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ANTINOCICEPTIVE EFFECTS OF [D-ALA^2]DELTORPHIN II, A HIGHLY SELECTIVE δ AGONIST *IN VIVO*

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Abstract. The present study has characterized the antinociceptive actions of [D-Ala^2]deltorphin II following intracerebroventricular (*i.c.v.*) administration in the mouse tail-flick test. [D-Ala^2]deltorphin II produced dose- and time-related antinociception, with maximal effects at +10 min and significant antinociception which lasted for 40-60 min. [D-Ala^2]deltorphin II was 13-fold more potent than *i.c.v.* [D-Pen^2, D-Pen^5]enkephalin (DPDPE), a second highly selective δ agonist, and approximately equipotent with *i.c.v.* morphine in producing antinociception. The antinociceptive effects of *i.c.v.* [D-Ala^2]deltorphin II and DPDPE, but not those of morphine, were antagonized by the selective δ antagonist, ICI 174,864. In contrast, pretreatment with the non-equilibrium μ antagonist, β-funaltrexamine blocked morphine antinociception, but failed to antagonize [D-Ala^2]deltorphin II and DPDPE antinociception. These data indicate that [D-Ala^2]deltorphin II produced its antinociceptive effects at a supraspinal δ receptor. [D-Ala^2]deltorphin II appears to be the most appropriate δ opioid agonist currently available for studies *in vivo* and support the involvement of δ receptors in supraspinal antinociception.

Introduction

Advances in the understanding of opioid receptor involvement in pharmacological endpoints *in vivo* have depended on the synthesis and availability of highly selective agonists and antagonists. This problem has been particularly significant in investigations of opioid δ receptor activity, as the presumed natural ligands for this receptor [Leu^5]enkephalin and [Met^5]enkephalin (1) are straight chain peptides that are highly susceptible to enzymatic destruction *in vivo* (2). Development agonists and antagonists for the δ receptor with sufficient selectivity and which retain sufficient stability for studies *in vivo* has been difficult. The synthesis of DPDPE(3), a highly selective δ agonist, and of ICI 174,864, a highly selective δ antagonist (4) has overcome some of these problems and made investigations of the δ receptor possible. However, in spite of the relatively high selectivity of DPDPE as a δ agonist, the possibility nevertheless exists of cross-

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reactivity at other (non-preferred) receptors; thus, compounds of even higher selectivity (specificity?) are desirable.

Recently, Erspamer and colleagues (5) have reported the isolation of the linear heptapeptides isolated from the skin of frogs from the genus *Phyllomedusa*. These peptides, named the deltorphins, (6) have been demonstrated to have extremely high affinity for the δ receptor and increased δ selectivity compared to DPDPE when evaluated using bioassay and radioligand binding techniques *in vitro*. The present study has evaluated and compared [D-Ala^2]deltorphin II (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2) with DPDPE in an antinociceptive assay *in vivo*.

MATERIALS AND METHODS

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 hr 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.

Injection techniques. Intracerebroventricular administrations were made directly into the lateral ventricle as previously described (7). 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.

Tail flick assay. The thermal nociceptive stimulus was 55 °C warm water with the latency to tail-flick or withdrawal taken as the endpoint. After the determination of control latencies, the mice received graded *i.c.v.* doses of vehicle, agonist alone, or with concurrent administration of antagonist. [D-Ala^2]deltorphin II was given *i.c.v.* as a single injection and testing took place 10, 20, 30, 40, and 60 min later. DPDPE was also given by the *i.c.v.* route and testing took place after 10 min, a time previously shown to result in a maximal response (8). The delta antagonist, ICI 174,864 (4) was always given concurrently with the agonist, 10 min prior to testing. In studies with the µ antagonist, β-funaltrexamine (β-FNA)(9), this compound was given as a single *i.c.v.* pretreatment 24 hr prior to testing. This time has previously been demonstrated to produce maximal antagonism of µ agonists (10).

A cutoff time of 15 sec 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 sec in the initial control trial were eliminated from the experiment. Antinociception at each time point was calculated according to the following formula: % antinociception = 100 x (test latency - control latency)/(15 - control latency).

Chemicals. DPDPE and [D-Ala^2]deltorphin II were synthesized as previously described (3,6). ICI 174,864 (N,N-diallyl-Tyr-*Aib*-*Aib*-Phe-Leu-OH, where *Aib* is α-aminoisobutyric acid) was purchased from Cambridge Research Biochemicals (Atlantic Beach, NY) and β-funaltrexamine (β-FNA) was purchased from Research Biochemicals Inc. (Natick, MA). All compounds were dissolved in distilled water just before using.

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 using the computer program described by Tallarida and Murray (11). 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.

RESULTS

Acute antinociceptive effects of [D-Ala^2]deltorphin II AND DPDPE

I.c.v. administration of [D-Ala^2]deltorphin II (0.38 - 12.78 nmol) produced a dose- and time-related antinociception (Fig. 1). The maximal antinociceptive response of [D-Ala^2]deltorphin II was seen after 10 min and effects were detected until 60 min after administration. DPDPE and morphine also produced antinociception when tested 10 min after *i.c.v.* administration. The A_{50} values for these agonists are shown in Table I, and indicate that [D-Ala^2]deltorphin II is approximately 13-fold more potent than DPPDE, and approximately equipotent with morphine (10).

Antagonist studies with ICI 174,864 and β-FNA

Co-administration of the δ antagonist, ICI 174,864 (4.4 nmol, *i.c.v.*) did not produce any measureable antinociception alone, but antagonized the antinociceptive actions of DPDPE (Table I) in agreement with previous reports (10,12) as well as those of [D-Ala^2]deltorphin II (Fig. 2, Table I). This dose of ICI 174,864, however, did not antagonize the antinociceptive actions of morphine, also in agreement with previous reports (10,12)(Table I). In contrast, pretreatment with β-FNA (18 nmol, *i.c.v.* at -24 hr), while not producing antinociception alone, blocked the antinociceptive effects of morphine (10), but not those of [D-Ala^2]deltorphin II (Fig. 2) or DPDPE (Table I).

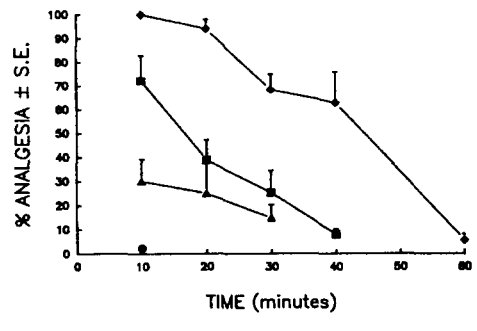

Figure 1. Antinociceptive time-response curves of graded *i.c.v.* doses [0.38 (circle), 1.28 (triangles), 3.83 (squares) and 12.77 nmol (diamonds)] of [D-Ala^2]deltorphin II in the mouse tail flick test.

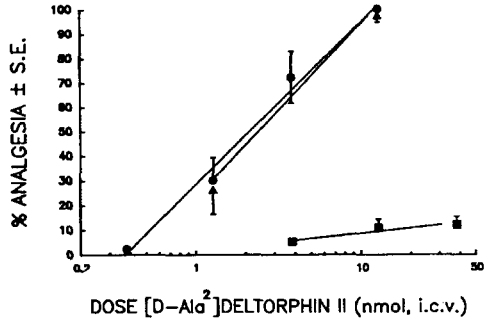

Figure 2. Dose-response lines for *i.c.v.* [D-Ala^2]deltorphin II (at +10 min) alone (circles), in the presence of ICI 174,864 (4.4 nmol, *i.c.v.*) (squares) or in mice pretreated with β-FNA (18 nmol at -24 hr)(triangles) in the mouse tail-flick test.

Table I. Effects of co-administration of the δ antagonist ICI 174, 864 or pretreatment with the μ antagonist β-FNA on the A_{50} (and 95% confidence limits) for antinociception produced by *i.c.v.* morphine, DPDPE or [D-Ala^2]deltorphin II in the mouse tail-flick test.

	A_{50} (95% C.L.)(*nmol*)	+ ICI 174,864 (4.4 *nmol*)	+ β-FNA (18 *nmol*, -24 hr)
Morphine	0.9 (0.6 - 1.4)	0.9 (0.6 - 1.3)	10.8 (7.3 - 16.8)
DPDPE	28.8 (21.1 - 39.2)	---	25.3 (23.8 - 28.6)
[D-Ala^2]deltorphin II	2.1 (1.7 - 2.7)	---	2.4 (1.6 - 2.5)

DISCUSSION

The discovery of the deltorphin family of peptides (5,6) is of particular significance in the strong leads offered by these novel structures into the design and synthesis of compounds which will interact specifically with opioid δ receptors. [D-Ala^2]deltorphin II has been characterized *in vitro* as one of the most highly selective δ ligands with the highest affinity for the δ receptor of all the δ-ligands known to date (6). [D-Ala^2]deltorphin II has been shown to have approximatley 13-fold higher affinity for the δ receptor than DPDPE and an increased μ/δ selectivity ratio of approximately 15-fold (6). Additionally, [D-Ala^2]deltorphin II shows potent agonist activity in the mouse isolated vas deferens (MVD)(13), a bioassay for opioid δ activity and minimal effects in the guinea pig isolated ileum (GPI), a bioasay for opioid μ activity (14). Recent studies from our group have indicated that [D-Ala^2]deltorphin II has a GPI/MVD potency ratio of approximately 32,000, approximately 10-16 fold higher than that of DPDPE (3) in these bioassays (Dr. Thomas H. Kramer, Department of Pharmacology, University of Arizona, personal communication). Thus, it is of particular interest that [D-Ala^2]deltorphin II produced antinociceptive effects *in vivo*, which were shown to be approximately 13-fold more potent than DPDPE. The increase in potency is in agreement with the increase in affinity for the δ receptor demonstrated in binding assays and with the increased potency seen in the MVD (IC_{50} = 0.8 nM). Further, the increase in potency *in vivo* is of interest in that some investigators have postulated that antinociceptive potency *in vivo* is correlated solely with affinity at μ receptors *in vitro* (15). The increase in potency of [D-Ala^2]deltorphin II demonstrated *in vivo* together with increased affinity for δ receptors reported *in vitro* argue against this hypothesis and strongly support the involvement of supraspinal δ receptors in antinociceptive processes. Additional support for this concept stems from the demonstration of differential antagonism that strongly supports the action of [D-Ala^2]deltorphin II and DPDPE at a receptor site distinct from that acted upon by morphine (16).

Based on the data from present studies as well as previous reports, it is suggested that [D-Ala^2]deltorphin II may be the most selective δ agonist currently available and the most appropriate for study *in vivo*. The suitability for studies *in vivo* of this peptide is indicated by the long-lasting antinociceptive time-course of 40-60 min. This antinociceptive time-course is similar to that seen with *i.c.v.* morphine in this test (7). This novel δ agonist should be of value in providing further insight into the pharmacological and physiological importance of the opioid δ receptor.

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