURRENCE) PRECIPITATED BY ICV-ADMINISTERED NALTRINDOLE IN BUTORPHANOL-DEPENDENT RATS

	Naltrinodole (nM/5 μl/rat)				
NTI	0	12	24	48	100
TC	0.3 ± 0.3	0.0 ± 0.0	2.5 ± 1.3*	3.9 ± 1.6*	14.8 ± 1.8†
WS	1.6 ± 0.9	0.1 ± 0.1	4.8 ± 1.7	4.7 ± 2.4	13.3 ± 3.5†
FT	0.4 ± 0.3	0.2 ± 0.1	1.6 ± 0.8	0.4 ± 0.2	0.7 ± 0.5
EJ	0.5 ± 0.4	0.1 ± 0.1	1.5 ± 0.6	0.7 ± 0.4	1.2 ± 0.7

TC, teeth-chattering; WS, wet shakes; FT, forepaw tremors; EJ, ejaculation.
*$p < 0.05$, †$p < 0.01$ (values are significantly higher than the control values as determined by Student's t-test).

dependent rats after 100 nM naltrindole ICV ($p < 0.05$) (Table 1). Diarrhea was not detected in dependent animals following both saline and naltrindole treatments (Table 1).

Both saline- and naltrindole-treated dependent rats showed hyperthermia (Table 1). No significant difference was seen in loss of body weight of dependent animals after saline or naltrindole treatment (Table 1).

Naltrindole, given ICV, significantly increased the incidents of teeth-chattering (24, 48 nM, $p < 0.05$; 100 nM, $p < 0.01$) (Table 2) and wet shakes (100 nM, $p < 0.01$) in butorphanol-dependent rats (Table 2).

DISCUSSION

Cowan et al. (4) examined opioid physical dependence by direct infusion of selective agonists for μ- [(D-Ala^2, MePhe^4, Gly-ol^5) enkephalin (DAGO)], κ- [3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide (U-50,488H)], and δ- [D-Pen^2, D-Pen^5] enkephalin (DPDPE)] opioid receptors into the cerebral aqueduct in rats. They found that a low to moderate dependence was associated with the δ-agonist, DPDPE, and maximal dependence was achieved by the infusion of morphine or DAGO (μ-agonists).

In addition to μ-opioid receptors (6), δ-opioid receptors have been implicated in mediating morphine dependence (1). Despite a lower efficacy at μ-receptors (16), relative to morphine, butorphanol has a higher affinity for μ- and δ-receptors (7). We therefore hypothesize that μ- and δ-opioid receptors may mediate butorphanol dependence.

Our results indicate that β-FNA seems to suppress withdrawal behaviors in the dose range tested. Further, only 100 nM naltrindole precipitated teeth-chattering, wet shakes, yawning (48 nM also), and urination (Table 1). These data tend to suggest that μ-receptors may not be involved while δ-receptors may be involved in mediating butorphanol dependence.

β-FNA was synthesized by Portoghese et al. (18) and determined to have reversible κ-agonist activities and irreversible μ-antagonist effects against morphine in vitro in guinea pig ileal longitudinal muscle and mouse vas deferens preparations (21,23). In vivo, β-FNA showed analgesic effects of brief duration that seemed to be mediated by κ-receptors and long-acting antagonist activities that were μ-receptor-selective (23). In vitro receptor binding and autoradiographic studies demonstrated that at low concentrations (1-10 nM) [^{3}H]β-FNA covalently binds to μ-receptors with high specificity (11,13). At concentrations greater than 10 nM, β-FNA also binds irreversibly to δ-receptors (22). Finally, brain membranes were incubated with 5 nM [^{3}H]β-FNA; specific irreversible binding plateaued in 60 min (12).

The interpretation of the present results has to take both in vivo and in vitro pharmacological properties of β-FNA into consideration. Because β-FNA has acute reversible in vivo κ-agonist effects (10), irreversible long-lasting μ-antagonist actions, and possible interactions with δ-receptors at doses greater than 10 nM (both μ- and δ-receptor effects take longer to develop; while κ-receptor effects are almost immediate), it is therefore possible that β-FNA, due to its acute κ-agonist effects, suppresses butorphanol withdrawal. In addition, we demonstrated that ICV nor-binaltorphimine (an irreversible κ-antagonist) can precipitate butorphanol withdrawal, qualitatively and quantitatively similar to that precipitated by naloxone (a nonspecific antagonist) (Jaw et al., in preparation).

The involvement of μ-receptors in butorphanol dependence cannot be ruled out by the present results. Further, experiments in our laboratories have demonstrated that β-FNA pretreatment can attenuate butorphanol dependence (14). Therefore, another μ-selective antagonist, cyclic somatostatin octapeptide (CTOP), may be a more useful tool with which to study and define the involvement of μ-receptors in butorphanol dependence.

Based upon the "message-address" concept, Portoghese et al. (19) developed naltrindole HCl, a nonpeptide δ-receptor antagonist. In vitro, naltrindole (K_e = 0.1- to 0.3-nM range) antagonized δ-selective agonists but not μ- and κ-agonists in the guinea pig ileum and in the mouse and rat vas deferens smooth muscle preparations (20). In mice, naltrindole (20 mg/kg) SC antagonized the analgesia produced by the δ-preferring agonists DSLET [(D-SER^2, Leu^5, The^6) enkephalin] and DPDPE, but not that produced by the κ-agonist, U-50,488H, or the μ-agonists, morphine and DAGO (3,20).

Our data indicate that only 100 nM naltrindole has a significant effect to precipitate butorphanol withdrawal. This tends to suggest that the δ-receptor is involved in butorphanol dependence. In support of this suggestion, we recently demonstrated that pretreatment with 0.1, 1, and 10 nM naltrindole blocked the development of butorphanol dependence (9).

In summary, our studies reveal that β-FNA and naltrindole are weak in eliciting butorphanol withdrawal behaviors. Our pretreatment studies with β-FNA and naltrindole indicate that μ- and δ-opioid receptors are involved in the development of butorphanol dependence (9,14). And, pretreatment with nor-binaltorphimine blocked the development of butorphanol dependence (9). Therefore, we propose that μ- and δ-opioid re-

ceptors have a permissive role in butorphanol dependence, while κ-receptors are the major opioid receptor subtype mediating butorphanol dependence, that is, animals have become dependent upon butorphanol, μ- and δ-receptor antagonists are less efficacious in precipitating withdrawal behaviors than are the κ-receptor antagonists or the nonspecific opioid receptor antagonists.

The actions of β-FNA and the selectivity (for different opioid receptor subtypes) of high concentrations (100 nM) of naltrindole used in the present study can be resolved when radioligand opioid receptor binding studies are carried out. These investigations are currently underway in our laboratories.

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REFERENCES

1. Abdelhamid, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. Selective blockage of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. J. Pharmacol. Exp. Ther. 258:299–303; 1991.
2. Brown, G. R. Stadol dependence: Another case. JAMA 254:910; 1985.
3. Calcagnetti, D. J.; Holtzman, S. G. Delta opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine. Pharmacol. Biochem. Behav. 38:185–190; 1991.
4. Cowan, A.; Zhu, X. Z.; Mosberg, H. I.; Omnaas, J. R.; Porreca, F. Direct dependence studies in rats with agents selective for different types of opioid receptor. J. Pharmacol. Exp. Ther. 246: 950–955; 1988.
5. Evans, W. S.; Bowen, J. N.; Giordano, F. L.; Clark, B. A case of stadol dependence. JAMA 253:2191–2192; 1985.
6. Gulya, K.; Krivan, M.; Nyolczas, N.; Sarnyai, Z.; Kovacs, G. L. Central effects of the potent and highly selective mu opioid antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) in mice. Eur. J. Pharmacol. 150:355–360; 1988.
7. Horan, P. J.; Ho, I. K. Comparative pharmacological and biochemical studies between butorphanol and morphine. Pharmacol. Biochem. Behav. 34:847–854; 1989.
8. Horan, P.; Ho, I. K. The physical dependence liability of butorphanol: A comparative study with morphine. Eur. J. Pharmacol. 203:387–391; 1991.
9. Jaw, S. P.; Oh, K. W.; Makimura, M., Hoskins, B.; Ho, I. K. Effects of β-funaltrexamine on naloxone-precipitated butorphanol-withdrawal. FASEB J. 6:1574; 1992.
10. Jiang, Q.; Heyman, J. S.; Sheldon, R. J.; Koslo, R. J.; Porreca, F. Mu antagonist and kappa agonist properties of β-funaltrexamine in vivo: Long-lasting spinal analgesia in mice. J. Pharmacol. Exp. Ther. 252:1006–1011; 1990.
11. Liu-Chen, L.-Y.; Li, S. X.; Tallarida, R. J. Studies on kinetics of [^{3}H] β-funaltrexamine binding to mu opioid receptor. Mol. Pharmacol. 37:243–250; 1990.
12. Liu-Chen, L.-Y.; Li, S.; Lewis, M. E. Autoradiographic study of irreversible binding of [^{3}H] β-funaltrexamine to opioid receptors in the rat forebrain: Comparison with mu and delta receptor distribution. Brain Res. 544:235–242; 1991.
13. Liu-Chen, L.-Y.; Li, S.; Wheeler-Aceto, H.; Cowan, A. Effects of intracerebroventricular β-funaltrexamine on mu and delta opioid receptors in the rat: Dichotomy between binding and antinociception. Eur. J. Pharmacol. 203:195–202; 1991.
14. Oh, K. W.; Makimura, M.; Jaw, S. P.; Hoskins, B.; Ho, I. K. Effects of β-funaltrexamine on butorphanol dependence. Pharmacol. Biochem. Behav. 42:29–34; 1992.
15. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. Orlando, FL: Academic Press; 1986.
16. Pickers, M. J.; Negus, S. S.; Craft, R. M. Butorphanol's efficacy at mu and kappa opioid receptors: Inferences based on the schedule-controlled behavior of nontolerant and morphine-tolerant rats and on the responding of rats under a drug discrimination procedure. Pharmacol. Biochem. Behav. 36:563–568; 1990.
17. Pircio, A. W.; Gylys, J. A.; Cavanagh, R. L.; Buyniski, J. P.; Bierwagen, M. E. The pharmacology of butorphanol, a 3,14-dihydroxymorphinan narcotic antagonist analgesic. Arch. Int. Pharmacodyn. 220:231–257; 1976.
18. Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E. A novel opioid receptor site directed alkylating agent with irreversible narcotic antagonistic and reversible agonistic activities. J. Med. Chem. 23:233–234; 1980.
19. Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. Application of the message-address concept in the design of highly potent and selective nonpeptide delta opioid receptor antagonists. J. Med. Chem. 31:281–282; 1988.
20. Portoghese, P. S.; Sultana, M.; Takemori, A. E. Naltrindole, a highly selective and potent nonpeptide delta opioid receptor antagonist. Eur. J. Pharmacol. 146:185–186; 1988.
21. Takemori, A. E.; Larson, D. L.; Portoghese, P. S. The irreversible narcotic antagonistic and reversible agonistic properties of the fumaramate methyl ester derivative of naltrexone. Eur. J. Pharmacol. 70:445–451; 1981.
22. Tam, S. W.; Liu-Chen, L.-Y. Reversible and irreversible binding of β-funaltrexamine to mu, delta and kappa opioid receptors in guinea pig brain membranes. J. Pharmacol. Exp. Ther. 239:351–357; 1986.
23. Ward, S. J.; Portoghese, P. S.; Takemori, A. E. Pharmacological characterization in vivo of the novel opiate, β-funaltrexamine. J. Pharmacol. Exp. Ther. 220:494–498; 1982.