Interaction of [D-Pen²,D-Pen⁵]enkephalin and [D-Ala²,Glu⁴]deltorphin with δ-opioid receptor subtypes in vivo

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Abstract

The interaction of [D-Pen²,D-Pen⁵]enkephalin (DPDPE) and [D-Ala²,Glu⁴]deltorphin with δ-opioid receptor subtypes was investigated. Pretreatment of mice with the δ_1-opioid receptor antagonist, [D-Ala²,Leu⁵,Cys⁶]enkephalin (DALCE), produced a virtually complete antagonism of the antinociceptive actions of DPDPE, but had no effect on those of [D-Ala²,Glu⁴]deltorphin. In DALCE pretreated mice (i.e., δ_1-opioid receptors blocked), DPDPE was able to significantly antagonize the antinociceptive effects of [D-Ala²,Glu⁴]deltorphin. Pretreatment of mice with the δ_2-opioid receptor antagonist, naltrindole-5'-isothiocyanate (5'-NTII) produced a virtually complete antagonism of the antinociceptive effects of [D-Ala²,Glu⁴]deltorphin, but had no effect on the antinociception produced by DPDPE. In 5'-NTII pretreated mice (i.e., δ_2-opioid receptors blocked), [D-Ala²,Glu⁴]deltorphin had no effect on the antinociception produced by DPDPE. These data suggest that [D-Ala²,Glu⁴]deltorphin is highly selective for the δ_2-opioid receptor in vivo, and that neither agonist nor antagonist actions can be demonstrated at δ_1-opioid receptors for this peptide. In contrast, under appropriate conditions, DPDPE can be shown to interact with both δ_1- and δ_2-opioid receptor subtypes; DPDPE may have limited efficacy (i.e., is a partial agonist) at the δ_2-opioid receptor.

Key words: Opioid; δ-Opioid receptor subtype; DPDPE ([D-Pen²,D-Pen⁵]enkephalin); [D-Ala²,Glu⁴]Deltorphin; Antinociception; (Mouse)

1. Introduction

Recently, subtypes of δ-opioid receptors have been identified, and termed δ_1- and δ_2-opioid receptors (Sofuoglu et al., 1991; Jiang et al., 1991; Mattia et al., 1991). [D-Pen²,D-Pen⁵]Enkephalin (DPDPE) and [D-Ala²,Glu⁴]deltorphin are highly selective peptidic δ-opioid receptor agonists which produce antinociception following intracerebroventricular (i.c.v.) administration to mice (e.g., Jiang et al., 1990). The antinociceptive effects of DPDPE are antagonized by [D-Ala²,Leu⁵, Cys⁶]enkephalin (DALCE) but not by 5'-naltrindole isothiocyanate (5'-NTII) (Jiang et al., 1991); neither DALCE nor 5'-NTII produces antagonism of the antinociceptive effects of μ- or κ-opioid receptor agonists (Jiang et al., 1991). In contrast, the antinociceptive effects of [D-Ala²,Glu⁴]deltorphin are antagonized

by 5'-NTII, but not by DALCE (Jiang et al., 1991). On this basis, DPDPE has been suggested to produce its antinociceptive effects via a δ_1-opioid (i.e., DALCE-sensitive) receptor subtype, while [D-Ala²,Glu⁴]deltorphin appears to be selective for a δ_2-opioid (5'-NTII) receptor subtype.

In addition to the direct antinociceptive properties of δ-opioid receptor agonists, these compounds have been repeatedly demonstrated to produce a modulatory (i.e., increase or decrease in potency and efficacy) action on effects of μ-opioid receptor agonists such as morphine (Vaught and Takemori, 1979; Lee et al., 1980; Heyman et al., 1989a,b; Horan et al., 1992) in a variety of endpoints including antinociception in mice and rats. The interactions between δ-opioid receptor agonists and μ-opioid receptor agonists such as morphine are mediated via δ-opioid receptors as the modulation, but not the direct antinociception of morphine, is antagonized by δ-opioid receptor-selective antago-

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nists such as ICI 174,864 (Heyman et al., 1989a,b). Further, the selective δ-opioid receptor agonists DPDPE and [D-Ala^2,Glu^4]deltorphin (given at sub-effective doses) have been shown to positively modulate morphine antinociception, and further, to interact with morphine in a synergistic fashion (Horan et al., 1992). Based on data such as these, as well as on the basis of substantial evidence using radioligand binding approaches in vitro, one hypothesis to explain the modulatory actions of δ-opioid receptor agonists on μ-opioid receptor-mediated effects is that these interactions occur via a μ-δ-opioid receptor complex (see Rothman et al., 1988, for review), though other interpretations of such data are possible.

The observation that both DPDPE and [D-Ala^2,Glu^4]deltorphin can be demonstrated to produce an ICI 174,864-sensitive modulatory effect on morphine antinociception (Heyman et al., 1989a,b) might be taken to suggest that both the δ_1 and δ_2 subtypes of δ-opioid receptors are involved in the observed modulatory actions. However, recent studies using subtype-selective antagonists have demonstrated that the modulatory actions of both DPDPE and [D-Ala^2,Glu^4]deltorphin are sensitive to antagonism by 5'-NTII, but not by DALCE (Porreca et al., 1992), leading to the conclusion that only the δ_2-opioid receptor is involved in the modulatory effects of δ-opioid receptor agonists on μ-opioid receptor effects.

The observation that the δ_1-opioid receptor agonist, DPDPE, as well as the δ_2-opioid receptor agonist, [D-Ala^2,Glu^4]deltorphin, both produce modulatory actions on morphine antinociception apparently via a δ_2-opioid receptor does not appear consistent with the observed selectivity of these agonists for δ-opioid receptor subtypes as identified using studies of direct antinociception (Jiang et al., 1991; Sofuoglu et al., 1991; Mattia et al., 1991). Thus, the present study sought to explore the possibility that DPDPE and [D-Ala^2,Glu^4]deltorphin may interact with one, or both, subtypes of δ-opioid receptors. The approach focused on the possibility that these peptides might be capable of interaction at more than one subtype of δ-opioid receptor if the preferred subtype were selectively blocked. The data indicate that while [D-Ala^2,Glu^4]deltorphin appears highly selective for the δ_2-opioid receptor, DPDPE can act at both δ-opioid receptor subtypes.

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

All compounds were given by the intracerebroventricular (i.c.v.) route. I.c.v. administrations were made directly into the lateral ventricle as previously described (Jiang et al., 1991). 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. Animals were tested by gently holding them by hand in a vertical position and recording the baseline latency. Control baseline latencies (i.e., prior to injection of test substances) ranged between 2–4 s. Mice not responding within 5 s ($<$ 10%) during baseline testing were discarded from the experimental groups. After the determination of baseline latencies, the mice received graded doses of δ-opioid agonist alone in control mice, or in DALCE- or 5'-NTII-treated mice in combination with the alternate δ-opioid receptor agonist (i.e., DPDPE or [D-Ala^2,Glu^4]deltorphin). Tail-flick latencies were determined after 10 min, a time previously shown to result in a maximal antinociceptive response for both compounds (Heyman et al., 1989a,b; Jiang et al., 1990, 1991). In antagonist studies employing irreversible agents, DALCE (4.5 nmol) or 5'-NTII (17.5 nmol) were given as a single pretreatment dose, 24 h prior to testing. These doses and times have been previously established to be the times of peak agonist and antagonist actions (Jiang et al., 1990, 1991).

A cut-off 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. Chemicals

Cyclic Tyr-D-Pen-Gly-Phe-D-Pen (where Pen is D-penicillamine, DPDPE), [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE), [D-Ala^2,Glu^4]deltorphin and naltrindole-5'-isothiocyanate (5'-NTII) were synthesized as previously described. [D-Ala^2,Glu^4]Deltorphin was suspended in 20% Tween-80/water solution immediately prior to use. All other compounds were dissolved in distilled water just before using.

2.5. Statistics

Regression lines, A_{50} values (i.e., the dose producing a 50% antinociceptive response) and 95% confidence limits (C.L.) were determined with each individual data point as previously described (Jiang et al., 1990, 1991). 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 standard error.

3. Results

Pretreatment of mice with DALCE (4.5 nmol i.c.v.) or 5′-NTII (17.5 nmol i.c.v.) 24 h prior to testing produced no changes in basal tail-flick latency. DALCE pretreatment produced a significant blockade of the antinociceptive effects of a submaximal dose of i.c.v. DPDPE (46.5 nmol), but had no effect on the antinociceptive effects of [D-Ala^2,Glu^4]deltorphin (fig. 1A). In contrast, pretreatment with 5′-NTII significantly antagonized the antinociceptive effects of a submaximal dose of [D-Ala^2,Glu^4]deltorphin (12.6 nmol) but had no effect on antinociception produced by DPDPE (fig. 1B). The dose-response lines for i.c.v. [D-Ala^2,Glu^4] deltorphin in control, or in DALCE-pretreated mice are shown in fig. 2. Co-administration of DPDPE (46.5 nmol) with [D-Ala^2,Glu^4]deltorphin in DALCE-pretreated mice resulted in a marked rightward displacement of the [D-Ala^2,Glu^4]deltorphin dose-response line (fig. 2). The A_{50} values (and 95% confidence limits) for [D-Ala^2,Glu^4]deltorphin in control, DALCE-pretreated, and DALCE-pretreated plus DPDPE mice were 3.9 (3.36–4.53), 3.9 (3.28–4.73) and 31.7 (26.25–38.28) nmol, respectively. These data show that under δ_1-opioid receptor blocked conditions, DPDPE produced an approximately 8-fold rightward displacement
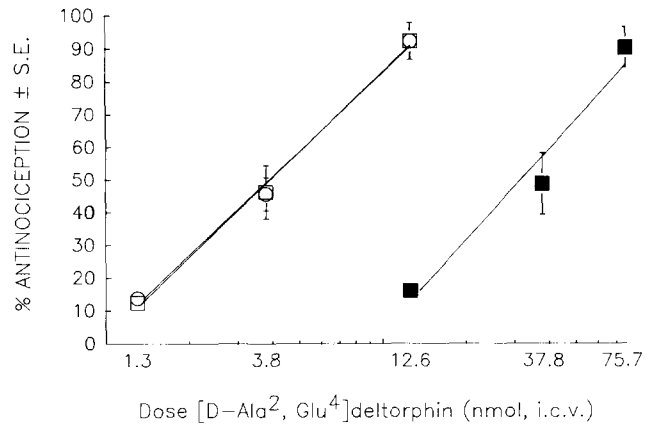

Fig. 2. Antinociceptive dose-response curves of graded i.c.v. doses of [D-Ala^2,Glu^4]deltorphin in control mice (open circles), DALCE-pretreated mice (open squares), or in DALCE-pretreated mice in the presence of a sub-maximal dose of DPDPE (closed squares); DPDPE produced virtually no effect in DALCE-pretreated mice.
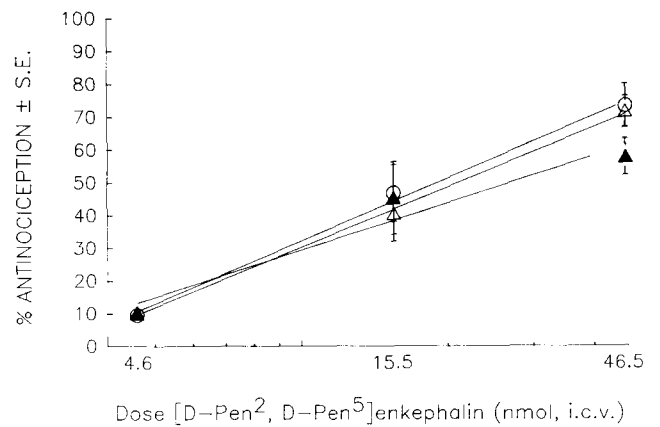

Fig. 3. Antinociceptive dose-response curves of graded i.c.v. doses of DPDPE in control mice (open circles), 5′-NTII-pretreated mice (open triangles), or in 5′-NTII-pretreated mice in the presence of a sub-maximal dose of [D-Ala^2,Glu^4]deltorphin (closed triangles); [D-Ala^2,Glu^4]deltorphin produced virtually no effect in 5′-NTII-pretreated mice.
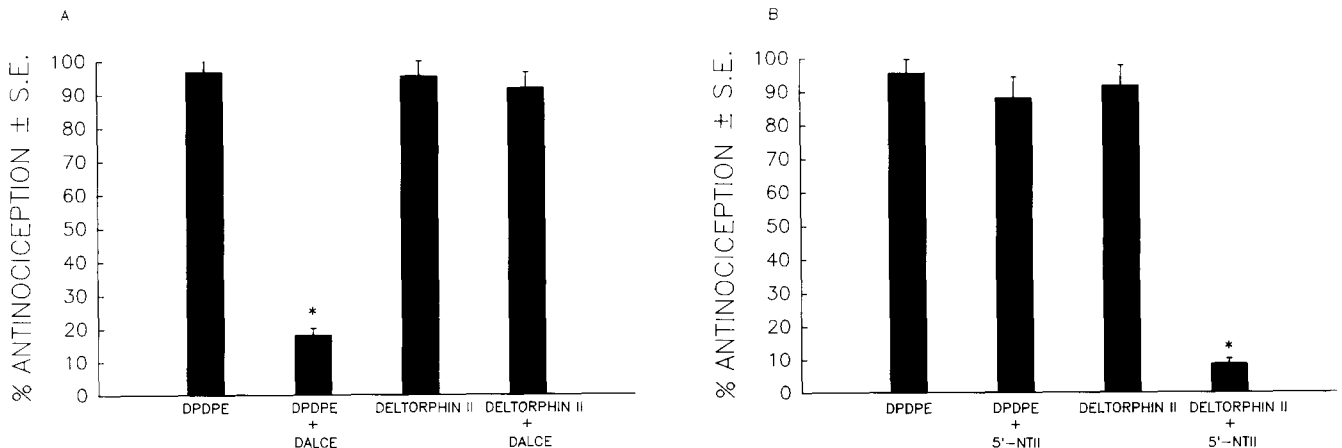

Fig. 1. Antinociceptive effect of DPDPE or [D-Ala^2,Glu^4]deltorphin in control mice, or following pretreatment with DALCE (A) or 5′-NTII (B); neither DALCE nor 5′-NTII had any direct antinociceptive effect.

of the [D-Ala^2,Glu^4]deltorphin dose-response curve. The dose-response line for [D-Ala^2,Glu^4]deltorphin in DALCE-pretreated mice was not different from that in controls. The dose-response lines for DPDPE in control, or in 5′-NTII-pretreated mice are shown in fig. 3. Co-administration of [D-Ala^2,Glu^4]deltorphin (12.6 nmol) with DPDPE in 5′-NTII-pretreated mice had no significant effect on the DPDPE dose-response line (fig. 3). The A_{50} values (and 95% confidence limits) for DPDPE in control, 5′-NTII-pretreated, and 5′-NTII-pretreated plus [D-Ala^2,Glu^4]deltorphin mice were 19.3 (14.55–25.58), 25.38 (18.72–34.41) and 29.03 (18.48–45.61) nmol, respectively.

4. Discussion

Recent studies of the direct antinociceptive properties of δ-opioid receptor agonists have provided strong pharmacological evidence for the existence of subtypes of δ-opioid receptors. Through the use of novel and selective δ-opioid receptor antagonists, i.c.v. DPDPE and [D-Ala^2,Glu^4]deltorphin have been suggested to produce their antinociceptive effects in mice via subtypes of δ-opioid receptors, termed δ_1 and δ_2, respectively (Sofuoglu et al., 1991; Jiang et al., 1990, 1991). This finding is supported by the observation of a two-way lack of cross-tolerance between DPDPE and [D-Ala^2,Glu^4]deltorphin, as well as to the μ-opioid receptor agonist [D-Ala^2,NMPhe^4,Gly-ol]enkephalin (DAMGO) (Mattia et al., 1991; Sofuoglu et al., 1991). It should also be noted that the antinociceptive effects of DPDPE and [D-Ala^2,Glu^4]deltorphin are not sensitive to μ- or κ-opioid receptor antagonists in this model, and the δ-opioid receptor-selective antagonists, DALCE and 5′-NTII, do not antagonize the antinociceptive actions of μ-opioid receptor agonists such as morphine or DAMGO (Jiang et al., 1990, 1991).

In addition to a direct role for δ-opioid receptors in the mediation of antinociception, it has long been recognized that agonists at δ-opioid receptors can produce either a positive (i.e., increase in potency and efficacy) or negative (i.e., decrease in potency and efficacy) effect on μ-opioid receptor agonists. Interestingly, two endogenous ligands of the δ-opioid receptor produce opposite modulatory actions: [Leu^5]enkephalin produces a positive, while [Met^5]enkephalin produces a negative, effect on μ-opioid receptor-mediated antinociception (Vaught et al., 1979; Lee et al., 1980). Both the positive and the negative modulatory actions of [Leu^5]enkephalin and [Met^5]enkephalin (but not the direct μ-opioid receptor-mediated antinociception) are sensitive to antagonism by the δ-opioid receptor antagonist, ICI 174,864, leading to the conclusion that the modulatory effect is mediated via δ-opioid receptors; such receptors have been hypothesized to be a part of

a functional or physical μ-δ-opioid receptor complex (see Rothman et al., 1988 for review).

Using the same approach as taken for direct antinociceptive studies, recent data have demonstrated that the δ-opioid receptor modulatory effect is sensitive only to δ_2-opioid receptor antagonists, suggesting the participation only of this receptor subtype in μ-opioid receptor modulation (Porreca et al., 1992). Additionally, induction of tolerance at the δ_2-, but not the δ_1-opioid, receptor prevents the modulatory action (Vanderah and Porreca, unpublished observations). The finding that both the positive and negative modulatory effects of δ-opioid receptor agonists (Heyman et al., 1989a,b) could be antagonized by ICI 174,864 together with the identified subtypes of δ-opioid receptors initially suggested that these positive and negative modulatory effects might be mediated through different δ-opioid receptor subtypes. This concept was reinforced by the observation that DPDPE and [D-Ala^2,Glu^4]deltorphin produced their direct antinociceptive effects via subtypes of δ-opioid receptors. Notably, however, both DPDPE and [D-Ala^2,Glu^4]deltorphin produced a positive modulatory effect, and further, this action was selectively sensitive to antagonism by δ_2-opioid receptor antagonists (Porreca et al., 1992). Such observations appeared inconsistent with the concept that these selective δ-opioid receptor agonists acted at subtypes of δ-opioid receptors. This paradox was investigated in the present study by blocking the preferred receptor at which DPDPE and [D-Ala^2,Glu^4]deltorphin are hypothesized to act using irreversible δ-opioid receptor subtype-selective antagonists, DALCE and 5′-NTII. Possible interactions of these ligands with the non-preferred receptor might be revealed under such δ_1- and δ_2-opioid receptor blocked conditions.

Following blockade of the δ_1-opioid receptor using DALCE, the direct antinociceptive effects of DPDPE were almost completely blocked while those of [D-Ala^2,Glu^4]deltorphin were unaltered. Construction of the [D-Ala^2,Glu^4]deltorphin dose-effect curve in the presence of a dose of DPDPE (which produced a sub-maximal effect under control conditions) resulted in a marked rightward displacement of the [D-Ala^2,Glu^4]deltorphin dose-response curve. This observation suggests that DPDPE can bind to the δ_2-opioid receptor, but apparently does not produce antinociception directly at this site. In contrast, the converse experiment under δ_2-opioid receptor blocked conditions suggested that [D-Ala^2,Glu^4]deltorphin does not interact with the δ_1-opioid receptor site. No antagonism of the DPDPE dose-effect curve was observed in the presence of a sub-maximal dose of [D-Ala^2,Glu^4] deltorphin under conditions in which the effects of [D-Ala^2,Glu^4]deltorphin were almost completely blocked by 5′-NTII. The possibility that supramaximal

doses of [D-Ala^2,Glu^4]deltorphin might eventually interact with the δ_1-opioid receptor cannot be excluded and was not investigated in the present study.

These observations suggest that DPDPE has actions at both δ-opioid receptor subtypes. Apparently, DPDPE mediates its direct antinociceptive actions via the δ_1-opioid receptor while its modulatory effects are mediated via the δ_2-opioid receptor (i.e., is a partial agonist at this site). In contrast, [D-Ala^2,Glu^4]deltorphin produces both actions via the δ_2-opioid receptor subtype. It is also of particular interest that the direct antinociceptive actions of these substances appear to be mediated predominantly, if not exclusively, through its own receptor subtype, even though both compounds compete for sites labelled by δ-opioid receptor-selective ligands. A possible explanation of this observation may relate to the efficacy of each compound at each receptor subtype. Thus, though DPDPE may effectively bind to the δ_2-opioid receptor site, it may have only limited efficacy and be unable to transduce direct antinociception at this site. Such a view would be supported by demonstrations of effective modulatory actions of [Leu^5]enkephalin (Vaught and Takemori, 1979) and [Met^5]enkephalin (Lee et al., 1980) on morphine antinociception, even though it is virtually impossible to demonstrate direct antinociceptive effects of these compounds in this test (Horan et al., 1992). On this basis, it would be reasonable to suggest that the production of a modulatory action on μ-opioid receptor-mediated antinociception would occur with greater efficiency than that required for direct production of antinociception, and would appear to account for the actions of DPDPE at both subtypes of δ-opioid receptors, as well as the significant differences in the direct antinociceptive pharmacology of DPDPE and [D-Ala^2,Glu^4]deltorphin.

5. References

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