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β-Funaltrexamine Antagonizes the Analgesic Effects of Mu and Kappa Agonists in the Formalin Test

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ABBOTT, F. V. *β-Funaltrexamine antagonizes the analgesic effects of mu and kappa agonists in the formalin test.* PHARMACOL BIOCHEM BEHAV **37**(4) 713–716, 1990.—The formalin test assesses the behavioral response of an animal to minor tissue injury-induced pain. Opioid antinociception in this test has been suggested to depend largely on activation of kappa receptors but mu agonists are also potent in reducing pain behavior. The present study used the irreversible mu antagonist, β-funaltrexamine (β-FNA), to examine the role of mu receptor activation in this test. β-FNA given intracranially 4 h before testing fully blocked the effects of morphine and attenuated the effects of ethylketocyclazocine and U50,488H. The results do not support a role for kappa receptors in antinociception in the formalin test. Instead, mu and, possibly, delta receptors are involved.

β-Funaltrexamine Formalin test Analgesia Morphine Etylketocyclazocine U50,488

THE formalin test differs from tests of heat pain threshold along several dimensions. First, it involves continuing pain associated with tissue injury rather than a brief threshold level noxious stimulus. Humans describe formalin-induced pain as poorly localized with prominent burning and throbbing qualities (16). The mechanisms of both pain and opioid antinociception also differ in the formalin and heat pain tests. While the heat pain threshold is raised by depletion of peptides in peripheral nerves with capsaicin, the behavioral response to formalin is not altered (7,8). In heat pain tests, opioid antinociception is attenuated by lesions of the descending bulbospinal 5HT systems (5,19) that are activated under situations of acute stress (18,24). Opioid antinociception in the formalin test (3, 4, 29) and in surgical pain in humans (17) is antagonized by 5HT.

The receptor mechanisms underlying opioid antinociception are also different in formalin and heat pain tests. In particular, opioid antinociception in the formalin test is 2- to 7-fold less sensitive to naloxone antagonism (2). This led us to propose that the test was particularly sensitive to kappa-mediated antinociceptive effects as opposed to mu-mediated effects in heat pain tests. Despite these data, the antinociceptive effects of clonidine in the formalin test are partially dependent on activation of mu receptors (25) and Fanselow et al. (14) found stress analgesia but not analgesia produced by U50,488 to be antagonized by the putative mu antagonist, $Cyr^2Tyr^3Orn^5Pen^7$-amide in the formalin test. These data suggest that mu receptors may be important in antinociception in the formalin test.

The present experiments were conducted to determine the extent to which mu receptor activation plays a role in antinociception in the formalin test using β-funaltrexamine (β-FNA), an irreversible antagonist (34). β-FNA selectively alkylates mu receptors and does not discriminate between mu_1 and mu_2 receptor subtypes in that both respiratory depression and antinociception are blocked (30,36). Three agonists with varying specificity for mu and kappa receptors were used, morphine as a mu agonist, ethylketocyclazocine (EKC) as a nonspecific mu and kappa agonist (20,37) and U50,488 as a kappa agonist (35). The latter two agents, EKC and U50,488, both label the putative $kappa_1$ and $kappa_2$ receptors, the affinity of U50,488 being about 10-fold greater for $kappa_1$ relative to $kappa_2$ than EKC (26,39). It should also be noted that the specificity of agonists is relative at best and as dose increases, specificity cannot be assumed (2,31). These agonists, therefore, can presumably activate all subtypes of mu and kappa receptors.

METHOD

Male Long Evans rats, 275–300 g, Charles River Quebec, were prepared with a guide cannula implanted above the lateral ventricle [coordinates 1.3 posterior to bregma, 1.8 lateral, and 3.0 below the skull (27)] 5 to 8 days before testing using standard stereotaxic technique under 30 mg/kg pentobarbital supplemented with ketamine:zylazine 10:1 mg/kg. Rats were housed in group cages of 2–3 in the colony room on a 12:12 light dark cycle with food and water available ad lib. Rats were used only

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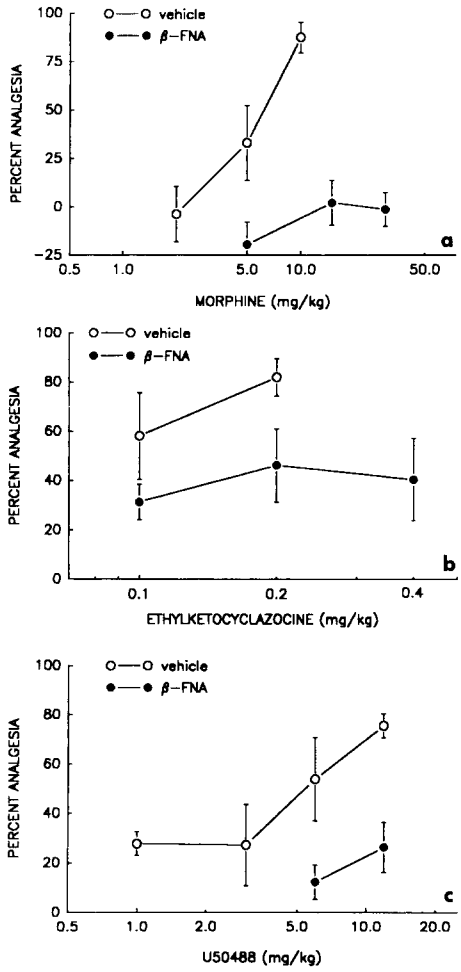

FIG. 1. Dose-response curves for morphine (A), EKC (B) and U50,488 (C) with and without pretreatment with β-FNA in the formalin test. Mean scores for 5–7 rats at each data point are shown. In all cases where doses overlap, Mann-Whitney U-tests comparing β-FNA-treated rats with controls indicate significant attenuation of antinociception except at 0.1 mg/kg EKC (p's<0.05).

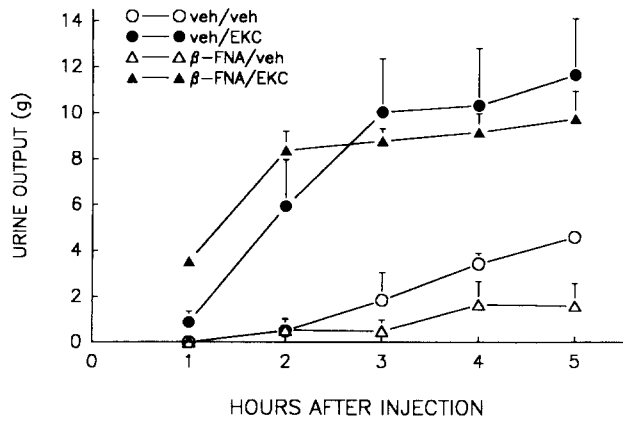

FIG. 2. Urination in response to 2.5 mg/kg EKC with and without pretreatment with β-FNA. ANOVA of the cumulated urine production at 5 h indicated a main effect of EKC, $F(1,8)=26.9$, $p<0.01$, and no interaction, $F(8,11)=0.1$, $p>0.1$.

once with the exception of those used for the diuresis test. These rats were used in a pain test 1 week prior to urine collection.

The formalin test (16) was conducted in $30\times30\times30$ Plexiglas chambers with a mirror mounted under the floor at a 45° angle to allow an unobstructed view of the rat's paws. The procedure was as follows. Three and one-half hours after β-FNA, 0.05 ml of 2.5% formalin was injected SC into the plantar surface of one rear paw and the rat was placed in the observation chamber. The formalin test was developed using the dorsal surface of a forepaw (12). The use of a rear paw eliminates the problem of how to classify rearing and normal grooming. While some investigators use the dorsal surface of a rear paw [e.g., (13, 14, 23)], we have always used the plantar surface because we began using the rear paw in the context of investigation of inflammation and did not want to use a hairy surface that obscured the visibility of the skin (7). The behavioral response to formalin injection rises rapidly after the injection, decreases between 5 and 20 min and then becomes stable for 30 min. Pain scores were rated from 30 to 50 min after formalin in the case of morphine and U50,448 and from 30 to 40 min for EKC because of its short time-effect curve (2).

Momentary pain scores were entered into a computer to record the time spent in each of the following behavioral categories: 0—weight is born evenly on both rear paws; 1—limps when locomoting or rests injected paw lightly on the floor; 2—elevates injected paw with at most the nails touching the floor; 3—chews or grooms the injected paw. A pain score was calculated using the following formula.

$$\text{Pain Score} = \frac{[(0*t_0)+(1*t_1)+(2*t_2)+(3*t_3)]}{(t_0+t_1+t_2+t_3)}$$

where t_0–t_3 are the number of seconds spent in each of the behavioral categories. Antinociception was expressed as % of the maximum possible effect (MPE) using the formula:

$$\%\text{MPE} = \frac{E - E_{min}}{E_{max} - E_{min}} * 100$$

where E_{max} is defined as a pain score of 0 (i.e., complete abolition of pain behavior) and E_{min} is the mean score of control rats. Controls were run with vehicle injections (β-FNA vehicle plus agonist vehicle and β-FNA plus agonist vehicle) at the same times as each agonist. β-FNA did not alter baseline pain scores (p's>0.2) and the scores for both controls were pooled to calculate the %MPE for each agonist. The mean control scores ranged from 1.53 to 1.91 for the various agonists and each drug was compared to its own control.

On the test day, 5 μg β-FNA in 5 μl of the distilled water vehicle was injected into the lateral ventricle using a 30-gauge cannula that projected 1.5 mm below the guide cannula. Pain testing was begun 4 h later. These parameters were based on time/dose relations given in (36). Other drugs were given subcutaneously in a volume of 1 ml/kg at the following times: morphine sulphate (gift of Sabex Canada) 50 min before testing in doses of 2, 5, 15 and 30 mg/kg; U50,448H (gift of Upjohn Canada) 40 min before testing in doses of 1, 3 and 12 mg/kg; ethylketocyclazocine methanesulfonate (gift of Sterling-Winthrop, Rensselaer, NY) 10 min before testing in doses of 0.1, 0.2 and 0.4 mg/kg. All doses are given as the salt. Drugs were not given blind because the effects of the opioid agonists are obvious so that blindness cannot be maintained.

To confirm that 5 μg β-FNA given intraventricularly did not block kappa receptors, nondeprived rats treated with either β-

FNA or vehicle 4 hours earlier were given a dose of EKC and placed in metabolic chambers. Urine was collected over the following 5 h and weighed hourly.

RESULTS

Figure 1 shows the dose-effect relations for morphine, U50,448 and EKC with and without β-FNA pretreatment. All three agents produced antinociception although U50,448 did not completely abolish the pain behavior at 12 mg/kg. At this dose, rats made persistent efforts to escape from the test chamber, suggesting that the drug was highly aversive. Higher doses of U50,448 were not tested since decreases in pain scores are uninterpretable when behavior is grossly distorted.

The antinociceptive effects of morphine up to 30 mg/kg were completely abolished by β-FNA pretreatment. Both U50,448 and EKC were partially antagonized by β-FNA such that at the higher doses, some antinociception was apparent. Attempts to escape were more prominent after β-FNA for U50,448 and also occurred in rats receiving EKC and β-FNA.

In order to confirm that the β-FNA pretreatment schedule used here did not attenuate the kappa receptor-mediated diuretic effects of EKC, urinary output was measured in 12 rats following pretreatment with β-FNA or its vehicle. As illustrated in Fig. 2, β-FNA did not block the diuretic effects of EKC. In fact, during the first hour after EKC, β-FNA increased the diuretic effect of EKC. This suggests that the β-FNA blocked a presumably mu-mediated antidiuretic effect of EKC (21,32).

DISCUSSION

The complete antagonism of morphine by β-FNA indicates that activation of central mu receptors plays an important role in antinociception in the formalin test. The partial antagonism of the two drugs with prominent kappa agonist activity, EKC and U50,488, could be interpreted as indicating that kappa receptor activation can also produce antinociception in the formalin test. However, at high doses, EKC acts by a peripheral mechanism (1) which would not be expected to be blocked by intraventricular β-FNA. Unlike EKC, U50,448 is not antagonized by systemic methylnaloxone which does not cross the blood-brain barrier (unpublished observations) but its low potency argues against a major role for kappa receptors. In addition, the marginal antinociceptive effect seen at 12 mg/kg of U50,448 with β-FNA is questionable since it was associated with almost continuous attempts to escape the test chamber. These results contrast with the failure of β-FNA to antagonize kappa agonists in the writhing test (38). The writhing test has also been shown to involve peripheral opioid receptors (6).

The data do not support the proposal (2) that opioid antinociception in the formalin test involves kappa receptor activation, even when agonists with preferential affinity for kappa receptors are used. Fanselow et al. (14) came to a similar conclusion using antagonists with putative selectivity for mu and kappa receptors although they did not find that U50,488 was blocked by the mu antagonist $Cys^2Tyr^3Orn^5Pen^7$-amide. The primary difference in their procedure that may explain the differences between the present results and theirs (i.e., that they obtained agonist and antagonist specific effects) is that their rats were subject to the stress of a novel environment even when not specifically stressed by shock. Such stressors change the predominant mechanism of morphine action both in terms of receptor subtype and neural basis (15,16).

Instead, the data implicate mu receptors. However, this conclusion depends on the assumption that β-FNA is specific in alkylating mu receptors and does not alter either delta or epsilon receptor function. This issue has not been definitively resolved (33). Most studies find that β-FNA has weak or negligible effects on the binding of delta ligands (28,33). Despite this, there appear to be physiological effects on delta receptor function. β-FNA antagonizes the ability of naloxone or ICI154,129 to ameliorate toxic shock without blocking the exacerbation caused by delta agonists (10,22). In the urinary bladder contraction model, β-FNA in the dose range used here blocked effects mediated by both mu and delta receptors (11). Rothman et al. (28) have proposed that this is a consequence of alteration of a mu-delta receptor complex.

The possibility of a mu-delta interaction in opioid antinociception in the formalin test is interesting and plausible. The naloxone dose-ratios we reported are consistent with delta as well as kappa mediation (20,37). Furthermore, delta-selective peptides are active in the formalin test (13) and ICI-174864, a partial delta agonist (9), potentiates the antinociceptive effects of clonidine (25). In clinical medicine analgesic agents with delta selectivity would have the advantage of producing less sedation and respiratory depression.

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