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Antinociceptive and behavioral effects of synthetic deltorphin analogs

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Abstract

A possible correlation of behavioral, antinociceptive and cataleptic responses with central δ- and μ-opioid receptor stimulation was tested for in the rat by i.c.v. injections of some synthetic deltorphin analogs. At doses ranging from 0.1 to 3.0 nmol/rat, the selective δ-opioid receptor agonist, [D-Ala^2,Glu^4]deltorphin (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2), induced a dose-dependent stereotyped pattern of locomotor activity, reaching the maximum in the first 30 min; doses higher than 30 nmol induced early and fleeting antinociception. The replacement of Glu^4 by Gly, Ala, Val, His or Asn yielded peptides with a lower δ-selectivity because of a gain in μ-affinity. [D-Ala^2,Ala^4]deltorphin (0.14–4.0 nmol) induced negligible behavioral stimulation but a rapidly appearing and long-lasting analgesia and catalepsy. The other four synthetic peptides induced biphasic effects: low dosages stimulated locomotion whereas higher doses initially suppressed, then increased locomotor activity. At doses ranging from 1 to 70 nmol all the peptides induced analgesia and catalepsy. In experiments examining the locomotor and antinociceptive effects induced by 14 nmol of [D-Ala^2,Gly^4]deltorphin in rats pretreated with μ and δ antagonists, the non-selective μ-opioid receptor antagonist, naloxone (1 mg/kg i.p.), reduced analgesia and abolished the initial hypolocomotion. The δ-selective antagonist, naltrindole (10 mg/kg i.p.), abolished locomotor activity without affecting analgesia. The μ_1-selective antagonist, naloxonazine (10 mg/kg i.v.), seemed to prolong analgesia and immobility. Hence this peptide appears to activate, in addition to δ-receptors, mainly the opioid receptor μ_2-subtype, which mediates catalepsy in the rat. We suggest that the μ_2- and δ-opioid receptors of the rat brain modulate locomotor behavior by activating functionally opposed responses. [D-Ala^2,Ala^4]deltorphin had an antinociceptive and cataleptic potency higher than would have been expected from its μ-affinity. A possible explanation might be a μ/δ-opioid receptor interaction.

Keywords: Deltorphin analog; Antinociception; Catalepsy; Locomotion

1. Introduction

Dermorphins and deltorphins are naturally occurring opioid heptapeptides from amphibian skin. Dermorphins are potent and selective μ-opioid receptor agonists (Montecucchi et al., 1981; Broccardo et al., 1981; Negri et al., 1992), whereas deltorphins bind with high affinity and selectivity to δ-opioid receptors (Erspamer et al., 1989; Kreil et al., 1989). Injections of the highly selective δ-opioid receptor agonists, deltorphins, into the rat brain ventricles, ventral tegmental area and nucleus accumbens invariably increase locomotor activity and induce stereotyped behavior (Longoni et al., 1991; Negri et al., 1991). Paakkari et al. (1990) reported that, in rats, dermorphin given at subanalgesic doses (lower than 100 pmol/kg i.c.v.) caused increased locomotor activity, which was blocked by pretreatment with the μ_1-opioid receptor-selective antagonist, naloxonazine. At higher doses, dermorphin and [Lys^7]dermorphin produced locomotor inhibition and catalepsy that were resistant to naloxonazine pretreatment. The recently synthesized [Trp^4,Asn^7]dermorphin, a μ_2-opioid receptor-preferring ligand (Negri et al., 1992), did the opposite: at low, subanalgesic doses, it induced a naloxonazine-resistant cataleptic state in the rat. The above findings suggest that μ_1-opioid receptor activation stimulates and μ_2-opioid receptor activation inhibits locomotor activity in the rat.

Dermorphins and deltorphins have in common the

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N-terminal sequence, Tyr-D-Xaa-Phe, where D-Xaa is a D-amino acid residue, but they differ in the sequence of their *C*-terminal tetrapeptide. We previously showed that, in a series of synthetic deltorphin analogs, replacement of the Asp or Glu residues in the fourth position of the sequence of deltorphins by an amino acid residue (such as Gly, Ala, Val, Asn or His) gave peptides with a δ-opioid receptor affinity approximately equal to that of the parent peptide but a μ-opioid receptor affinity many times higher (Melchiorri et al, 1991). The present study was designed to find whether the behavioral stimulation, antinociception and catalepsy evoked by these [D-Ala^2,Xaa^4]deltorphin analogs correlated with δ- and μ-opioid receptor activation in the rat brain. For this purpose, we compared the pharmacological activity of these analogs with their affinity for μ- and δ-sites.

2. Materials and methods

2.1. Animals and surgery

Male Sprague-Dawley rats weighing 240–260 g were used. Under light ethyl ether anesthesia each rat was implanted surgically with a plastic guide cannula (Linca, Tel-Aviv, Israel), stereotaxically inserted through a skull hole drilled over the left lateral ventricle of the brain (AP = −1 mm, L = +1.8 mm relative to the bregma, according to data from Paxinos and Watson, 1982). The cannula was screwed into the skull hole until it reached a depth of 1 mm below the external surface of the skull and was secured to bone with dental cement. After surgery, the rats were allowed to recover for 4–6 days in individual plastic cages. Food and water were available ad libitum and the animals were maintained on a natural day/night, light/dark cycle. The compounds were injected into the left lateral ventricle, using a Hamilton 10-μl syringe fitted with a 26-gauge needle that was inserted through the guide cannula to a depth of 4.2 mm below the external surface of the skull. Drugs and control solutions were injected in a constant volume of 5 μl. The needle was left in situ for 30 s to allow the drug to diffuse. 2 days before peptide testing, the proper position of the cannula was checked by measuring the drinking response of the rats to i.c.v. administration of 50 ng of angiotensin II and at the end of the experiments the proper position of the i.c.v. injections was ascertained by inspection of the brain ventricles after an injection of methylene blue.

2.2. Behavioral testing

During studies of locomotor activity and stereotyped behavior, the rats were housed in 40 × 40 × 30-cm cages. The bottom of each cage was divided into four equal sectors. A television camera was installed above the cages, the movements of the rats were videotaped and subsequently analyzed. Locomotion was measured by counting the number of sectors entered by the rat with its four limbs in 15-min periods. The exploratory component due to the novel environment was minimized by letting the animals adapt to the chambers in a 1-h daily session for 3 days before drug testing began. Test sessions were conducted between 9:00 am and 2:00 pm. The rats were adapted to the activity chamber for 30 min before injection and were returned to the same chamber immediately after injection. Each rat's behavior was observed for 120 min after the i.c.v. injection of the peptides (treated rats) or saline (controls). Locomotor activity was expressed as the cumulative score over a 120-min observation period (8 periods of 15 min). 20 groups of eight rats were used. Rats were used twice at an interval of one week. Every dose of each peptide was tested in eight rats. The data are expressed as means ± S.E.M. of cumulative activity. LcD_{50} was defined as the dose that produced 50% of the maximum locomotor stimulation (= the maximum cumulative score obtained with [D-Ala^2,Glu^4]-deltorphin over 2 h of observation).

2.3. Antinociception and catalepsy testing

Antinociception and catalepsy were evaluated in groups other than those used for locomotion. Every dose of each peptide was evaluated in groups of six to eight animals. Antinociception was determined by the tail-flick test (D'Amour and Smith, 1941). A cut-off time of 12 s was used to prevent blistering. The intensity of the thermal stimulus was adjusted to obtain a reaction latency ≤ 4 s, in untreated animals. Rats not withdrawing their tails within 12 s were assigned a maximum antinociceptive score. The degree of analgesia was expressed as percentage maximum possible effect (%MPE) according to the equation: %MPE = [(test latency − control latency)/(12 − control latency)] × 100. The tail-flick latency was measured before drug treatment (control) and every 15 min after drug injections, during the first hour, and every 30 min thereafter, until analgesia disappeared. The AD_{50} of each peptide was defined as the dose producing 50% of the MPE.

Catalepsy was evaluated (at 15, 30, 45, 60, 90, and 120 min after drug injection) by placing the rat's forelimbs over a 10 cm high horizontal bar and measuring the time the animal maintained this posture. Rats remaining more than 30 s on the bar were defined as cataleptic (positive bar test). The CD_{50} of each peptide was defined as the dose that produced a positive bar test in 50% of the animals tested. Five doses of each drug were used and six to eight rats were used for each dose.

2.4. Experiments with antagonists

To determine whether antinociceptive and locomotor effects resulted from opioid receptor activation, we studied the effect of three opioid antagonists against [D-Ala^2,Gly^4]deltorphin, chosen as representative of the deltorphins tested, because it has the lowest μ/δ selectivity. 12 groups of six rats were used. The μ-preferring antagonist, naloxone (1, 3 and 10 mg/kg), and the δ-selective antagonist, naltrindole (10 mg/kg), were administered intraperitoneally (i.p.) 20 min before i.c.v. injection of the peptides. The μ_1-opioid receptor antagonist, naloxonazine (Hahn et al., 1982), was given both at a dose of 10 mg/kg intravenously (i.v.) and of 50 mg/kg intraperitoneally (i.p.), 24 h before i.c.v. injection of the opioid agonists. Because the two administration routes provided similar results we report here only results obtained after the i.v. injection of naloxonazine.

2.5. Binding assay

Binding of the [D-Ala^2]deltorphin analogs to δ-, μ- and κ-sites was assayed on crude membrane preparations from rat brain (δ- and μ-sites) and from guinea-pig brain (κ-sites) as previously described (Melchiorri et al., 1991). The δ-binding sites were selectively labeled with [^{3}H][D-Ala^2,Asp^4]deltorphin (0.3 nM), the μ-sites with [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO, 0.5 nM) and the κ binding sites with [^{3}H]U-69,593 (0.5 nM). Competition curves were determined in triplicate. The inhibition constant (K_i) of the various peptides was calculated from competitive binding curves with the computer program, LIGAND (Munson and Rodbard, 1980). The δ-selectivity of each compound was defined as the ratio between the K_i for δ-sites and the K_i for μ-sites.

2.6. Drugs

The following peptides were used: [D-Ala^2,Glu^4]-deltorphin (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH$_2$, m.w. = 783), [D-Ala^2,Gly^4]deltorphin (m.w. = 710), [D-Ala^2,Ala^4]deltorphin (m.w. = 724), [D-Ala^2,Val^4]deltorphin (m.w. = 751), [D-Ala^2,His^4]deltorphin (m.w. = 860), [D-Ala^2,Asn^4]deltorphin (m.w. = 767) and dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$, m.w. = 804). All the peptides, synthesized as previously described (Melchiorri et al., 1991), were dissolved in 10% DMSO and injected i.c.v. in a 5-μl volume. Naloxone (Salars, Como, Italy) and naltrindole (Research Biochemicals, MA, USA) were dissolved in saline. Naloxonazine (Research Biochemicals, MA, USA) was dissolved in 0.01% acetic acid. [^{3}H][D-Ala^2,Asp^4]-deltorphin ([3,5-^{3}H-tyrosyl]-D-Ala-Phe-Asp-Val-Val-Gly-NH$_2$; 47 Ci/mmol) was custom synthesized by CRB (Cambridge, UK); [^{3}H]DAMGO ([^{3}H][D-Ala^2,MePhe^4, Gly-ol^5]enkephalin, 60 Ci/mmol) and [^{3}H]U-69,593 [(5a,7a,8b)-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl)-[3,4-^{3}H]benzeneacetamide], 53.7 Ci/mmol] were purchased from New England Nuclear, Du Pont, Florence, Italy.

2.7. Data analysis

Locomotor activity data are expressed as the means of the cumulative score ± S.E. Antinociceptive effect data are expressed as the means of %MPE ± S.E. Individual points in the dose- and time-effect curves were compared with each other and with baseline by two-way analysis of variance (ANOVA) and post-hoc Dunnett's *t*-test. All dose-response lines were analyzed with a linear regression method (Tallarida and Murray, 1986): AD_{50}, CD_{50} and LcD_{50} with confidence limits (C.L.) were calculated only from the linear portion of

Table 1
Pharmacological activities of [D-Ala^2,Glu^4]deltorphin (peptide N.1), of [D-Ala^2]deltorphin analogs (peptides N. 2–6) and of dermorphin (peptide N. 7), and their affinity for δ-, μ- and κ-opioid receptors.

N Peptide	LcD_{50} nmol/rat	AD_{50} nmol/rat	CD_{50} nmol/rat	K_i, nM		
				δ	μ	κ
(1) Tyr-ala-Phe-Glu-Val-Val-Gly-NH$_2$	0.49 (0.34–0.73)	45.0 (22.4–90.2) [b]	–	1.03 ± 0.15	2222 ± 233	> 20000
(2) Tyr-ala-Phe-**Gly**-Val-Val-Gly-NH$_2$	3.7 (2.0–6.9)	2.5 (2.1–2.9)	14.0 (9.3–19.9)	7.35 ± 0.91	14.2 ± 2.1	> 20000
(3) Tyr-ala-Phe-**Ala**-Val-Val-Gly-NH$_2$	– [a]	0.32 (0.09–1.09)	1.4 (0.9–2.0)	1.21 ± 0.14	21 ± 2.7	> 20000
(4) Tyr-ala-Phe-**Val**-Val-Val-Gly-NH$_2$	2.7 (1.0–7.3)	3.2 (2.8–3.6)	47.0 (31.0–70.3)	1.19 ± 0.17	56.6 ± 5.5	8000
(5) Tyr-ala-Phe-**His**-Val-Val-Gly-NH$_2$	2.5 (1.5–4.2)	6.9 (5.4–9.0)	23.0 (15.0–33.1)	4.64 ± 0.37	86.3 ± 5.7	> 20000
(6) Tyr-ala-Phe-**Asn**-Val-Val-Gly-NH$_2$	3.0 (1.3–6.7)	10.3 (7.1–14.9)	–	3.07 ± 0.31	280.6 ± 17	> 20000
(7) Tyr-ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$	– [c]	0.029 (0.013–0.069)	0.13 (0.08–0.25)	929 ± 65	1.19 ± 0.07	8160

LcD_{50}, AD_{50}, CD_{50} represent the ED_{50} [95% confidence limits] for locomotion, antinociception and catalepsy respectively; K_i = inhibition constant calculated from competitive binding curves with 0.3 nM [^{3}H][D-Ala^2,Glu^4]deltorphin (δ-opioid receptors), 0.5 nM [^{3}H]DAMGO (μ-opioid receptors) and 0.5 nM [^{3}H]U-69,593 (κ-opioid receptors); values are means ± S.E.M. from three experiments carried out in triplicate. [a] Maximum locomotion score (62 ± 10) was obtained with 1.4 nmol and corresponded to about an LcD_{30}. [b] Highest doses tested, from 60 to 130 nmol, were unable to produce an MPE > 70%. [c] Maximum locomotion score (40 ± 10) was obtained with 3.6 pmol and there was no evident dose-effect relationship.

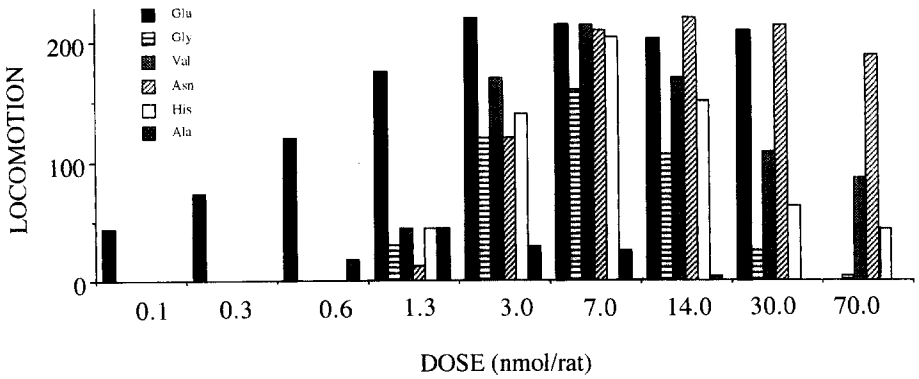

Fig. 1. Cumulative locomotion score (over 2-h observation period) induced by increasing doses of i.c.v. [D-Ala^2,Glu^4]deltorphin and its 4-substituted analogs.

the dose-response curves. The correlation between antinociceptive and cataleptic ED_{50} of the [D-Ala^2]deltorphin-analogs and the peptides' K_i values for μ- and δ- opioid receptors was assessed by linear regression analysis.

3. Results

3.1. Effects on motor activity

As previously reported (Negri et al., 1991), the selective δ-agonists [D-Ala^2,Glu^4]deltorphin (Table 1, peptide 1), injected i.c.v. in rats, induced a stereotyped pattern of locomotor activity. Ambulatory activity was intermittent and usually alternated with rearing events. The maximum increase in locomotion appeared within the first 15 min and the effect lasted for 30–90 min depending on the dose. The dose-response curve reached a plateau at 3.8 nmol; higher doses (6.4–38 nmol) did not further enhance locomotor activity. Doses higher than 13 nmol often caused a brief initial phase of behavioral depression, accompanied by frozen postures lasting from 1 to 8 min.

All the [D-Ala^2]deltorphin analogs tested produced a cumulative 2-h locomotion score that was significantly higher than that recorded after i.c.v. injection of drug vehicle (28 ± 5). Doses ranging from 0.6 to 7 nmol always yielded good dose-effect relationships; however, the calculated LcD_{50}s did not differ significantly (Table 1). The maximum increase in rat locomotion was obtained with the i.c.v. injection of 7 nmol of [D-Ala^2,Gly^4]deltorphin (peptide 2, 189 ± 21), 6.7 nmol of [D-Ala^2,Val^4]deltorphin (peptide 4, 214 ± 20), 6.5 nmol of [D-Ala^2,Asn^4]deltorphin (peptide 6, 210 ± 24) and 5.8 nmol of [D-Ala^2,His^4]deltorphin (peptide 5, 204 ± 27). Higher doses did not further enhance and sometimes even strongly reduced the locomotor score. The i.c.v. injection of 68 nmol of [D-Ala^2,His^4]deltorphin resulted in prominent and sustained immobility, which lasted throughout the first hour observation period and was accompanied by intense catalepsy. But the locomotion score after 65 nmol of [D-Ala^2,Asn^4]deltorphin did not differ significantly from that obtained after 6.5 nmol, whereas the locomotor score after 67 nmol of [D-Ala^2,Val^4]deltorphin was significantly lower than the score after 6.7 nmol. [D-Ala^2,Ala^4]deltorphin (peptide 3) induced a feeble behavioral stimulation. This peptide yielded the highest score (62 ± 10) at a dose of 1.4 nmol, whereas the i.c.v. injection of 14 nmol resulted in sustained immobility and catalepsy (Fig. 1). The locomotor activity induced by i.c.v. injection of the four synthetic peptides had a characteristic time pattern consisting of an early, dose-dependent hypoactivity, followed by increased activity. With small doses the excitatory effect prevailed. For example (Fig. 2), doses of 4.2 and 7 nmol of [D-Ala^2,Gly^4]deltorphin stimulated locomotion, whereas the dose of 14 nmol resulted in an initial suppression followed by hyperactivity. The dose of 70 nmol caused a prominent and sustained immobility during the first hour after injection. For this

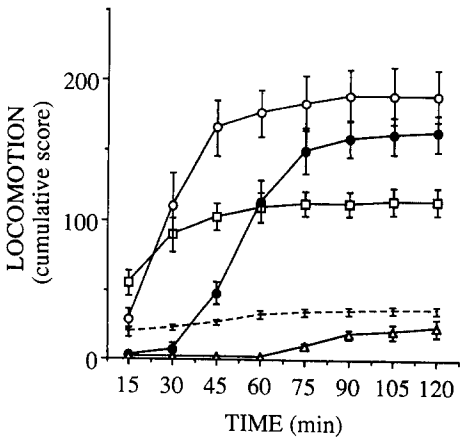

Fig. 2. Time course of the locomotor activity induced in rats by graded i.c.v. doses of [D-Ala^2,Gly^4]deltorphin: 4.2 nmol (□); 7 nmol (○); 14 nmol (●); 70 nmol (△); saline (dashed lines).

reason the cumulative, 2 h score, was significantly lower than the score after 7 nmol.

During the initial immobility phase, the bar test was positive in 50% of the animals at the dose of 1.4 nmol for [D-Ala^2,Ala^4]deltorphin, of 14 nmol for [D-Ala^2,Gly^4]deltorphin, of 23 nmol for [D-Ala^2,His^4]-deltorphin and only at the dose of 47 nmol for [D-Ala^2,Val^4]deltorphin. Catalepsy had a dose-related duration. [D-Ala^2,Asn^4]deltorphin induced catalepsy only at doses > 70 nmol (Table 1).

3.2. Antinociception

As expected, the μ-opioid receptor agonist, dermorphin (Table 1, peptide 7), caused a dose-related increase in antinociception (A_{50} = 29 (13–69) pmol/rat) that peaked at 20–30 min after injection and lasted 90–120 min depending on the dose. The selective δ-opioid receptor agonist, [D-Ala^2,Glu^4]deltorphin (Table 1, peptide 1) showed only limited antinociceptive efficacy. Doses lower than 13 nmol/rat never induced significant analgesia. In five of the 26 rats tested with 13 nmol, however, [D-Ala^2,Glu^4]deltorphin induced an analgesic effect that peaked at 5–15 min post-injection and disappeared in 30 min. A dose of 52 nmol produced 60 ± 23.8% MPE and doses up to 130 nmol did not further increase the %MPE (67 ± 18). The antinociceptive response peaked at 15 min and faded out in 30–45 min. All the [D-Ala^2]deltorphin analogs tested induced dose-dependent analgesia (Fig. 3), displaying a dermorphin-like time-response curve: antinociception reached a maximum in 30 min and lasted 90–120 min, depending on the dose. [D-Ala^2,Ala^4]deltorphin had the most intense antinociceptive effect (AD_{50} = 0.32 (0.09–1.0 nmol)), which peaked within the first 15–20 min and, at the highest dose, lasted for 3–4 h (Fig. 4).

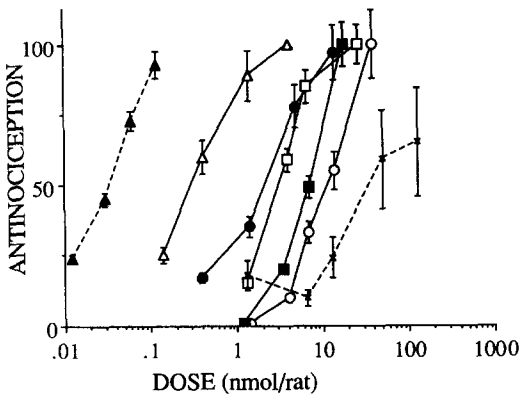

Fig. 3. Antinociceptive dose-response curves for graded i.c.v. doses of [D-Ala^2,Glu^4]deltorphin (×– – –×) and its substituted analogs, compared with that of dermorphin (▲– – –▲). [D-Ala^2,Ala^4]-deltorphin (△); [D-Ala^2,Gly^4]deltorphin (●); [D-Ala^2,Val^4]deltorphin (□); [D-Ala^2,Asn^4]deltorphin (○); [D-Ala^2,His^4]deltorphin (■).

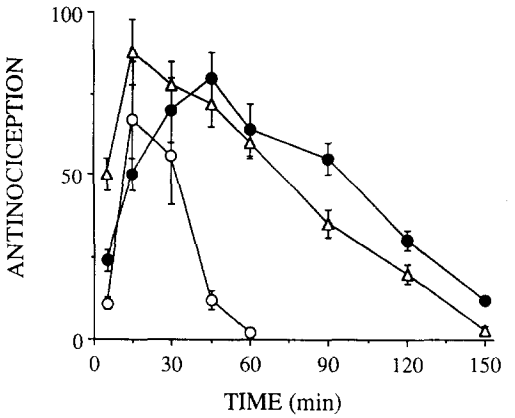

Fig. 4. Time course of the antinociception induced by 62 pmol of dermorphin (●), by 4 nmol of [D-Ala^2,Ala^4]deltorphin (△) and by 52 nmol of [D-Ala^2,Glu^4]deltorphin (○).

3.3. Correlation between receptor affinities and pharmacological effects

All the peptides had absolutely negligible κ-opioid receptor affinity (Table 1), thus excluding κ-opioid receptor involvement in antinociceptive or locomotor effects. Because the LcD_{50}s of the [D-Ala^2]deltorphin analogs did not differ statistically, no correlation could be found with the receptor affinities.

The regression analysis used to asses the correlation between antinociceptive and cataleptic ED_{50} of the [D-Ala^2]deltorphin-analogs and the peptides' affinities for μ- and δ-opioid receptors showed that the antinociceptive potency of these peptides increased with their μ-affinity (slope = 0.019 (0.016, 0.022); r = 0.99; $P <$ 0.00001). However, [D-Ala^2,Ala^4]deltorphin had an antinociceptive potency higher than would have been expected from its μ-affinity. No significant correlation was found between μ-opioid receptor affinity and catalepsy: [D-Ala^2,His^4]deltorphin had a cataleptic potency higher than would have been expected from its μ-affinity. The two analogs with the highest μ-affinity (peptides 2 and 3, Table 1) were also the most potent inducers of catalepsy and, at high doses, completely blocked locomotor activity (Fig. 1).

3.4. Opioid antagonists

The effect of i.c.v. injection of 14 nmol/rat of [D-Ala^2,Gly^4]deltorphin (taken as representative of the whole group because it had the lowest δ-μ selectivity) was studied in rats pretreated with naloxone, naloxonazine and naltrindole (Fig. 5A,B). The lowest dose of naloxone (1 mg/kg i.p.) reduced the antinociceptive response and abolished catalepsy, without significantly affecting the cumulative locomotion score over the 120 min observation period. But because this dose antagonized the initial hypoactivity, the locomotion score at

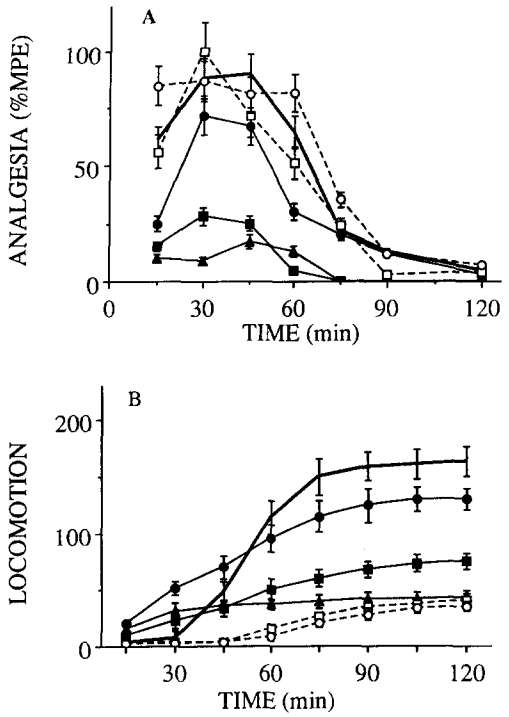

Fig. 5. Effect of naloxone 1 (●), 3 (■), 10 (▲) mg/kg i.p., naltrindole 10 mg/kg i.p. (□) and naloxonazine 10 mg/kg i.v. (○), on the antinociceptive (A) and locomotor (B) response to i.c.v. administration of 14 nmol of [D-Ala^2,Gly^4]deltorphin (thick solid line), in rats.

30 min (51 ± 9) was significantly higher ($F(1,1) = 9.57$; $P = 0.013$) than it was when the rats received [D-Ala^2,Gly^4]deltorphin alone (13 ± 5). At the dose of 3 mg/kg, naloxone significantly reduced ($F(1,7) = 18.35$; $P = 0.0001$) and, at the dose of 10 mg/kg, abolished the antinociceptive and locomotor responses. Naltrindole (10 mg/kg i.p.) abolished the late phase of locomotor stimulation but left unchanged the early phase of locomotor hypoactivity, antinociception and catalepsy. Naloxonazine (10 mg/kg i.v.) appeared to prolong antinociception (Fig. 5A) and the immobility of the rats (Fig. 5B). However the areas under the time-antinociception curve (AUC) induced by 14 nmol of [D-Ala^2,Gly^4]deltorphin (3570 ± 325) did not differ significantly from the AUC obtained after naloxonazine pretreatment (4050 ± 335). Analgesia induced by 0.4 nmol of [D-Ala^2,Ala^4]deltorphin (%MPE = 61 ± 8) was antagonized by naloxone (3 mg/kg s.c.) (%MPE = 14 ± 3), but left unchanged by naltrindole (10 mg/kg s.c.) (%MPE = 57 ± 7).

4. Discussion

The δ-selective agonist, [D-Ala^2,Glu^4]deltorphin, always produced a dose-related stimulation of locomotor activity of rats placed in a familiar open field. All the [D-Ala^2]deltorphin analogs tested had a dose-dependent, biphasic effect on locomotor activity: low doses mostly inducing stimulation, larger doses inducing an initial suppression followed by hyperactivity. The initial depressive phase was accompanied by antinociception and catalepsy. Comparing the time pattern of the phenomena, we observed that locomotor activity arose when catalepsy had faded and analgesia had begun to decrease. Antinociception and catalepsy were antagonized by naloxone but were left unchanged by the μ_1-opioid receptor-selective antagonist, naloxonazine (Ling et al., 1986), indicating a preferential involvement of the μ_2-opioid receptor subtype. Locomotor stimulation was antagonized by the δ-opioid receptor antagonist, naltrindole, thereby confirming intervention of the δ-opioid system. Low doses of naloxone did not significantly affect the cumulative locomotion score over a 120-min observation but, by removing the initial depression phase, allowed an earlier start of locomotor activity. Naloxonazine appeared to prolong the initial immobility. If μ_1-opioid receptors mediate locomotor stimulation (Paakkari et al., 1990), when naloxonazine had blocked the μ_1 receptors, the depressant effect of deltorphin analogs on the available μ_2-opioid receptors was presumably unopposed and therefore potentiated. Hence these peptides appear to activate mainly the μ_2-opioid receptor subtype, which has been shown to mediate catalepsy in the rat (Negri et al., 1992). Although the four analogs have a δ-opioid receptor affinity similar to that of [D-Ala^2,Glu^4]deltorphin, their locomotor activity-inducing potency is five to seven times lower, probably because it is weakened by their μ_2-opioid receptor-mediated depressive action.

Calenco-Choukroun et al. (1991a) demonstrated that, when rats were exposed to a fear-inducing environment (open-field), the injection of selective δ-opioid receptor agonists into the ventral tegmental area always induced hyperlocomotion that could be completely antagonized by naltrindole. In contrast, the μ-opioid receptor selective agonist, DAMGO, produced hypolocomotion that could be suppressed by naloxone but not by δ-opioid receptor-selective antagonists. These authors speculated that the hypoactivity observed in this test could be related to an enhanced emotionality produced by μ-opioid receptor stimulation (Daugé et al., 1988). Evidence that lesioning of the dopamine neurons in the nucleus accumbens blocks the increased rearing and locomotor activity induced by the δ-opioid receptor agonists but not the hypolocomotion induced by μ-opioid receptor agonists, suggests that this behavioral effect is independent of the integrity of the mesoaccumbens dopamine pathways (Calenco-Choukroun et al., 1991b). An involvement of distinct neuronal pathways in μ and δ effects that balance out, leading to the appropriate adaptation process is consistent with our hypothesis that the μ_2- and δ-opioid receptors, stimulated by the [D-Ala^2]-

deltorphin analogs, modulate the locomotion response by activating functionally opposed mechanisms.

[D-Ala^2,Ala^4]deltorphin had greater antinociceptive and cataleptic efficacy than its μ-affinity would suggest (its affinity for μ-opioid receptors being 18-fold lower than that of dermorphin and its analgesic and cataleptic potency only 11 times less than that of dermorphin). It also had a surprisingly poor locomotion-inducing efficacy. Pharmacokinetic factors, such as rapid accessibility to brain areas that mediate antinociception and catalepsy, could help to explain the mismatch in receptor affinities and in vivo activity. Accordingly, [D-Ala^2,Ala^4]deltorphin was the most lipophilic of the analogs tested. The anomalous behavior of [D-Ala^2,Ala^4]deltorphin could also be attributed to self-modulation (Vaught and Takemori, 1979; Horan et al., 1993). Horan et al. (1992) demonstrated a synergistic antinociceptive action between [D-Ala^2,Glu^4]-deltorphin and morphine when i.c.v. administered in mice at a suitable fixed dose ratio. Rossi et al. (1994) demonstrated that co-administration of DAMGO in rat RVM (rostral ventral medulla) with [D-Ala^2, Glu^4]deltorphin in PAG (periaqueductal grey) resulted in a synergistic analgesic response. Data from our laboratory (Negri et al., 1995) showed that, in the rat tail-flick test with radiant heat, combined subanalgesic doses of dermorphin and of [D-Ala^2,Glu^4]-deltorphin produce [D-Ala^2,Glu^4]deltorphin dose-dependent antinociception and confer to the δ-opioid agent the profile of a full analgesic agonist. Might the same agonist activate μ-opioid and δ-opioid receptors concurrently and in the proportion appropriate for increasing its analgesic efficacy? [D-Ala^2,Ala^4]deltorphin and [D-Ala^2,Gly^4]deltorphin (peptides 3 and 2, Table 1) had similar μ-opioid receptor affinities, but the first peptide – whose δ-opioid receptor affinity was about 6 times higher – displayed an analgesic and cataleptic potency that was 8–10 times higher than that of the second.

Also, the lack of correlation between μ-opioid receptor affinity and CD_{50} values might be explained by a μ/δ-opioid receptor interaction: indeed peptide 3, in spite of having a μ-receptor affinity only 3–4 times higher than peptide 4 and 5, nevertheless displayed a cataleptic potency about 20 times higher.

Intrinsic activity or efficacy in the agonist-receptor interaction can be more relevant to selectivity in vivo than the affinity ratio computed from in vitro studies. In producing supraspinal antinociception, μ-opioid receptors appear to interact in vivo with several classes of G transducer proteins. The unequal efficacy of opioid agonists could be interpreted as their diverse efficiency to activate these G-proteins (Garzon et al., 1994).

In conclusion, this study shows that substitution of the negative charge in the *C*-terminal domain of [D-Ala^2]deltorphins produces peptides that retain a high affinity for δ-opioid receptors but gain increased affinity for a naloxonazine-insensitive μ-opioid receptor subtype. Whereas δ-opioid receptor activation appears to be responsible for the locomotor stimulation in rats, their increased μ-affinity may explain the antinociception, catalepsy and hypolocomotion. The degree of early hypolocomotion depends upon the greater or lesser dominance of the inhibitory μ_2-opioid receptor system over the excitatory δ-opioid receptor system.

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