Pharmacology Biochemistry and Behavior, Vol. 57, No. 4, pp. 771–777, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(96)00395-4

Antagonism of Morphine-Like Discriminative Effects by β-Funaltrexamine

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Received 15 October 1996; Accepted 28 July 1996

HOLTZMAN, S. G. *Antagonism of morphine-like discriminative effects by β-funaltrexamine.* PHARMACOL BIOCHEM BEHAV **57**(4) 771–777, 1997.—β-Funaltrexamine (β-FNA), an irreversible antagonist at the *mu*-opioid receptor, was administered intracisternally to rats discriminating between subcutaneous injections of saline and 3.0 mg/kg of morphine in order to reduce the size of the receptor reserve. β-FNA alone (10 μg) occasioned substantial morphine-appropriate responding for at least 6 h but mainly saline-appropriate responding 24 h after administration, the pretreatment interval for most experiments. β-FNA (3.0–30 μg) dose-dependently shifted to the right stimulus-generalization curves for morphine and fentanyl; 10 μg also shifted to the right the curves for meperidine and buprenorphine. In all cases, antagonism was fully surmounted by higher doses of the agonist, even after inactivation of more than 75% of *mu*-opioid receptors. This antagonist effect of β-FNA is smaller than that reported previously in tests of analgesia, suggesting that the receptor reserve for the discriminative effects of morphine-like drugs is larger than the receptor reserve for their analgesic effects. β-FNA produced larger rightward displacements of the morphine and buprenorphine curves than of the fentanyl curve and inactivated a larger fraction of the receptors acted upon by those drugs compared to fentanyl. Results with meperidine were intermediate. This suggests that the receptor population mediating morphine-like discriminative effects of fentanyl is not identical to the receptor population mediating these effects of morphine and buprenorphine. © 1997 Elsevier Science Inc.

Morphine Fentanyl Buprenorphine Meperidine β-Funaltrexamine *mu*-Opioid receptors Drug discrimination Receptor reserve

THE discriminative stimulus effects of morphine and morphine-like opioids, like most of their other effects, are mediated by *mu*-opioid receptors (7). However, the plasticity of the receptor population that underlies stimulus control of behavior by morphine-like drugs appears to differ from that of the receptor population that underlies certain other effects of the drugs, such as analgesia. This is exemplified by the results of experiments on drug tolerance and cross-tolerance. Rats that received a continuous 7-day SC infusion of either morphine or the *mu*-opioid receptor agonists fentanyl or meperidine developed tolerance to the analgesic effect of the infused drug and cross- tolerance to the analgesic effect of other *mu*-opioid agonists (12). The extent of the tolerance and cross-tolerance was an inverse function of the intrinsic efficacy both of the drug used to induce tolerance and the drug tested for antinociceptive activity. Thus, among the three drugs, fentanyl, which has the highest intrinsic efficacy, induced the least amount of tolerance and displayed the least amount of cross-tolerance. Meperidine, which has the lowest intrinsic efficacy, induced the most tolerance and displayed the greatest amount of cross-tolerance. An infusion of any of the three drugs induced complete cross-tolerance to buprenorphine, which has lower intrinsic efficacy than even meperidine and functions as only a partial-morphine-like agonist in many bioassay systems (3). In contrast, a 7-day continuous SC infusion of those same doses of morphine, fentanyl or meperidine induced relatively little tolerance or cross-tolerance to the morphine-like discriminative stimulus effects of these drugs (13). Even the morphine-like discriminative effects of buprenorphine were reduced only slightly after a 7-day infusion of morphine.

Tolerance to morphine-like drugs may reflect a reduction in the *mu*-opioid receptor reserve (2,10). Accordingly, one possible reason for the difference in the extent of tolerance development to the analgesic and discriminative effects of morphine-like drugs is the size of the receptor reserve for the two effects. If the receptor reserve for discrimination is larger than the one for analgesia, a greater number of *mu*-opioid receptors would have to be inactivated to induce tolerance to discriminative effects than would have to be inactivated to induce analgesic tolerance. The discriminative effects of morphine-like drugs appear to be intimately related to subjective effects (6), which are major determinants of potential for abuse (9). Therefore, a large receptor reserve for discriminative effects relative to other drug effects has important impli-

cations for the persistence of abuse potential during chronic drug intake.

Mu-opioid receptor reserve also can be reduced *in vivo* by administering an irreversible receptor antagonist. Intracerebroventricular (ICV) injection of β- funaltrexamine (β-FNA), an irreversible antagonist of the *mu*-opioid receptor (17), reduced the analgesic effect of morphine and fentanyl when they were tested 24 h later (1). The maximum analgesic effect of morphine was reduced after pretreatment with 5.0 μg of β-FNA and that of fentanyl after 20 μg, consistent with the higher intrinsic efficacy of fentanyl compared to morphine. There are no reports of comparable studies of the effects of irreversible antagonists on the discriminative effects of morphine-like drugs. Therefore, the purpose of the present study was to examine the ability β-FNA to antagonize the discriminative effects of morphine and related drugs in rats discriminating between SC injections of morphine and saline. Fentanyl, meperidine, and buprenorphine, drugs that have either higher or lower intrinsic efficacy than morphine has, were tested before and after the administration of one or more doses of β-FNA. β-FNA was administered by the intracisternal (IC) route in order to ensure adequate delivery of the drug to the brain. Multiple IC injections can be given without the need to implant an intracerebral cannula, which usually is necessary for multiple ICV injections. This eliminates the potential problems attendant with performing surgery on highly trained animals and in maintaining patent cannulae and healthy and behaviorally- stable subjects during long experiments.

METHODS

Subjects

The subjects were 19 adult male rats of Sprague–Dawley descent (Charles River, Inc., Raleigh, NC), all experimentally-naive at the start of the study. Between experiments, the rats were housed in pairs in standard laboratory cages where they had continuous access to food and water. The cages were kept in a temperature-controlled animal facility in which a 12 h light/dark cycle was maintained (lights on at 0700 h).

Drug Discrimination Training

The rats were trained in a discrete-trial avoidance/escape procedure to discriminate between SC injections of saline and 3.0 mg/kg of morphine, which were injected on alternate days, 30 min before a 20-trial session (16). Trial onset was signalled by illuminating the house light of the experimental chamber and concurrently turning on a white noise. Five sec later a constant current of 1.0–1.5 mA was distributed to the grid floor of the chamber in 1.0-sec pulses every 3.0 sec until the animals completed the following two-response chain: pressing the single "observing" lever that was mounted in one wall of the chamber and then pressing one of the two "choice" levers that were mounted in the opposite wall. The observing response turned off the white noise, and the choice response, if appropriate for what the animal had been injected with before the session (i.e., saline or 3.0 mg/kg of morphine), turned off the house light, and ended the trial, which was recorded as correct. A trial also ended after the response sequence of observing lever, inappropriate choice lever, appropriate choice lever (recorded as incorrect), or after 30 sec had elapsed without the required sequence of responses (recorded as incomplete). Another trial began 50 sec later. Half of the rats were trained to press the left choice lever in sessions that followed an injection of morphine and the right choice lever in sessions that followed an injection of saline; the designation of choice levers was the reverse for the other half of the animals. The behavior of a rat was considered to be under the stimulus control of morphine and saline when the animal could reliably complete 18 or more trials out of 20 on the choice lever that was appropriate for the substance injected before the session (i.e., ≥ 90% correct trials) in four consecutive training sessions and two consecutive test sessions (see below).

Experimental Design

Drugs were tested by a cumulative-dosing procedure (13). Saline was injected SC 20 min before the first test session of the day. Upon completion of the session, the first drug dose was injected SC and 20 min later another test session was conducted. Upon completion of that session, a second drug dose was injected that was 0.5 log-unit higher cumulatively than the first, and a third test session was conducted 20 min later. This procedure was repeated until 3–5 drug doses had been tested. Thus, in order to establish a stimulus-generalization curve for morphine under base-line conditions, the testing sequence was saline, 0.3, 0.7, and 2.0 mg/kg for cumulative doses of 0, 0.3, 1.0, and 3.0 mg/kg. As each session typically lasted 20–25 min, the interval between successive doses was 40–45 min. Test sessions were similar to training sessions with the exception that a trial was terminated by the first response on either choice lever following a response on the observing lever regardless of what the animal had been injected with before the session.

A separate control curve was determined (day 1) for each drug for each test with β-FNA. If the animal completed ≤ 10% of the trials on the morphine-appropriate choice lever in the session that followed the saline injection and ≥ 90% of the trials on the morphine-appropriate lever in the session that followed the highest drug dose, the test sequence proceeded as follows. A single training session preceded by an injection of either saline or 3.0 mg/kg of morphine was conducted on day 2 and again on day 3. If the rat completed at least 90% of the trials correctly on both days, β-FNA was injected on day 4. The stimulus-generalization curve was re-determined on day 5 (or on days 4 or 6 in the time-course experiment). No sessions were conducted on the next 5–7 days, after which training sessions resumed. The rats spent at least two weeks in training sessions, with morphine and saline being given on alternate days. If the performance of the animals was at 90% correct trials in at least the last four training sessions, the testing sequence was repeated. Thus, at the minimum, successive injections of β-FNA were given 3–4 weeks apart, each injection preceded three days earlier by the determination of a control stimulus-generalization curve. Each animal was tested with β-FNA a median of 3 times (range: 2–7). Drugs were not tested in a systematic sequence.

Drugs

The drugs used were morphine sulfate (Penick Corp., Newark, NJ) and the hydrochloride salts of fentanyl, meperidine, buprenorphine, and β-FNA (National Institute on Drug Abuse, Rockville, MD). Morphine, fentanyl, and meperidine were dissolved in 0.9% sodium chloride solution, and buprenorphine in distilled water, and were injected SC in a volume of 1.0 ml/kg of body weight (2.0 ml/kg for high doses of morphine and meperidine). β-FNA was dissolved in distilled water and injected intracisternally in a volume of 10 μl per rat while the animal was anesthetized briefly with either halothane or methoxyflurane. All drug doses refer to the free base.

Data Analysis

Discrimination data are presented as the average number of trials completed on the choice lever appropriate for morphine; the remaining trials of the 20-trial session were completed on the saline-appropriate choice lever. The dose of a drug that resulted in selection of the morphine-appropriate choice lever in 10 trials per session (ED_{50}) was estimated for each rat by linear regression of the stimulus-generalization curve where at least three points were available. In the few instances where only two points were usable, the ED_{50} was estimated by simple interpolation. The individual ED_{50}s were used to calculate average ED_{50}s and 95% confidence limits for the group. A ratio of the ED_{50} after β-FNA/ED_{50} before β-FNA was derived for each animal; a two-tailed Student's *t*-test was used to determine if the ratio for the group was significantly different from 1.0.

The fraction of receptors that remained available to the agonists 24 h after pretreatment with β-FNA, q (5), was calculated from double-reciprocal plots of equieffective doses of the agonist with and without β-FNA pretreatment (1,18). Under these conditions, q is the inverse of the slope of the linear regression line, and its 95% confidence limits the inverse of the confidence limits of the slope. The regression line was based upon doses calculated to result in completion of 5, 8, 10, 12, and 15 trials (*i.e.*, 25–75%) on the morphine-appropriate choice lever. All linear regression analyses were performed with the InStat™ computer program (GraphPad Software, San Diego, CA).

RESULTS

The control stimulus-generalization curves for morphine that were derived by cumulative dosing before each injection of β-FNA did not vary significantly across the six determinations that were made to generate the data depicted in Figs. 1 and 2: morphine ED_{50}s ranged from 0.95 (0.50–1.78) to 1.35 (0.69–2.57) mg/kg (Tables 1 and 2). An intracisternal injection of saline had no effect on the morphine generalization curve determined 24 h later (Fig. 1, Table 1). However, 24-h pretreatment with 3.0, 10 or 30 μg of β-FNA resulted in a dose-dependent significant rightward displacement of the stimulus-generalization curve for morphine (Fig. 1); morphine ED_{50}s increased 2.4-, 4.9-, and 12.3-fold, respectively (Table 1). Following the highest dose of β-FNA, a 100 mg/kg cumulative dose of morphine was required to achieve the completion of ≥ 90% of the session trials on the morphine-appropriate choice lever.

β-FNA also reduced dose-dependently the fraction of the receptor population that remained available for morphine to interact with. The lowest dose of β-FNA reduced q to less than 0.5 and the highest dose reduced it to only 0.11 (Table 1), indicating inactivation of 90% of the receptor pool targeted by morphine. In this and in the other sets of experiments, there was no evidence of residual effects of β-FNA when training of animals resumed 5–7 days after an injection.

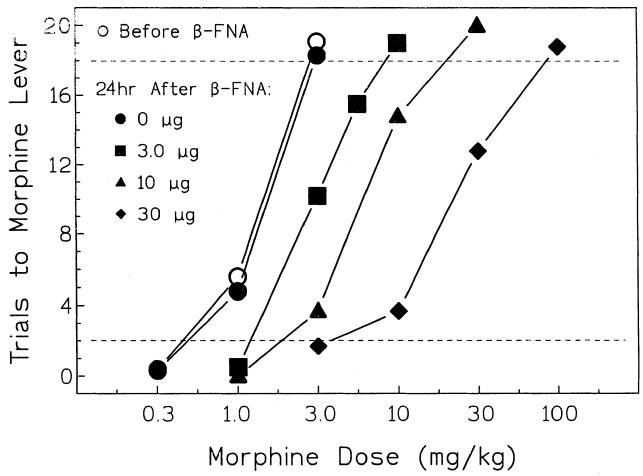

FIG. 1. Dose-dependent antagonism of the discriminative stimulus effects of morphine by β-FNA. Stimulus-generalization curves for morphine were determined by cumulative dosing before and 24 h after an IC injection of the indicated dose of β-FNA or saline (0 μg). The curve Before β-FNA is based upon four separate determinations in each of 6 rats; the curves After β-FNA are each based upon one determination in each of 6 rats. The same set of rats was not used for each curve. Each point is the mean number of trials completed on the morphine-appropriate choice lever in a 20-trial session; the remaining trials of the session were completed on the choice lever appropriate for saline. The upper and lower horizontal dashed lines indicate the minimum levels at which discrimination performance was maintained in training sessions that followed the SC administration of 3.0 mg/kg of morphine or saline, respectively.

Three different pretreatment intervals were examined, using a 10 μg dose of β-FNA (Fig. 2, Table 2). At the shortest pretreatment interval, 4 h, 2 out of 6 rats responded primarily on the choice lever appropriate for saline, even at a cumulative morphine dose of 100 mg/kg. Therefore, an ED_{50} for the group could not be calculated. Within the subgroup of 4 rats that did complete at least 18 trials on the morphine-appropriate lever at some dose of morphine, the 4-h pretreatment with β-FNA increased the ED_{50} for morphine to 6.17 mg/kg from a

TABLE 1

DOSE-DEPENDENT ANTAGONISM OF THE DISCRIMINATIVE STIMULUS EFFECTS OF MORPHINE BY 24 H PRETREATMENT WITH β-FUNALTREXAMINE

β-FNA Dose (μg IC)	Morphine ED_{50} (95% Confidence Limit, mg/kg)		Ratio	q% (95%) CL)
	Before β-FNA	After β-FNA		
0	1.35 (0.69–2.57)	1.48 (0.87–2.51)	1.1	
3.0	1.35 (1.04–1.74)	3.24 (1.38–7.59)	2.4*	0.42 (0.39–0.45)
10	1.20 (0.79–1.86)	5.89 (3.55–10.0)	4.9**	0.27 (0.24–0.30)
30	1.33 (0.95–1.91)	16.6 (5.5–51.3)	12.3**	0.11 (0.09–0.15)

%Fraction of receptors available to morphine after β-FNA (5).
*Significantly different from 1.0; $p < 0.05$.
**Significantly different from 1.0; $p < 0.01$.

TABLE 2

TIME-DEPENDENT ANTAGONISM OF THE DISCRIMINATIVE STIMULUS EFFECTS OF MORPHINE BY PRETREATMENT WITH 10 μg β-FUNALTREXAMINE

Hours after β-FNA	Morphine ED_{50} (95% Confidence Limit, mg/kg)		Ratio
	Before β-FNA	After β-FNA	
0#	1.35 (0.69–2.57)	1.48 (0.87–2.51)	1.1
4	0.93 (0.50–1.78)	>6.17	>6.6
24#	1.20 (0.79–1.86)	5.89 (3.55–10.0)	4.9**
48	1.23 (0.83–1.78)	2.34 (1.32–4.17)	1.9*

#Reproduced from Table 1.
*Significantly different from 1.0; $p < 0.05$.
**Significantly different from 1.0; $p < 0.01$.

control value of 0.95 mg/kg. The morphine-antagonist action of β-FNA remained evident 48 h after administration, but the extent of antagonism was less than half that measured after 24-h pretreatment (Fig. 2, Table 2).

Following the 4-h pretreatment with 10 μg of β-FNA, some of the rats completed more than 10% of the trials on the morphine-appropriate choice lever in the first test session of the cumulative-dosing procedure, which followed an SC injection of saline. They were excluded from further testing in accordance with the performance criteria described in Methods. To examine this occurrence more thoroughly, a time-effect curve was determined for IC injections of either saline or 10 μg of β-FNA. Subjects were tested in a single session immediately before the IC injection (time 0, Fig. 3), and again in single sessions 1, 2, 4, 6, and 24 h after the IC administration of saline or β-FNA. An SC injection of saline preceded each test session. β-FNA engendered a substantial amount of responding on the morphine-appropriate choice lever 1–6 h after its injection (Fig. 3). The peak effect occurred 4 h after the injection of β-FNA, at which time the group of 6 rats completed an average of 9.8 trials on the morphine-appropriate choice lever, based upon individual responses of 0, 2, 7, 14, 16, and 20 trials. At 24 h after β-FNA administration, the rats responded almost exclusively on the choice lever appropriate for saline, as they also did at all time points after the IC injection of saline (Fig. 3).

Fentanyl was approximately 70 times more potent than morphine in occasioning selection of the morphine-appropriate choice lever during cumulative dosing. As in the case of morphine, the control stimulus-generalization curve for fentanyl was reproducible from one determination to another (Table 3). Pretreatment with β-FNA had a consistently smaller effect on the stimulus-generalization curve for fentanyl than it did on the generalization curve for morphine. IC administration of 3.0 μg of β-FNA 24-h earlier failed to displace the fentanyl curve, whereas 10 and 30 μg resulted in rightward curve shifts of 2.3- and 4.2-fold, respectively (Fig. 4, Table 3). Each dose of β-FNA appeared to inactivate a smaller fraction of the receptors acted upon by fentanyl than those acted upon by morphine: 40% of receptors still remained available to fentanyl after the administration of 30 μg of the alkylating agent (Table 3).

Meperidine and buprenorphine were tested for morphine-like discriminative effects before and 24 h after a single 10 μg

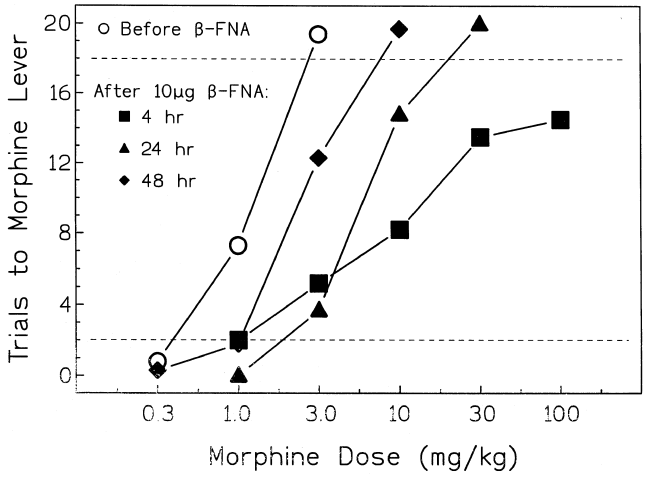

FIG. 2. Time-dependent antagonism of the discriminative stimulus effects of morphine by 10 μg of β-FNA. Stimulus-generalization curves for morphine were determined by cumulative dosing before and at the indicated times after the IC injection of β-FNA. The curve Before β-FNA is based upon three determinations in each of 6 rats; the curves After β-FNA are each based upon one determination in each of 6 rats. The same set of rats was not used for each curve. The curve at 24 h is the same one that appears in Fig. 1. Other details as in Fig. 1.

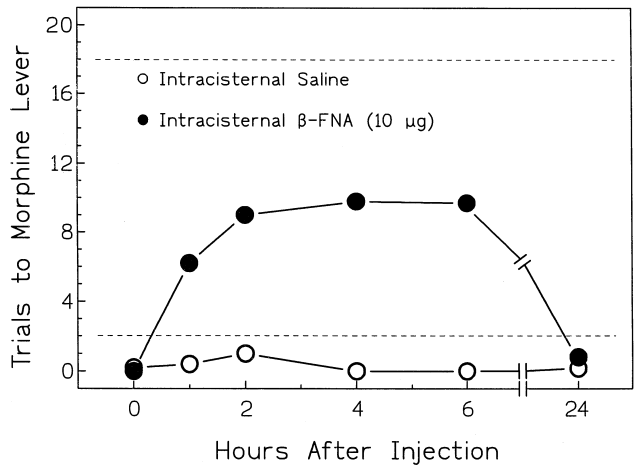

FIG. 3. The IC injection of 10 μg of β-FNA resulted in appreciable morphine- appropriate responding for at least 6 h. Each of 6 rats was tested in a single experimental session before the administration of either saline or β-FNA (0 h) and in single sessions at the indicated times after the IC injection; an SC saline injection was given 20 min before each of the experimental sessions. Different sets of rats were used to derive each curve. Other details as in Fig. 1.

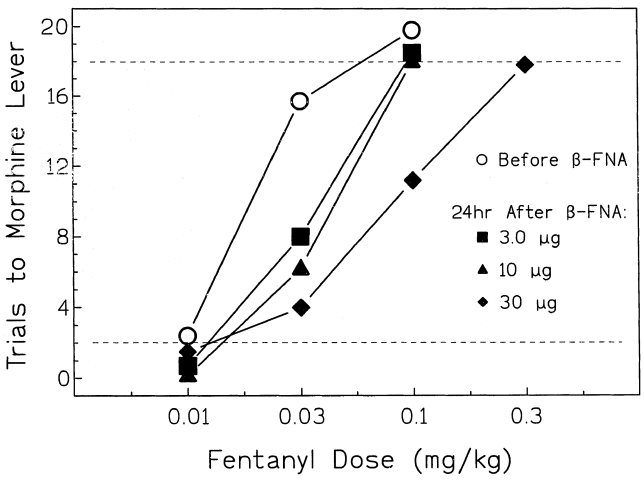

FIG. 4. Dose-dependent antagonism of the morphine-like discriminative stimulus effects of fentanyl by β-FNA. Stimulus-generalization curves for fentanyl were determined by cumulative dosing Before and 24 h aAfter an IC injection of the indicated dose of β-FNA. The curve Before β-FNA is based upon three separate determinations in each of 6 rats; the curves After β-FNA are each based upon one determination in each of 6 rats. The same set of rats was not used for each curve. Other details as in Fig. 1.

dose of β-FNA. Under control conditions, both drugs occasioned dose-dependent increases in trials completed on the morphine-appropriate choice lever; buprenorphine was as potent as fentanyl, and meperidine was an order of magnitude less potent than morphine (Fig. 5, Table 4). The stimulus-generalization curves for both drugs were displaced to the right significantly by 24-h pretreatment with β-FNA, 2.8-fold in the case of meperidine and 6.5-fold in the case of buprenorphine (Fig. 5, Table 4).

The effects of pretreatment with 10 μg of β-FNA on the ED_{50} of each of the four opioid drugs are compared in Table 4. This dose of β-FNA had a 2.8-fold greater effect on the buprenorphine ED_{50} than it did on the fentanyl ED_{50}. Analysis of variance confirmed a significant difference among the four ED_{50} ratios: $F(3, 20) = 4.43$; $p = 0.015$. In addition, this dose of β-FNA inactivated 78% of the receptors that mediate the morphine-like discriminative effects of buprenorphine but only 37% of the receptors that mediate those same effects of meperidine.

DISCUSSION

β-FNA administered IC to rats discriminating between morphine and saline antagonized the discriminative effects of morphine and three other morphine-like drugs, fentanyl, meperidine, and buprenorphine, 24 h later. However, the antagonist effect of β-FNA at the doses tested was smaller than its antagonist effect in tests of analgesia and was fully surmountable by higher doses of the agonists. Inactivation of almost 75% of the population of *mu*-opioid receptors that mediate the discriminative effects of morphine resulted in only a 5-fold increase in the morphine ED_{50} (Table 4). In contrast, inactivation of a similar percentage of the receptor population that mediates the analgesic effect of morphine in the rat tail-flick test increased the ED_{50} of morphine by a full order of magnitude and reduced the maximum effect obtained (1). The failure of β-FNA to reduce the maximum level of morphine-appropriate responding precluded an assessment of the relative efficacies of the four opioid agonists.

The differential effects of β-FNA on the analgesic and discriminative effects of morphine-like opioids resemble differences in the outcomes of tolerance and cross- tolerance experiments. SC infusions of agonists sufficient to induce significant tolerance and cross-tolerance to analgesic effects (12) often failed to induce tolerance or cross-tolerance to the morphine-like discriminative effects of the same drugs (13). Indeed, a 7-day SC infusion of morphine that essentially eliminated the analgesic effect of buprenorphine in the rat tail-flick test resulted in less than a 3-fold increase in the ED_{50} of buprenorphine for morphine-like discriminative effects.

Of course, procedures for evaluating the analgesic and the discriminative effects of drugs differ in many ways in addition to the endpoints that were used. For example, drug histories of subjects used in the two assays often differ markedly. Subjects in analgesia assays usually are tested with drugs a limited number of times or only once, whereas those in discrimination assays are tested repeatedly over long periods. It is not clear how these differences across procedures might influence estimates of relative receptor reserve and agonist efficacy. Nevertheless, when taken together, the results of the antagonism and tolerance studies suggest that in the rat the receptor reserve for morphine-like discriminative effects is larger than the receptor reserve for analgesic effects. There appears to be a similar relationship between the receptor reserves for the reinforcing and analgesic effects of morphine-like opioids in the rhesus monkey. The results of experiments with the irreversible *mu*-opioid antagonist clocinnamox indicate that the receptor reserves for the reinforcing effects of alfentanil and nalbuphine are significantly larger than the receptor reserves for the analgesic effects of the drugs (20,21). If comparable differences in receptor reserves occur in humans, effects of *mu*-opioid agonists that underlie their abuse could persist under conditions, such as drug tolerance, that might minimize therapeutic effects, notably analgesia.

TABLE 3

DOSE-DEPENDENT ANTAGONISM OF THE MORPHINE-LIKE DISCRIMINATIVE EFFECTS OF FENTANYL BY 24 H PRETREATMENT WITH β-FUNALTREXAMINE

β-FNA Dose (μg IC)	Fentanyl ED_{50} (95% Confidence Limit, mg/kg)		Ratio	q% (95% CL)
	Before β-FNA	After β-FNA		
3.0	0.026 (0.018–0.036)	0.037 (0.019–0.071)	1.4	0.75 (0.73–0.77)
10	0.017 (0.011–0.026)	0.039 (0.018–0.082)	2.3*	0.49 (0.47–0.51)
30	0.018 (0.013–0.026)	0.077 (0.026–0.222)	4.2*	0.40 (0.30–0.57)

%Fraction of receptors available to fentanyl after β-FNA (5).
*Significantly different from 1.0; $p < 0.05$.

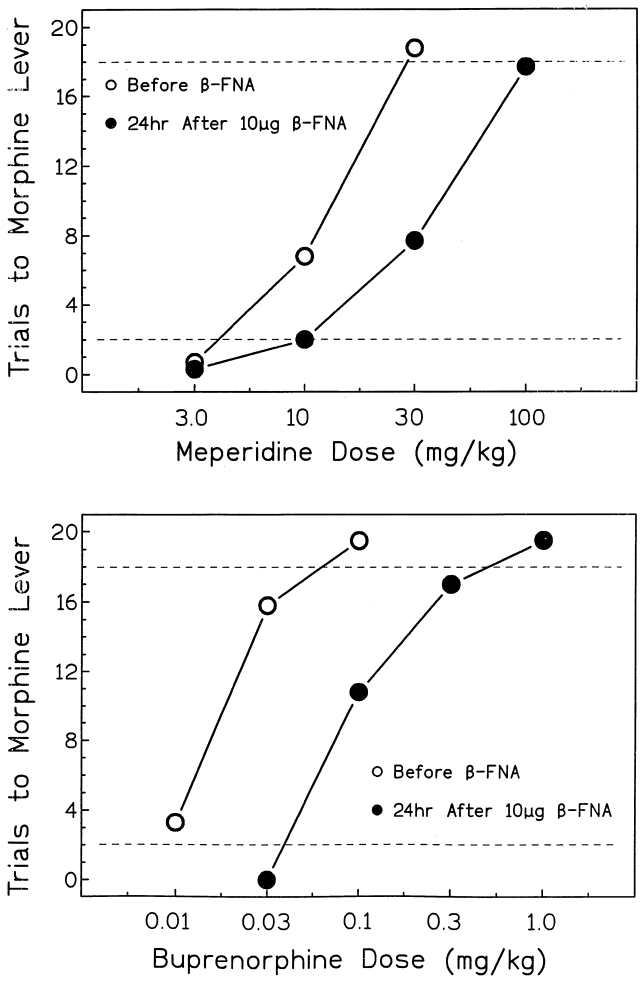

FIG. 5. Antagonism of the morphine-like discriminative stimulus effects of meperidine (top) and buprenorphine (bottom) by β-FNA. Stimulus-generalization curves for meperidine and buprenorphine were determined by cumulative dosing before and 24 h after an IC injection of 10 μg of β-FNA. Each curve is based upon one determination in each of 6 rats. The same set of rats was used to derive the "before" and "after" curves for each drug. Other details as in Fig. 1.

The interaction of β-FNA with fentanyl and, perhaps, meperidine appeared to be different from its interaction with morphine and buprenorphine. The rightward shifts of the stimulus-generalization curves for fentanyl and meperidine produced by 10 μg of β-FNA were smaller than those of the curves for morphine and buprenorphine. In addition, this dose of β-FNA inactivated a smaller fraction of the receptors that mediate the morphine-like discriminative effects of fentanyl than it did in the case of the receptors that mediate those same effects of morphine and buprenorphine (Table 4). The results with meperidine were intermediate. The outcomes with fentanyl and morphine are similar to those observed in the rat tail-flick procedure. This suggests that, as in the case of the analgesic effects of the two drugs, the population of receptors mediating the morphine-like discriminative effects of fentanyl is not identical to the receptor population mediating these effects of morphine. If there are multiple forms and/or subtypes of the *mu*-opioid receptor (8,15), fentanyl might interact with more of them than morphine or buprenorphine do. It is difficult to determine where meperidine falls on this spectrum on the basis of the available data.

β-FNA engendered an unexpected amount of morphine-appropriate lever selection for at least the first 6 hours after the injection of 10 μg. The effect varied considerably across animals and was almost never observed 24 h after the administration of β-FNA, even at the 30 μg dose. Although it is an irreversible antagonist of *mu*-opioid receptors, β-FNA has reversible agonist activity mediated by the *kappa*-opioid receptor that can persist for up to several hours (4,19). *Kappa*-opioid agonists can sometimes occasion a small amount of morphine-appropriate responding in rats (14). Whether or not the *kappa*-agonist effects of β-FNA can account for the present results is unclear in the absence of experiments to determine if the morphine-appropriate responding induced by β-FNA can be blocked by an antagonist that is selective for the *kappa*-opioid receptor. Regardless of the underlying mechanism, this activity of β-FNA made it impractical to use shorter pretreatment intervals where, as the data in Fig. 2 suggest, it might have been possible to reduce the maximum level of morphine-appropriate responding occasioned by the opioid agonists.

ACKNOWLEDGEMENTS

Technical assistance throughout the study was provided by Christine M. Engels. This study was supported, in part, by Grant DA00541 and by Research Scientist Award KO5 DA00008, both from the National Institute on Drug Abuse, National Institutes of Health.

TABLE 4

ANTAGONISM OF THE MORPHINE-LIKE DISCRIMINATIVE EFFECTS OF *MU*-OPIOID AGONISTS BY 24 H PRETREATMENT WITH 10 μg β-FUNALTREXAMINE

Drug	ED_{50} (95% confidence limit, mg/kg)		Ratio	$q^{\%}$ (95% CL)
	Before β-FNA	After β-FNA		
Fentanyl[#]	0.017 (0.011–0.026)	0.039 (0.018–0.082)	2.3*	0.49 (0.47–0.51)
Meperidine	11.2 (6.53–19.1)	31.5 (15.6–63.7)	2.8**	0.37 (0.34–0.41)
Morphine[#]	1.20 (0.79–1.86)	5.89 (3.55–10.0)	4.9**	0.27 (0.24–0.30)
Buprenorphine	0.017 (0.009–0.031)	0.114 (0.072–0.179)	6.5**	0.22 (0.20–0.24)

[#]Fraction of receptors available to agonist after b-FNA (5).
[#]Reproduced from previous tables.
*Significantly different from 1.0; $p < 0.05$.
**Significantly different from 1.0; $p < 0.01$.

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