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Effects of β-Funaltrexamine on Butorphanol Dependence

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OH, K. W., M. MAKIMURA, S. P. JAW, B. HOSKINS AND I. K. HO. *Effects of β-funaltrexamine on butorphanol dependence.* PHARMACOL BIOCHEM BEHAV **42**(1) 29–34, 1992.—The present experiments were performed to investigate the effects of the selective μ opioid receptor antagonist, β-funaltrexamine (β-FNA), on the physical dependence liability of butorphanol (a mixed agonist/antagonist opioid analgesic). Butorphanol (26 nmol/μl/h) was continuously infused via osmotic minipumps into the lateral cerebral ventricle of male Sprague-Dawley rats for 72 h. β-FNA (12, 24, and 48 nmol/5 μl/rat) was administered ICV 3 h prior to and 48 h after initiation of the butorphanol infusion. Treatment with β-FNA significantly diminished naloxone-induced escape behavior, hypothermia, and loss of body weight in a dose-dependent manner, while naloxone-induced teeth-chattering, forepaw tremors, and urination were also reduced, but in a dose-independent manner. These results suggest that the μ opioid receptor is partially involved in the development of physical dependence upon butorphanol.

β-Funaltrexamine Butorphanol Abstinence signs Opioid receptors

BUTORPHANOL was introduced as a potent synthetic analgesic agent with a lower physical dependence liability than other opioid analgesics (9,19,21). However, marked physical dependence has been observed in animals after continuous administration of this compound (17,18). In addition, in a primary dependence study in man in which large doses of butorphanol were administered for a prolonged period of time a severe physical dependence syndrome was reported (11). A recent study in our laboratory demonstrated that large doses of butorphanol given by continuous (ICV) infusion to rats resulted in substantial physical dependence. Furthermore, the nature of the physical dependence syndromes produced by butorphanol under these conditions was similar to that induced by an equimolar dose of morphine (10).

Much work has been done in an attempt to define the mechanisms involved in the development of physical dependence on opioids, and although several neurotransmitters have been implicated in the production of physical dependence on these drugs (22), little information is available concerning their effects on opioid receptors. A few studies have investigated the role(s) of opioid receptors in physical dependence on opioids (5,6,12), and it is now widely believed that μ and δ receptors mediate physical dependence. The role of the κ receptor in physical dependence is minor since negligible levels of abstinence signs were precipitated by naloxone after rats were infused with some selective κ ligands for 70 h via SC-implanted osmotic minipumps (5).

The pharmacology of butorphanol is complex due to its apparent multiplicity of actions on the opioid receptor systems. Butorphanol has high affinity in vitro and in vivo, and can act as an agonist or an antagonist at μ, δ, and κ opioid receptors depending upon conditions employed (13,25). The question then arises as to which receptor or receptors mediate(s) physical dependence upon butorphanol.

β-Funaltrexamine (β-FNA) has been described as a selective, long-acting, and irreversible opioid antagonist at the μ receptor (3). Because of these properties, β-FNA has become an important tool for characterizing μ opioid receptors (23,24). The present experiments utilized β-FNA to investigate the involvement of μ opioid receptors in physical dependence upon butorphanol.

METHOD

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 251–300 g, were used in these studies. They were kept in a room maintained at an ambient temperature of 21 ± 2°C and on a 12L:12D cycle for at least 7 days upon arrival.

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Surgical Procedures

Rats were anesthetized with equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4$ $7H_2O$, 0.972 g pentobarbital Na, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to bring the solution to 100 ml), 0.3 ml/100 g body weight, IP and then placed in a stereotaxic frame.

A permanent, bevelled, indwelling stainless steel guide cannula (26 ga, 10 mm long) was then implanted into the right lateral ventricle (AP: −0.5, LAT: +1.3, and DV: −4.5) (20). Cranioplast (Lang's jet acrylic) was applied to the surface of the skull and a protective cap placed around the cannula (to prevent its destruction by the rat upon awakening from anesthesia). After the cranioplast had firmly hardened, the animal was removed from the stereotaxic instrument. A stylet (32-ga stainless steel tubing) was placed into the guide cannula to allow the cannula to remain patent. Correct placement was determined by the presence of CSF in the guide cannula. After surgery, rats were administered 300,000 units of penicillin G procaine (Pfizerpen-AS, Pfizer Corp., New York, NY), SC, and allowed at least a 1-week recovery period prior to the induction of physical dependence upon butorphanol (a generous gift from Bristol-Myers Corp., Evansville, IN).

Administration Schedule and Induction of Butorphanol Dependence

Animals were infused with butorphanol tartrate (26 nmol/μl/h) for 3 days via osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA). This dose and period of infusion was determined through preliminary experiments to be optimal for the study of butorphanol dependence using this model. The minipumps were implanted SC between the scapulae of animals while they were under ether anesthesia. A 4-cm piece of Tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL) was used to attach the minipump to a piece of "L"-shaped stainless steel injector tubing (32 ga, 30 mm long) cut to the length of the guide cannula. Butorphanol solution was passed through a 0.2-mm Acrodisk filter (Gelman Scientific, Ann Arbor, MI) prior to introduction into the pumps, and the pumps were assembled using sterile techniques. The minipumps were primed overnight at room temperature in normal saline so their optimal flow rate (1 μl/h) was achieved. Four groups of butorphanol-infused rats received β-FNA (RBI., Natick, MA) as follows: 12 nmol/5 μl/rat, 24 nmol/5 μl/rat, 48 nmol/5 μl/rat, and saline (5 μl/rat) ICV through an indwelling stainless steel guide cannula 3 h prior to and 48 h after initiation of butorphanol infusion.

Measurement of Abstinence Signs

After 3 days of ICV infusion, rats weighed and placed in 1 gallon mayonnaise jars. To aid in visualizing abstinence-related urination and diarrhea, the jar was lined on the bottom with a piece of white filter paper. After a 10-min acclimation period, rats were challenged with naloxone (1 mg/kg, SC) (Sigma Chemical Corp., St. Louis, MO). Ten different signs associated with precipitated withdrawal were observed for 30 min on a quantal basis, that is, number of animals exhibiting more than two episodes of teeth-chattering, wet shakes, rearing, penis-licking, and forepaw tremors, and number of animals exhibiting a single episode of escape behavior, yawning, ptosis, diarrhea, and urination. Loss of body weight (number of animals exhibiting >3% body weight loss) and rectal temperatures were monitored over a 1-h period after naloxone administration. In addition, the incidence [total number of occurrences of each abstinence sign (teeth-chattering, wet shakes, rearing, penis-licking, and forepaw tremors per 30 min)] was determined to demonstrate the severity of abstinence syndromes. Autopsies after experiments revealed that animals received the drug solutions directly into the right lateral ventricle.

Statistics

Data were analyzed by the χ^2 test (number of animals exhibiting each abstinence sign) and by Student's *t*-test (mean ± SE of total number of occurrences of each abstinence sign).

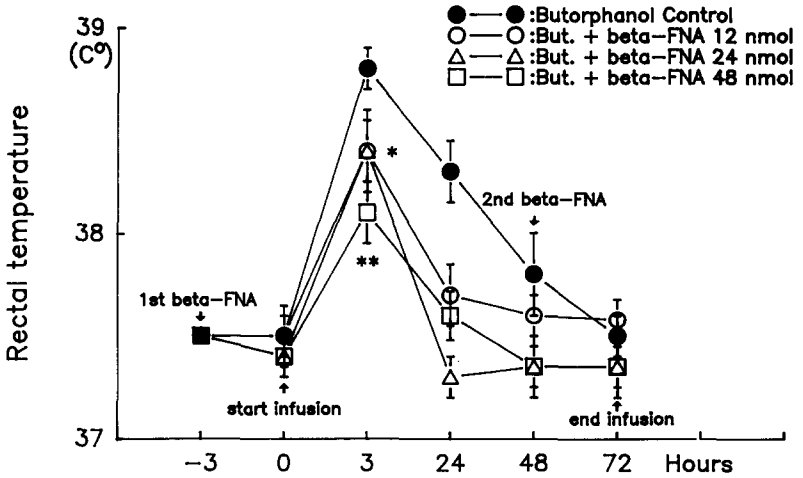

FIG. 1. Effects of β-FNA on butorphanol-induced rectal temperature changes. Animals were administered continuous ICV infusions of butorphanol. β-FNA was injected ICV 3 h prior to and 48 h after initiation of butorphanol infusions. *$p < 0.05$, **$P < 0.01$ (values are significantly lower than after 3 h of butorphanol infusion). Data are expressed as the mean ± SE for each treatment group.

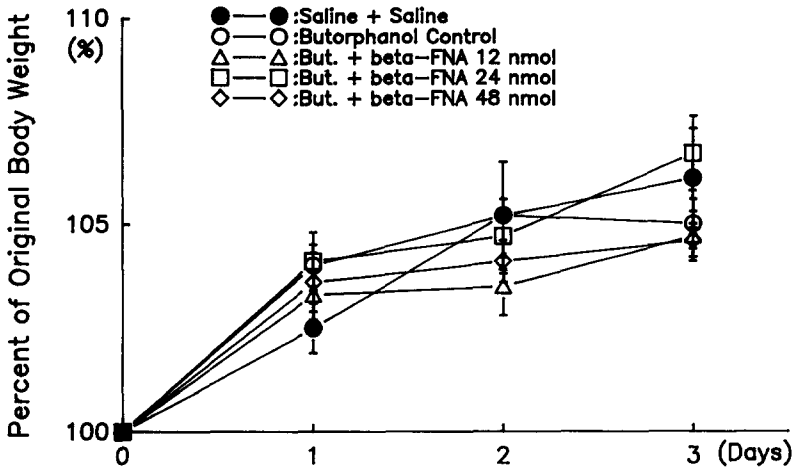

FIG. 2. Effect of β-FNA on butorphanol-induced body weight changes. Animals were administered continuous ICV infusion of saline or butorphanol for 3 days. β-FNA was injected ICV 3 h prior to and 48 h after initiation of butorphanol infusions.

RESULTS

Rectal temperatures were elevated 3 h after initiation of butorphanol ICV infusion and returned to normal after 3 days of butorphanol infusion. Treatment with β-FNA 3 h prior to initiation of butorphanol infusion significantly inhibited its hyperthermic effect. However, β-FNA alone had no effect on rectal temperatures of rats (Fig. 1).

There was no significant difference in body weights between saline- and butorphanol-infused rats. Furthermore, β-FNA had no effect upon body weights in butorphanol-infused rats (Fig. 2).

Although some behaviors were observed in a few saline-infused rats (vehicle) challenged with naloxone, penis-licking was significantly increased in the groups treated with 24 and 48 nmol of β-FNA (Table 1).

Furthermore, in butorphanol-infused rats various and severe abstinence signs precipitated by naloxone were observed. The symptoms observed after naloxone were qualitatively similar to those reported in morphine-infused rats (1).

Administration of two ICV injections of each of the three doses of β-FNA during the butorphanol infusion reduced naloxone-induced escape behavior, teeth-chattering, diarrhea, forepaw tremors, weight loss, and urination on a quantal basis (all or none, by χ^2 test) (Table 2). Escape behavior, hypothermia, and weight loss (>3%) were reduced in dose-dependent manner. Escape behavior was completely blocked by 48 nmol β-FNA. Teeth-chattering, forepaw tremors, and urination were reduced by treatment with β-FNA in a dose-independent manner (within the range of doses tested). Forepaw tremors and diarrhea were reduced by 24 and 48 nmol β-FNA, respectively. Urination was also reduced in these experiments. However, penis-licking was increased at doses of 24 nmol and higher.

On the other hand, the incidences of all butorphanol abstinence signs (except rearing and penis-licking) were significantly reduced by β-FNA (Table 3).

DISCUSSION

Continuous ICV infusions were chosen for these experiments for a variety of reasons. First, administration of compounds by this route requires minimal pharmacokinetic considerations, such as drug metabolism and protein binding, so that effects observed are primarily those of the parent compound. Second, since the majority of the effects of opioids is mediated by the CNS (16), the ICV route delivers the drug directly to its site of action, thus providing a rapid development of and a more definitive nature of the endpoint of interest. Finally, continuous ICV administration of opioids has been shown to be a more efficient means of producing dependence than continuous systemic administration (2). The naloxone-induced abstinence signs observed in butorphanol-infused rats were similar to those reported in morphine-dependent rats (1). In addition, Horan and Ho (10) found that the nature of the abstinence signs produced by butorphanol under these conditions was similar to that induced by an equimolar dose of morphine.

The dose-related decreases in naloxone-induced escape behavior and weight loss in rats treated with the irreversible μ opioid receptor antagonist, β-FNA, suggest that both these abstinence signs may be mediated via the μ opioid receptor. Cowan et al. (5) demonstrated that weight loss predominantly reflected abstinence at the μ (DAGO, morphine) receptor in rats, and escape behavior (jumping) was only associated with withdrawal from these ligands, that is, jumping was not observed in δ and κ agonist-dependent rats.

Withdrawal-induced rearing and wet shakes appear to be characteristic signs of abstinence from δ agonists; whereas jumping, diarrhea, and loss of body weight appear to be unrelated to withdrawal from δ agonists (5). We observed that withdrawal-induced rearing was much more frequent in butorphanol-infused rats than in saline-infused rats. Furthermore, DPDPE- (δ agonist) induced rearing has been shown to be selectively reduced by injection of the δ receptor antagonist, ICI-174,864 (4). Our results, in which β-FNA did not reduce rearing, are additional evidences that withdrawal-induced rearing in butorphanol-dependent rats does not involve mediation by μ receptors. Lee et al. (15) reported that DPDLE- (μ and δ agonist) induced wet shakes were antagonized by β-FNA, but not by ICI-174,864, suggesting this behavior is mediated by activation of μ rather than δ receptors in the ventral hippocampus.

TABLE 1

EFFECTS OF β-FNA ON BEHAVIORS CHALLENGED WITH NALOXONE (1 mg/kg, SC) AFTER ICV INFUSION OF SALINE FOR 72 h

	Treatment with β-FNA (nmol/5 μl/rat)			
	0	12	24	48
Escape behavior	0/19*	0/8	0/8	0/8
Teeth-chattering	2/19	1/8	2/8	1/8
Wet shakes	6/19	3/8	1/8	3/8
Rearing	15/19	7/8	6/8	6/8
	(3.1 ± 1.4)†	(7.0 ± 1.4)	(6.0 ± 1.8)	(3.9 ± 1.0)
Yawning	0/19	0/8	0/8	0/8
Ptosis	0/19	0/8	0/8	0/8
Penis-licking	1/19	1/8	4/8‡	5/8‡
Diarrhea	0/19	0/8	0/8	2/8
Forepaw tremors	2/19	2/8	3/8	1/8
Weight loss (>3%)	1/19	1/8	0/8	1/8
Urination	4/19	4/8	3/8	1/8
Rectal Temp. (°C)	0.0 ± 0.1§	0.2 ± 0.1	0.1 ± 0.2	−0.2 ± 0.1

*Numerator values are number of animals displaying this behavior; denominator values are the total number of animals per group.

†Values are mean ± SE of the total number of occurrences of rearing.

‡$p < 0.05$ (values are significantly higher than the control values as determined by the χ^2 test).

§Values are mean ± SE of changes of rectal temperatures.

Studies have attempted to clarify thermic responses to opioid receptor activation. It appears that the μ and κ opioid receptors are involved in the hyper- and hypothermic responses, respectively. In addition, Hayes et al. (7) found that hypothermia could be mediated by both μ and κ receptors. Our studies showed that naloxone-induced hypothermia in butorphanol-dependent rats was inhibited by β-FNA, suggesting mediation via the μ receptor because β-FNA has been shown to antagonize μ opioid receptor-mediated effects while having no antagonistic effect on κ receptors both in vitro (23) and in vivo (24). On the other hand, 3 h of butorphanol infusion resulted in hyperthermia. Since β-FNA at the doses used in these studies partially inhibited this butorphanol-induced hyperthermia, it would appear that butorphanol-induced hyperthermia is mediated by the μ receptor but that other opioid receptors may also be involved. Butorphanol administration also resulted in a profound diuresis. Opiate- and opioid-mediated diuresis is thought to be a result of ADH inhibition via κ opioid receptor activation (14). We found that urination in butorphanol-dependent rats was reduced by β-FNA. The significance of this finding is still unknown. However, since others (8,26) have shown that diuresis produced by κ agonists is

TABLE 2

EFFECTS OF β-FNA ON NALOXONE-PRECIPITATED ABSTINENCE SIGNS (ALL OR NONE) IN BUTORPHANOL-DEPENDENT RATS

	Treatment with β-FNA (nmol/5 μl/rat)			
	0	12	24	48
Escape behavior	15/45	1/22*	1/35†	0/25†
Teeth-chattering	43/45	11/22†	24/35†	18/25†
Wet shakes	38/45	17/22	24/35	19/25
Rearing	40/45	21/22	24/35	19/25
Yawning	4/45	0/22	4/35	3/25
Ptosis	21/45	17/22	13/35	12/25
Penis-licking	28/45	13/22	32/35‡	22/25‡
Diarrhea	18/45	11/22	8/35	2/25*
Forepaw tremors	24/45	9/22	6/35†	9/25
Weight loss (>3%)	30/45	8/22*	13/35*	7/25†
Urination	27/45	9/22	3/35†	3/25†
Hypothermia (°C)	0.5 ± 0.1	0.2 ± 0.1§	0.1 ± 0.1*	0.1 ± 0.1*

*$p < 0.05$, †$p < 0.005$ (values are significantly lower than the control values as determined by the χ^2 test).

‡$p < 0.05$ (values are significantly higher than the control values as determined by the χ^2 test).

§$p < 0.05$ (values are significantly lower than the control values as determined by Student's t-test).

TABLE 3

EFFECTS OF β-FNA ON NALOXONE-PRECIPITATED ABSTINENCE SIGNS (TOTAL OCCURRENCE) IN BUTORPHANOL-DEPENDENT RATS

	Treatment with β-FNA (nmol/5 μl/rat)			
	0	12	24	48
Teeth-chattering	11.7 ± 1.2*	3.1 ± 0.8†	5.0 ± 0.8†	4.6 ± 0.9†
Wet shakes	9.7 ± 1.4	4.2 ± 0.7†	5.2 ± 0.8‡	6.4 ± 1.2‡
Rearing	14.2 ± 1.9	12.5 ± 1.8	10.2 ± 1.6	11.8 ± 1.5
Penis-licking	5.0 ± 0.7	4.2 ± 1.0	7.3 ± 1.1	7.6 ± 1.1
Forepaw tremors	5.8 ± 1.3	2.5 ± 0.6‡	1.7 ± 0.5†	2.6 ± 0.6‡
Weight loss (g)	8.7 ± 0.8	5.2 ± 0.1†	6.0 ± 0.7‡	5.2 ± 0.1†

*Values are mean ± SE of the total number of occurrences in each treated group.
†$p < 0.005$, ‡$p < 0.05$ (values are significantly lower than the control values as determined by Student's t-test).

not antagonized by β-FNA, we propose that μ receptors must also be involved in opioid receptor modulation of urination in butorphanol-dependent animals.

Naloxone-induced teeth-chattering, diarrhea, and forepaw tremors appear to be mediated via the μ receptor. Teeth chattering was reduced at the three doses of β-FNA tested. However, forepaw tremors are more complicated in that they have been observed in morphine-dependent rats but not in DAGO-(μ-agonist) dependent rats (5).

Penis-licking was increased by treatment with β-FNA. Although the behavioral effects of β-FNA have not been established, it is of interest that β-FNA induced penis-licking in these experiments. Therefore, we cannot exclude some synergistic antagonist action of β-FNA at opioid receptors.

In summary, our studies have demonstrated that butorphanol exerts a multiplicity of actions at opioid receptors. Butorphanol's antinociceptive activity is related to its actions at μ, δ, and κ opioid receptors. Taken in toto, these data demonstrate that physical dependence on butorphanol may involve multiple opioid receptors because treatment with β-FNA (a selective μ receptor antagonist) diminished some opioid withdrawal signs. Further studies will be required to clarify the involvement of δ and κ receptors in the development of butorphanol dependence.

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REFERENCES

1. Bean, A. J.; Vaught, J. L. Physical dependence produced by chronic intracerebroventricular infusion of [D-Arg] kyotorphin or thiorphan to rats. Eur. J. Pharmacol. 105:333–337; 1984.
2. Cheney, D. L.; Goldstein, A. Tolerance to opioid narcotics: Time course and reversibility of physical dependence in mice. Nature 232:477–478; 1971.
3. Corbett, A. D.; Kosterlitz, H. W.; McKnight, A. T.; Paterson, S. J.; Robson, L. E. Pre-incubation of guinea-pig myenteric plexus with β-funaltrexamine: Discrepancy between bindings assays and bioassays. Br. J. Pharmacol. 85:665–673; 1985.
4. Cowan, A.; Rance, M. J.; Blackburn, T. P. In vitro studies on δ opioid receptors. Natl. Inst. Drug Abuse Res. Monogr. Ser. 75: 473–476; 1986.
5. 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.
6. Gulya, K.; Krivan, M.; Nyolczas, N.; Sarnyai, Z.; Kovacs, G. L. Central effects of the potent and highly selective μ opioid antagonist *D*-Phe-Cys-Tyr-*D*-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) in mice. Eur. J. Pharmacol. 150:355–360; 1988.
7. Hayes, A. G.; Skingle, M.; Tyers, M. B. Effect of β-funaltrexamine on opioid side-effects produced by morphine and U-50, 488H. J. Pharm. Pharmacol. 37:841–843; 1985.
8. Hayes, A. E.; Skingle, M.; Tyers, M. B. Evaluation of the receptor selectivities of opioid drugs by investigating the block of their effect on urine output by beta-funaltrexamine. J. Pharmacol. Exp. Ther. 240:984–988; 1986.
9. Heel, R. C.; Brogden, R. N.; Speight, T. M.; Avery, G. S. Butorphanol: A review of its pharmacological properties and therapeutic efficacy. Drugs 16:473–505; 1978.
10. Horan, P. J.; Ho, I. K. The physical dependence liability of butorphanol: A comparative study with morphine. Eur. J. Pharmacol. 203:387–391; 1991.
11. Jasinski, D. R.; Pevnick, J. S.; Griffith, J. D.; Gorodetzky, C. W.; Cone, E. J. Progress report from the clinical pharmacology section of the Addiction Research Center. 40th Annual Meeting, Committee on Problems of Drug Dependence: National Academy of Sciences; 1976:112–120.
12. Kovacs, G. L.; Nyolczas, N.; Krivan, M.; Gulya, K. Analgesic and tolerance-inducing effects of the highly selective delta opioid agonist [*D*-Pen^2, *D*-Pen^5] enkephalin in mice. Eur. J. Pharmacol. 150:347–353; 1988.
13. Lahti, R. A.; Mickelson, M. M.; McCall, J. M.; Von Voightlander, P. F. [^{3}H]U-69,593, a highly selective ligand for the opioid κ-receptor. Eur. J. Pharmacol. 109:281–284; 1985.
14. Leander, J. D.; Hart, J. C.; Zerbe, R. L. Kappa agonist-induced diuresis: Evidence for stereoselectivity, strain differences, independence of hydration variables and a result of decreased plasma vasopressin levels. J. Pharmacol. Exp. Ther. 242:33–39; 1987.
15. Lee, P. H.; Obie, J.; Hong, J. S. Opioid induced convulsions and wet dog shakes in rats. Mediation by hippocampal mu, but not delta or kappa opioid receptors. J. Neurosci. 9:692–697; 1989.
16. Martin, W. R. Pharmacology of opioids. Pharmacol. Rev. 35: 283–323, 1984.
17. McCarthy, P. S.; Howlett, G. J. Physical dependence induced by opiate partial agonists in the rat. Neuropeptides 5:11–14; 1984.
18. McCarthy, P. S.; Metcalf, G.; Howe, S. J. Continuous infusion in rats as a method of assessing morphine-like physical dependence for opiates. Pharmacol. Biochem. Behav. 16:725–729; 1982.

19. Monkovic, I.; Conway, T. T.; Wong, H.; Perron, Y. G.; Pachter, I. J.; Belleau, B. Total synthesis and pharmacological activities of *N*-substituted 3,14-dihydroxymorphans. J. Am. Chem. Soc. 95:7910–7912; 1973.
20. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates, 2nd ed. Orlando, FL: Academic Press; 1986.
21. Pircio, A. W.; Gylys, J. A.; Cavanagh, R. L.; Buyniski, J. P.; Bierwagen, M. E. The pharmacology of butorphanol, a 3,14-dihydroxymorphan narcotic antagonist analgesic. Arch. Int. Pharmacodyn. 220:231–257; 1976.
22. Takemori, A. E. Biochemistry of drug dependence. Annu. Rev Biochem. 43:15–33; 1974.
23. 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.
24. 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.
25. Wood, P. L.; Charleston, S. E.; Lane, D.; Hudgin, R. L. Multiple opiate receptors: Differential binding of μ, κ and δ agonists. Neuropharmacology 20: 1215–1220; 1981.
26. Zimmerman, D. M.; Hart, J. C.; Ree, J. K.; Leander, J. D. Effects of beta-funaltrexamine (beta-FNA) on the diuretic actions of kappa agonists and the antidiuretic actions of mu agonist. Fed. Proc. 43:966; 1984.