Short communication

Selective modulation of morphine antinociception, but not development of tolerance, by δ receptor agonists

Qi Jiang, Henry I. Mosberg [1] and Frank Porreca

*Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, U.S.A. and [1] College of Pharmacy,
University of Michigan, Ann Arbor, MI 48109, U.S.A.*

Co-administration of δ opioid agonists at doses which do not produce measurable antinociception were demonstrated to produce an increase in the antinociceptive potency of morphine in the mouse tail-flick test. In contrast, co-administration of equi-antinociceptive combinations of a δ agonist plus morphine for three days resulted in the development of less tolerance to morphine antinociceptive actions. The data indicate that while acute antinociceptive effects of opioid μ agonists are modulated by δ agonists, the development of antinociceptive tolerance is not.

Antinociception; Morphine; δ-Opioid receptor agonists; Tolerance

1. Introduction

Previous reports from our laboratory (Heyman et al., 1989a,b; Jiang et al., in press; Porreca et al., 1990) and others have shown that δ opioid agonists can modulate the potency and efficacy of opioid μ agonists such as morphine. The modulatory action (i.e. increases or decreases in potency and/or efficacy), but not the direct antinociceptive effect of morphine, was blocked by pretreatment with the selective δ opioid antagonist, ICI 174,864 (Cotton et al., 1984), and this compound given alone did not produce antinociception nor modulation of morphine effects. These studies, and others (see Rothman et al., 1989 for review) have supported the hypothesis that some opioid μ and δ receptors may exist in a functionally associated state. In this hypothesis, opioid receptors thought to functionally interact have been termed $\mu_{complexed}$ (μ_{cx}) and

$\delta_{complexed}$ (δ_{cx}) receptors to designate their existence within the μ-δ receptor complex (Rothman et al., 1989) while those μ and δ receptors not interacting were termed the $\mu_{non-complexed}$ (μ_{ncx}) and $\delta_{non-complexed}$ (δ_{ncx}) receptors.

The possibility that occupation of the opioid δ_{cx} site could increase the potency (Heyman et al., 1989a,b; Porreca et al., 1990) and efficacy (Jiang et al., in press) of opioid μ agonists was of interest, for example, in using μ agonists of lower efficacy to produce equi-effective pain relief clinically. Such a possibility was particularly interesting in that the route of administration of the modulating δ agonist seemed to be unimportant. In this regard, i.c.v. administration of the highly selective δ agonist, [D-Pen²,D-Pen⁵]enkephalin (DPDPE) (Mosberg et al., 1983) or i.p. administration of [Leu⁵]enkephalin produced an increase in the antinociceptive potency of morphine given by the same route.

In spite of these findings, however, it remained unclear whether all of the pharmacological actions of morphine would be similarly subjected to modulation by δ opioid agonists, or whether selective

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Correspondence to: F. Porreca, Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, U.S.A.

modulation of specific effects could be achieved. The present study has addressed whether DPDPE or [Leu[5]]enkephalin would increase the rate of development of tolerance to morphine antinociceptive actions following chronic administration of equi-antinociceptive combinations. We now report that although these compounds increase morphine antinociceptive potency, the rate of development of antinociceptive tolerance depends only on the amount of morphine administered.

2. Materials and methods

2.1. Animals

Male, ICR mice (20-30 g) were used for all experiments. Animals were kept in groups of five in a temperature-controlled room with a 12 h light-dark cycle (lights on 7:00 a.m. to 7:00 p.m.). Food and water were available ad libitum until the time of the experiment.

2.2. Injection techniques

I.c.v. administrations were made directly into the lateral ventricle as previously described (Heyman et al., 1989a,b). The mouse was lightly anesthetized with ether, an incision was made in the scalp, and the injection was made 2 mm lateral and 2 mm caudal to bregma at a depth of 3 mm using a 10 μl Hamilton microliter syringe with a 26-gauge needle. I.c.v. injections were made at a volume of 5 μl.

2.3. Tail flick assay

The thermal nociceptive stimulus was 55°C warm water with the latency to tail-flick or withdrawal taken as the endpoint (Heyman et al., 1989a,b). After the determination of control latencies, the mice received graded i.c.v. doses of vehicle, morphine, or concurrent combination of morphine plus DPDPE or [Leu[5]]enkephalin. Morphine and DPDPE were given by the i.c.v. route and testing took place after 10 min, a time previously shown to result in a maximal response for both compounds (Heyman et al., 1987;

1989a,b; Jiang et al., in press), while morphine and [Leu[5]]enkephalin were given i.p. and testing took place after 20 min, a time shown to result in a maximal response for the combination (Porreca et al., 1990).

A cutoff time of 15 s was employed; if the mouse failed to respond within this time, the tail was removed from the water and that animal was assigned a maximum score. Mice not responding within 5 s in the initial control trial were eliminated from the experiment. Antinociception at each time point was calculated according to the following formula: % antinociception = 100 × (test latency-control latency)/(15-control latency).

2.4. Induction of antinociceptive tolerance

In the tolerance experiments, mice were injected twice daily (8:00 a.m. and 5:p.m.) for 3 days, and testing took place on the morning of day 4. For the DPDPE/morphine experiments, animals received i.c.v. saline, morphine alone (1.2 or 6 nmol), DPDPE alone (1.55 nmol) or morphine plus DPDPE (1.2 and 1.55 nmol), respectively. This combination of i.c.v. morphine plus DPDPE was shown to produce an equivalent degree of antinociception as i.c.v. morphine given at the 6 nmol dose. After each i.c.v. injection, the scalp incision was closed using wound clips. No signs of distress were noted in these animals over the 3 days (six injections) period. In the [Leu[5]]enkephalin/morphine experiments, mice received i.p. saline, morphine alone (9 or 24 μmol/kg), [Leu[5]]enkephalin (18 μmol/kg) or morphine plus [Leu[5]]enkephalin (9 and 18 μmol/kg), respectively. The combination of i.p. morphine plus [Leu[5]]enkephalin was shown to produce an equivalent degree of antinociception as i.p. morphine given at the 24 μmol/kg dose.

2.5. Chemicals

Morphine sulfate was purchased from Mallinckrodt Chemical Co. (St. Louis, MO) while [Leu[5]]enkephalin was purchased from Sigma Chemical Company (St. Louis, MO). DPDPE was synthesized as previously described (Mosberg et

al., 1983). All compounds were dissolved in distilled water just before using.

2.6. Statistics

Regression lines, A_{50} values (i.e. the dose producing a 50% antinociception response) and 95% confidence limits (C.L.) were determined with each individual data point using the computer program described by Tallarida and Murray (1986) (procedure 8). For the fitting of regression lines and calculation of the A_{50} values, only the linear portion of the dose-effect curve was used. Relative potencies were calculated by comparison of the regression line A_{50} values. All data points shown are the mean of 10 mice and error bars represent the S.E.

3. Results

I.c.v. morphine produced a dose-related antinociceptive effect in the mouse tail-flick test with an A_{50} value of 0.92 (0.65-1.3) nmol. When morphine and DPDPE were co-administered i.c.v., the morphine dose-response line was displaced to the left; the A_{50} of this combination was calculated as 0.21 (0.12-0.34) indicating an approximately 4.4-fold increase in potency. Similarly, i.p. morphine produced a dose-related antinociceptive repsonse with an A_{50} value of 15.3 (12.99-18.02) μmol/kg and when co-administered with i.p. [Leu^5]enkephalin the dose-response line for the combination was to the left of that for morphine alone, with an A_{50} of 4.25 (3.41-5.31) μmol/kg, a shift of approximately 3.6-fold. From these pairs of curves, equi-antinociceptive combinations were calculated and given twice daily in the tolerance experiments.

Construction of the i.c.v. morphine dose-response line in animals pretreated twice daily with saline showed no change from non-injected animals with an A_{50} of 1.15 (0.87-2.09) nmol, and similarly, pretreatment i.c.v. with the modulating dose of DPDPE (1.55 nmol) did not affect the i.c.v. dose-response line for morphine resulting in an A_{50} of 1.27 (0.86-1.86) nmol (fig. 1). Pretreatment with i.c.v. morphine at 1.2 nmol for 3 days
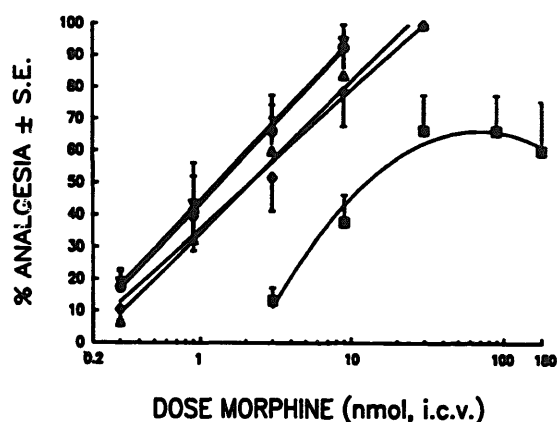

Fig. 1. Dose-response lines for i.c.v morphine in mice pretreated with saline (circles), DPDPE (1.55 nmol, inverted triangles), morphine (1.2 nmol, triangles), morphine (6 nmol, squares) and morphine (1.2 nmol) plus DPDPE (1.55 nmol) (diamonds).

resulted in a slight tolerance to the antinociceptive actions of morphine as shown by the 1.85-fold rightward displacement of the dose-response line; the A_{50} for morphine in animals pretreated with morphine at 1.2 nmol was calculated to be 2.13 (1.44-3.15) nmol (fig. 1). Pretreatment with i.c.v. morphine at 6 nmol produced a greater degree of tolerance resulting in approximately a 12.84-fold rightward displacement of the i.c.v. morphine dose-response line. Additionally, the maximum antinociceptive effect of i.c.v. morphine in animals pretreated with this higher dose of morphine was shown to be approximately 60%; the calculated A_{50} value for these pretreated mice was 14.77 (9.21-23.68) nmol, indicating severe antinociceptive tolerance (fig. 1). In contrast to the degree of tolerance seen with the higher dose of morphine, administration of the lower morphine dose plus DPDPE, a combination which produced an equi-antinociceptive effect, resulted in a much lower degree of tolerance. I.c.v. co-administration of morphine (1.2 nmol) plus DPDPE (1.55 nmol) for 3 days produced a rightward displacement of the i.c.v. morphine dose-response line of only 1.85-fold (fig. 1). The morphine A_{50} value in mice pretreated i.c.v. with the combination was 2.13 (1.55-2.93) nmol.

Construction of the i.p. morphine dose-response line in animals pretreated twice daily with
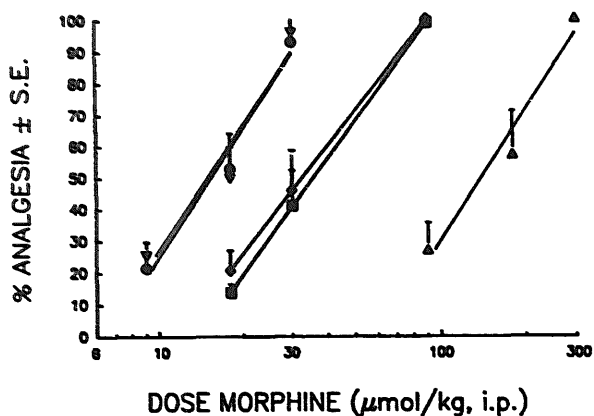
Fig. 2. Dose-response lines for i.p. morphine in mice pretreated with saline (circles), [Leu^5]enkephalin (18 μmol/kg, inverted triangles), morphine (9 μmol/kg, squares), morphine (24 μmol/kg, triangles) and morphine (9 μmol/kg) plus (Leu^5]enkephalin (18 μmol/kg) (diamonds).

saline showed no change from non-injected animals with an A$_{50}$ of 15.49 (13.41-19.03) μr.ol/kg, and similarly, pretreatment i.p. with the modulating dose of [Leu^5]enkephalin (18 μmol/kg) did not affect the i.p. dose-response line for morphine resulting in an A$_{50}$ of 14.91 (13.01-18.71) μmol/kg (fig. 2). Pretreatment with i.p. morphine at 9 μmol/kg for 3 days resulted in a slight tolerance to the antinociceptive actions of morphine as shown by the 2.29-fold rightward displacement of the dose-response line; the A$_{50}$ for morphine in animals pretreated with i.p. morphine at 9 μmol/kg was calculated to be 35.52 (30.65-41.16) μmol/kg) (fig. 2). Pretreatment with i.p. morphine at 24 μmol/kg produced a greater degree of tolerance resulting in approximately a 8.96-fold rightward displacement of the i.p. morphine dose-response line. The calculated A$_{50}$ value for these mice pretreated with i.p. morphine at 24 μmol/kg was 138.77 (114.47-168.22) μmol/kg (fig. 2). In contrast to the degree of tolerance seen with i.p. pretreatment with this higher dose of morphine, administration of the lower morphine dose plus [Leu^5]enkephalin, a combination which produced an equi-antinociceptive effect, resulted in a much lower degree of tolerance. I.p. co-administration of morphine (9 μmol/kg) plus [Leu^5]enkephalin (18 μmol/kg) for

3 days produced a rightward displacement of the i.p. morphine dose-response line of only 2.1-fold (fig. 2). The i.p. morphine A$_{50}$ value in mice pretreated i.p. with this combination was 32.48 (26.71-39.51) μmol/kg.

4. Discussion

Previous studies had reported that the antinociceptive potency of morphine could be enhanced by compounds acting at the δ receptor, such as [Leu^5]enkephalin (Vaught and Takemori, 1979) suggesting that the endogenous enkephalins might have an indirect, modulatory role in antinociception. The present study, and our previous work (Heyman et al., 1989a,b; Jiang et al., in press; Porreca et al., 1990) continue to support this hypothesis. Though the enhancement by δ agonists of desirable μ effects such as antinociception was attractive, the possibility remained that non-desirable effects such as the development of antinociceptive tolerance would also be simultaneously enhanced. In the study of Vaught and Takemori (1979) mice were pretreated with an acute s.c. injection of morphine alone, or a combination of s.c. morphine plus i.p. [Leu^5]enkephalin and the degree of antinociceptive tolerance to morphine established after a 3 h period. In this experiment, the degree of tolerance was increased by co-administration of morphine plus the δ agonist. However, it should be noted that this experimental protocol emphasized the phenomenon of acute tolerance. The present studies have re-evaluated this issue by administration of μ-δ combinations over a 3 days period.

Our results clearly demonstrate that using a chronic tolerance paradigm, that co-administration of μ-δ combinations result in a smaller degree of tolerance to morphine than administration of an equi-antinociceptive (higher) dose of morphine alone. This result was demonstrated regardless of whether both compounds were given by the i.c.v. or the i.p. routes. From the results of these experiments it would appear that the degree of antinociceptive tolerance depends only on the amount of morphine administered. These results are in agreement with the observation of a lack of cross-

tolerance between morphine and DPDPE (Porreca et al., 1987; Kovács et al., 1988).

The failure of the δ agonists to modulate the development of antinociceptive tolerance while enhancing the direct antinociceptive actions of the μ agonists is of interest when interpreted in light of the hypothesis of functionally complexed μ and δ receptors. In this hypothesis, μ agonists such as morphine can produce antinociceptive effects by acting at either μ_{ncx} or μ_{cx} sites. In such a scenario, it would seem possible that while the modulation of direct antinociceptive effects occurs via the δ_{cx} receptor, and the development of tolerance is more closely related to the μ_{ncx} site. In any case, it would appear that the direct mediation of antinociceptive actions and the development of antinociceptive tolerance can be separated, at least in terms of the modulatory actions of δ agonists. The present results continue to support the concept of functionally coupled μ and δ receptors and offer the possibility that such combinations may be of clinical use.

Acknowledgements

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