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Opioid Antinociceptive Effects of Delta-Receptor Antagonists

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LEANDER, J. D., P. D. GESELLCHEN AND L. G. MENDELSOHN. *Opioid antinociceptive effects of delta-receptor antagonists*. PHARMACOL BIOCHEM BEHAV **29**(2) 351–355, 1988.—The antinociceptive effects of delta opioid receptor antagonists (ICI 154129 and ICI 174864) have been studied using the mouse writhing assay. When administered intracerebroventricularly (ICV), ICI 154129 and ICI 174864 produced dose-related inhibition of writhing with respective ED50's of 97 μg/mouse and 1.4 μg/mouse. Inhibition of writhing by ICI 174864 (3 μg, ICV) was antagonized by subcutaneous (SC) naloxone doses of 0.1 mg/kg and greater. Pretreatment of mice with 80 mg/kg (SC) of beta-funaltrexamine (β-FNA), an irreversible mu-receptor antagonist, 28 hr before ICV injection of ICI 174864 shifted the dose-effect curve for ICI 174864 to the right (ED50 of 7.3 μg/mouse). When administered SC, ICI 174864 inhibited writhing with an ED50 of 8.5 mg/kg. Maximal inhibition occurred 30 min after SC administration and decreased 50% by 2 hr. After β-FNA pretreatment, doses of ICI 174864 as high as 80 mg/kg (SC) did not inhibit writhing. There was no antinociceptive effect of ICI 174864 in mice chronically maintained on morphine, i.e., chronic morphine produced cross-tolerance to the delta antagonist. These results show that delta-selective receptor antagonists produced antinociception which was related to the mu-receptor, but was probably not a result of direct agonist action.

Beta-funaltrexamine (β-FNA) Delta antagonists Antinociception Opioid Mice ICI 174864 ICI 154129 Analgesia

THE existence of multiple types of opioid receptors, mu, kappa and delta, in neural tissue has been documented by behavioral [21], pharmacological [15], and biochemical research (for reviews, see [28] and [35]). Delineation of the functions and possible interactions of these sites, however, remains as a primary challenge in the field of opioid research. While it is clear that the mu-receptor can mediate analgesia [10,21] and stimulation at the kappa site can produce analgesia and increased urination, as well as other behavioral effects [16–18, 29, 32], the *in vivo* role of the delta-receptor is less clear. Recent biochemical [25,26], as well as pharmacological [14,31], studies suggest that delta-receptors may interact with other opioid sites to modify the responses mediated by those receptors.

The recent development of selective ligands, both agonists [23,24] and antagonists [4, 5, 27] for the delta-receptor has facilitated exploration of the function of this site. In a recent study, we found that the delta antagonist, ICI 174864, produced an antinociceptive effect in the writhing test in mice [19]. In the studies reported here, the delta-selective opioid receptor antagonists ICI 154129 [27] and ICI 174864 [5] have been used to explore this antinociceptive effect.

METHOD

Behavioral Measurements

The mouse writhing response was defined as a contraction of the abdominal musculature followed by the extension of the hind limbs [3]. Writhing was induced by intraperitoneal administration of 0.6% acetic acid in a volume of 20 ml/kg. Five CF-1 male mice (Charles River, Portage, MI), weighing approximately 20 g after being fasted overnight, were observed for the writhing response. The observation period was 10 min in duration, beginning 5 min after acetic acid administration. The percent inhibition of writhing was calculated from the average number of writhes in the appropriate control group. The mean number of writhes for different groups of control-treated mice ranged between 30 and 40 writhes during the 10-minute observation period. Each data point is the mean±S.E.M. of 5 mice. The dose required to inhibit writhing by 50% was defined as the ED50 and was calculated by linear regression of log dose vs. percent inhibition of writhing. When the antagonist action of naloxone was being studied, an antagonist dose 50 (AD50) was calculated. The AD50 was defined as the dose of naloxone which antagonized the effect of an analgesic dose of ICI 174864 by 50%, and was calculated by linear regression. The writhing test has been previously used by this laboratory to study the antinociceptive effects of a variety of opioid-related compounds [19, 20, 36].

Drug Administration

Naloxone was obtained from Endo Laboratories, Garden City, NY. The ICI 154129 was obtained from ICI Phar-

maceuticals, England. ICI 174864 and β-FNA were prepared by Drs. Manohar Tilak and Dennis Zimmerman, respectively, Lilly Research Laboratories, Indianapolis, IN.

For the studies using ICV injection, Hamilton microsyringes, bearing 27-gauge needles with stops at 2.5 mm from the needle tip, were used. The mice were gently restrained, the scalp was quickly incised, the needle was inserted 1.5 mm to the side of the sagittal sutures, and 10 μl of saline or drug solution was slowly administered into a lateral ventricle. In mice of this present size, this is an easy procedure, and after such ICV injections, the mice show no behavioral signs of discomfort or irritation around the site of injection. The ICV doses were expressed as μg/mouse. The injection of acetic acid occurred 5 min after the ICV injection.

For parenteral administration of drugs, injections were administered SC in a volume of 10 ml/kg, and doses were expressed as mg/kg. L-Arginine was used to solubilize ICI 174864 in distilled water. The highest concentration needed of ICI 174864 was 8 mg/ml of distilled water. The addition of L-arginine in a concentration of 2 mg/ml solubilized the ICI 174864. Solutions necessary for doses lower than 80 mg/kg of ICI 174864 were derived by dilution with distilled water from this stock solution (8 mg of ICI 174864 + 2 mg of L-arginine/ml). The injection of acetic acid occurred 20 min after the parenteral injection of ICI 174864. All naloxone injections occurred 20 min before injection of acetic acid.

β-FNA has been considered to be a selective, long-lasting antagonist at the mu opioid receptor [33,34]; however, others have reported that β-FNA does have antagonist activity at the delta-receptor [9,13]. β-FNA as the HCl salt was administered in a dose of 80 mg/kg 28 hr before dosing with ICI 174864. This dose and pretreatment time resulted in marked shifts of the dose-effect curves for mu opioid agonists, but have little effect on kappa agonists under these test conditions [36]. The effects of 80 mg/kg of ICI 174864 were also studied in a group of mice that had been chronically maintained on morphine for several weeks. Morphine was provided in a concentration of 1 g/liter of 0.01 M Na saccharin as the sole source of fluid for these animals. This regimen maintains healthy mice that are massively tolerant to the mu-receptor agonist morphine, shifting the morphine dose-effect curve at least 10-fold to the right [19].

RESULTS

There was no difference in the number of writhes between the control mice given saline injections by the ICV route (37.2±4.1 and 42.6±3.0; two groups, n=5 each) and those by the SC route (41.2±4.8, 32.0±5.1 and 38.8±1.6; three groups n=5 each).

Figure 1 shows the antinociceptive activity in the mouse writhing assay of both delta antagonists ICI 154129 (a) and ICI 174864 (b). Both compounds produced dose-related inhibition of writhing, with ED50's of 97 μg and 1.4 μg/mouse, respectively, after ICV administration. These antinociceptive effects were not associated with classical behavioral signs produced by mu agonists, such as Straub tail and locomotor activity. The lower frame shows that pretreatment (28 hr) of the mice with β-FNA (80 mg/kg SC) produced approximately a 20% increase in writhing alone (shown above β) and a 5-fold rightward shift of the dose-effect curve for ICI 174864.

Subcutaneously administered naloxone antagonized, in a dose-related fashion, the antinociceptive effect of 3 μg of ICI 174864 (Fig. 2). The calculated dose of naloxone which re-

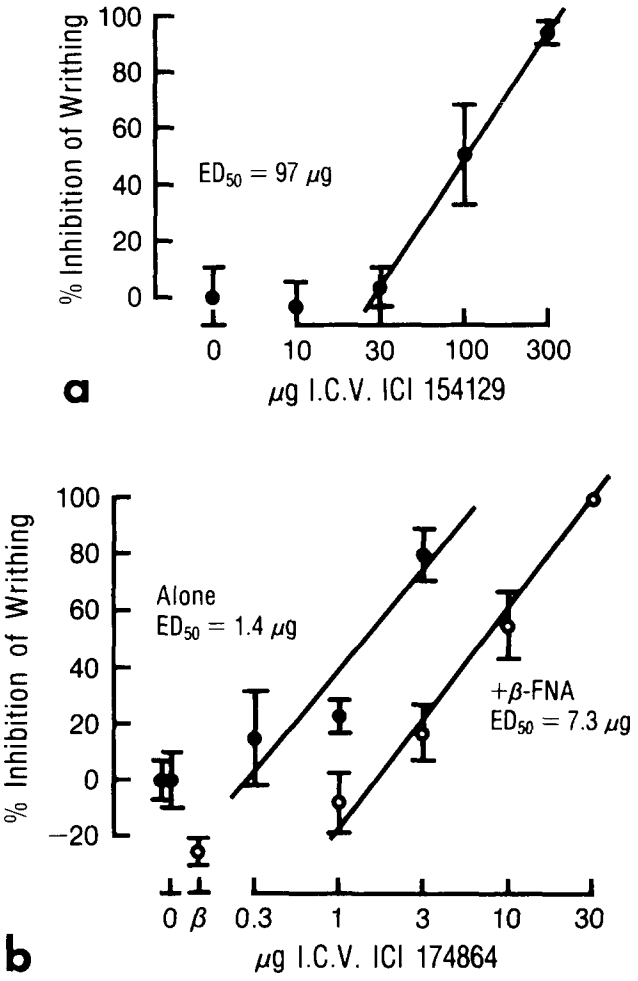

FIG. 1. Inhibition of mouse writhing by delta opioid antagonists. The percent inhibition of writhing was measured 5 min after acetic acid injection and 15 min after ICV administration of the peptide. (a) Dose-effect curve for ICI 154129. (b) Dose-effect curve for ICI 174864 alone (●) and after pretreatment of the mice with β-FNA (○). Each point is the mean±S.E.M. for 5 mice.

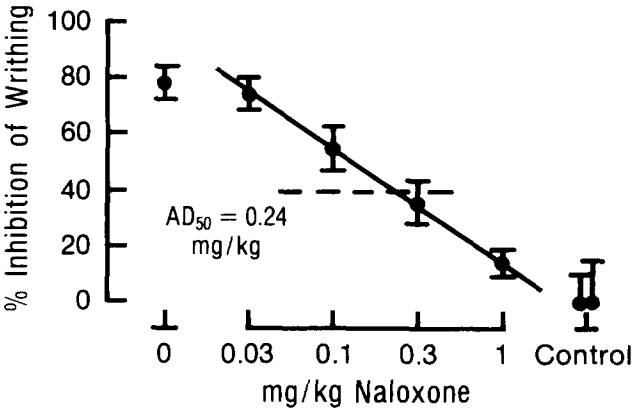

FIG. 2. Antagonism of 3 μg (ICV) ICI 174864 by subcutaneously administered naloxone. Controls were mice which received neither naloxone nor ICI 174864.

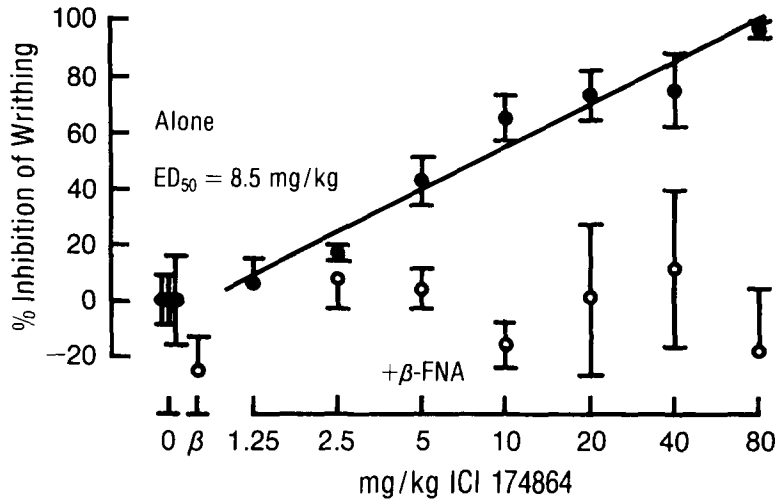

FIG. 3. Inhibition of mouse writhing by subcutaneously administered ICI 174864. Dose-effect curve for ICI 174864 alone (●) and after pretreatment of the mice with β-FNA (○).

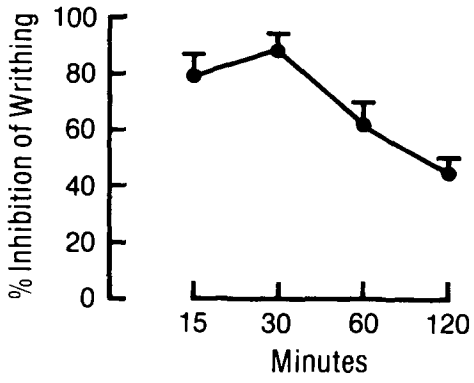

FIG. 4. Time course of analgesia produced by an 80 mg/kg SC dose of ICI 174864.

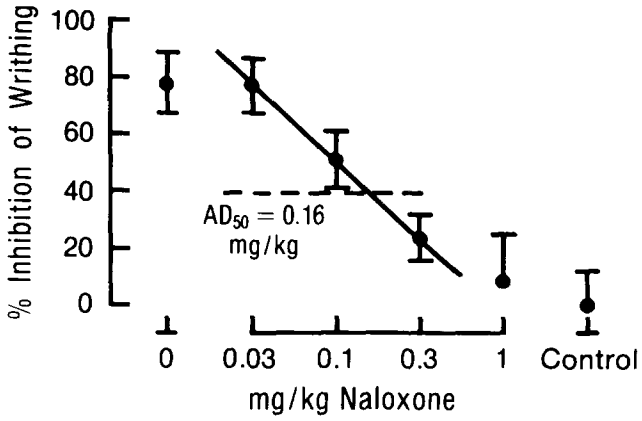

FIG. 5. Antagonism of 20 mg/kg (SC) ICI 174864 by subcutaneously administered naloxone. Controls were mice which received neither naloxone nor ICI 174864.

duced the antinociceptive effect by 50% (AD50) was 0.24 mg/kg.

ICI 174864 also produced a dose-related antinociceptive effect when administered by the subcutaneous route (Fig. 3). The ED50 was 8.5 mg/kg for ICI 174864 alone. Treatment of a group of mice with L-arginine (20 mg/kg) had no antinociceptive effect alone (36.0±1.5 writhes compared to control of 36.8±3.4). Pretreatment of the animals with β-FNA completely blocked the antinociceptive effects of ICI 174864. Even the 80 mg/kg dose of ICI 174864 had no effect in the β-FNA-pretreated animals. This dose is 16 times greater than the minimal dose (5 mg/kg) of ICI 174864 alone that produced a significant antinociceptive effect. Thus, β-FNA pretreatment appeared to shift the dose-effect curve for ICI 174864 greater than 16-fold. L-Arginine alone (20 mg/kg) had no effect in β-FNA-treated mice (data not shown).

The time course of the antinociceptive effect after the 80 mg/kg (SC) dose of ICI 174864 is shown in Fig. 4. Peak inhibition of writhing occurred at 30 min and decreased to 50% inhibition by 120 min after administration of ICI 174864.

In order to compare the ability of naloxone to antagonize the effects of SC administration of ICI 174864 to those of ICV administration (shown in Fig. 2), a SC dose of ICI 174864 was selected (20 mg/kg) which produced the approximate percent inhibition of writhing as the ICV dose of 3 μg. The antinociceptive effect of this subcutaneous dose of ICI 174864 was antagonized over the same naloxone dose range as was the 3 μg ICV dose (Fig. 5). The naloxone AD50 against 20 mg/kg (SC) of ICI 174864 was 0.16 mg/kg.

In the mice maintained on the morphine drinking solution, 80 mg/kg of ICI 174864 had no statistical effect. The mean (±S.E.M.) number of writhes in the control-treated mice on the morphine solution was 42.4 (±4.6), whereas the mean for the ICI 174864-treated mice was 36.0 (±6.4).

DISCUSSION

In the course of evaluating the behavioral effects of a selective delta opioid peptide, the antinociceptive effect of ICI 174864 was noted [20]. The present paper reported more

extensively on the antinociceptive effects of ICI 154129 and ICI 174864. These effects occurred in the dose ranges that have been used to demonstrate specific delta antagonist activity [6, 9, 11, 30]. Antinociceptive activity of these antagonists has also been noted by other investigators [6]. The antinociceptive activity produced by the delta-receptor antagonist ICI 174864 was antagonized by low doses of naloxone and by pretreatment of the mice with β-FNA and exhibited cross-tolerance in morphine-maintained mice. The dose range over which the effects of ICI 174864 were antagonized by naloxone in the present study is similar to the dose range (0.02–0.32 mg/kg) for antagonizing the effects of an equally antinociceptive dose of morphine (unpublished observations, J. D. Leander), and compares favorably with doses used by others for morphine and other mu opioid agonists in analgesic studies [1,12]. Likewise, the pretreatment regimen of β-FNA used in the present experiments has been demonstrated to produce a marked shift to the right for dose-effect curves of mu-receptor agonists, but not for kappa-receptor agonists [36]. These observations are consistent with the present suggestion that mu-receptor integrity (or activation) is required for expression of the antinociceptive activity produced by these delta-receptor antagonists.

The data presented here are also complementary with the results of other studies reporting *in vivo* agonist-like effects of the delta antagonists. For example, β-FNA blocked the usual therapeutic effect of delta antagonists on endotoxic shock [14]. Both β-FNA and low doses of naloxone antagonized the rise in shock threshold produced by ICI 154129 [30]. Dray *et al.* [7] observed inhibition of urinary bladder contractions with both delta antagonists. This inhibition was reversible with naloxone, but the required dose was greater than that necessary to antagonize the activity of a mu agonist.

Direct agonist activation of the mu-receptor by either of these delta opioid antagonists is highly unlikely. In membrane binding assays, these antagonists display low affinities for the mu sites, on the order of 10 μM or greater (personal observation, L. G. Mendelsohn; [4]). More importantly, in smooth muscle assays *in vitro*, these compounds antagonize delta agonists at low concentrations of approximately 30 nM [2,5], whereas, at higher concentrations of approximately 5000 nM, they antagonize mu agonists [5,27] as well. Moreover, a recent study utilizing the mouse vas deferens and guinea pig ileum preparations has demonstrated partial agonist activity of ICI 174864 *in vitro* which was attributed to its interaction solely with the delta- rather than the mu-receptor [2]. Thus, the present results cannot be considered to be a result of direct mu agonist activity.

An alternative source of agonist activity might be from potential metabolites of these delta antagonists. The two antagonists are pentapeptides, and carboxypeptidase A digestion of ICI 174864 can produce a Des leucine tetrapeptide analog which has been shown to have direct-acting mu agonist activity in smooth muscle preparations [2]. Whether this conversion occurs sufficiently in brain tissue *in vivo* to produce analgesia is not known.

It has recently been hypothesized that mu-receptors and delta-receptors coexist in an opioid-receptor complex [25,26]. Antinociception is thought to arise from activation of the mu-receptor [31]. According to this model, Leu-enkephalin-like peptides interact with the delta-receptor to facilitate the mu-receptor-mediated antinociception, whereas Met-enkephalin-like peptides attenuate the antinociception. Our results suggest that antagonist action at the delta-receptor of such an opioid complex may be sufficient to enhance antinociceptive responses mediated by endogenous ligands acting at the mu-receptor. We have previously shown that delta agonist activity appears to attenuate mu agonist analgesic activity in the writhing test [20]. Interactions of endogenous ligands with the mu-receptor may be tonically present or may be acutely initiated by release of these compounds in response to the stressful stimuli of the acetic acid. It is interesting to note that prior exposure to β-endorphin greatly enhanced the agonist-like effects of ICI 174864 (ICV) to inhibit bladder contractions [9]. All of these observations, taken together, suggest a close interaction of the mu- and delta-receptors in effecting antinociception.

The present data report that the two pentapeptides which are relatively selective delta opioid receptor antagonists produce antinociceptive effects in the mouse writhing test. These effects appear to be dependent upon the integrity of mu opioid receptors, although a direct agonist action of these compounds on the mu-receptor is unlikely. Thus, the exact mechanism by which this effect is produced is yet to be determined.

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