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Uncompetitive NMDA receptor antagonists potentiate morphine antinociception recorded from the tail but not from the hind paw in rats

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Abstract

We investigated the effects of pretreatment with low-affinity, uncompetitive NMDA receptor antagonists on morphine-induced antinociception in rats using the same intensity of thermal stimulus applied to the tail and the paws. Similar baseline responses to thermal stimuli of the same intensity were recorded from tails and hind paws. However, morphine produced equal antinociception from the tail and hind paw when used at doses of 2.5 and 6 mg/kg, respectively. These doses were used in further experiments. Thirty minutes before morphine, rats were administered the NMDA receptor antagonists dextromethorphan (2.5–30 mg/kg), memantine (2.5–15 mg/kg) and MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane HCl) (1.25–10 mg/kg). All three compounds significantly and dose-dependently potentiated morphine-induced antinociception recorded from the tail. However, none of these NMDA receptor antagonists affected morphine antinociception recorded from the paw. These findings suggest that low-affinity NMDA receptor antagonists modulate differently morphine antinociceptive activity recorded from the tail and hind paws.

Keywords: Morphine; Opiate; Antinociception; NMDA receptor antagonist; Memantine; Dextromethorphan

1. Introduction

Converging lines of evidence indicate the involvement of glutamate NMDA (*N*-methyl-D-aspartate) ionotropic subtype receptors in the acute antinociceptive effects of μ-opioids, because these effects are modulated by antagonists of the NMDA receptor complex. However, data in this respect are not uniform, as potentiation, inhibition and no effect have been reported, perhaps due to a variety of methodological differences and animal species and strains used (see Table 1).

The function of the NMDA receptor complex may be affected at several recognition sites including the glutamate competitive site as well as the glycine (Gly/NMDA) binding site. Additionally, its function may also be inhibited by uncompetitive NMDA receptor antagonists (NMDA channel blockers) such as the prototypical antagonist, MK-801 (dizocilpine), which inhibits the NMDA receptor complex at nanomolar concentrations (Wong et al., 1988). Some antagonists of NMDA receptor complex demonstrate therapeutic potential in several central nervous system disorders, as suggested by a number of preclinical studies (for review, see Parsons et al., 1998). Unfortunately, numerous undesirable "side-effects", including ataxia, psychotomimetic actions, cognitive impairment, and neurotoxicity in the retrosplenial cortex, accompany the administration of MK-801 and compounds with similar pharmacological characteristics, which precludes their potential clinical use (Tzschentke and Schmidt, 1998). In addition, the combination of MK-801 with morphine was reported to increase the mortality of laboratory animals as compared with morphine treatment alone (Trujillo and Akil, 1991b). At present, only the NMDA channel blockers with a relatively low, micromolar affinity and characterized by fast binding kinetics are considered as potential therapeutics. Among others, these include memantine (1-amino-3,5-dimethyladamantane), which has been used for the last 15 years in Europe in the treatment of dementia and spasticity (Ambrozi and Danielczyk, 1988) and was reported to be effective in Parkinson's disease (Parsons et al., 1999b). Another low-affinity NMDA channel blocker, dextromethorphan (Chou et al., 1999), is widely used as

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Table 1
Presented are details of the experiments investigating the influence of a single dose of uncompetitive NMDA receptor antagonist on the antinociception produced by an opiate in the hot-plate and the tail-flick tests in rats

Hot plate

Effect of NMDA receptor antagonist co-administrator	Strain of rats	Dose, NMDA receptor antagonist used, route and time	Morphine dose and route	Test parameters: (1) ~ baseline/cut-off (in [s], MPE or % of baseline latency), (2) how data are expressed	Temperature of the hot plate	Time [min] of the measurement after morphine administration	References
↑	Sprague–Dawley	0.1 mg/kg MK-801 i.p., or 30 mg/kg dextromethorphan, i.p., 30 min prior to morphine	5 mg/kg, s.c.	(1) NR, (2) data shown as "percent of baseline latency"	54 °C	0, 30, 60, 120, 180[a]	(Grass et al., 1996)
↑	Sprague–Dawley	30 mg/kg dextromethorphan (route: NR), 10 min prior to morphine	5 mg/kg s.c.	(1) ~ 5 s/30 s, (2) latencies in s	54 °C	0, 30, 60, 120, 180[a]	(Hoffmann and Wiesenfeld-Hallin, 1996)
↑	Sprague–Dawley	5, 15, 45 mg/kg dextromethorphan s.c., or 0.3, 1, 3 mg/kg ketamine s.c., 15 min prior to morphine	5 mg/kg s.c.	(1) ~ 4 s /30 s, (2) latencies in s	54 °C	0, 30, 60, 120, 180, 240[b,c]	(Plesan et al., 1998)
−	Wistar	5 mg/kg memantine i.p., simultaneously with morphine	5.1 mg/kg (ED_{50}), i.p.	(1) NR, (2) data expressed as ED_{50}	NR	30–60	(Malec and Langwinski, 1981)
−	Sprague–Dawley	0.05 mg/kg MK-801 i.p., 20 min prior to morphine	15 mg/kg i.p.	(1) NR/60 s, (2) latencies in s	52 °C	30, 60, 120 min	(Marek et al., 1991)
↑, − or ↓ depending on the strain and the dose of dextromethorphan[d]	Sprague–Dawley, SHR, Wistar-Kyoto, Dark Agouti	5, 15, 45 mg/kg dextromethorphan s.c., 15 min prior to morphine	5 mg/kg s.c.	(1) ~ 4 s /30 s, (2) latencies in s	54 °C	30, 60, 90, 120, 180, 240, 300 (depending on the strain of the rats)[e]	(Plesan et al., 1999)
−	Sprague–Dawley	10 μg i.t. or 10 μg i.c.v. MK-801 5 min prior to morphine	1 μg i.t. or 4 μg i.c.v.	(1) NR/60 s, (2)data expressed as AUC (MPE x min) and PEAK effect (MPE)	52 °C	5, 15, 30, 60, 90, 120	(Luger et al., 1995)

Tail-flick

Effect of NMDA receptor co-administration	Strain of rats	Dose, NMDA receptor antagonist used, route and time	Dose of opiate (morphine if not indicated otherwise)	Test parameters: (1) ~ baseline/cut-off ([s], MPE or % baseline latency), (2) How data are expressed	Time [min] of the measurements after morphine administration	References
↑ (heroin)	Sprague–Dawley	0.1, 0.15, 0.3 mg/kg MK-801, s.c., 30 min prior to heroin	1 mg/kg s.c. of heroin	(1) 2–3 s/10 s, (2) latencies in s and AUC	Every 15 min until the end of heroin antinociceptive effect (up to ~60 min when given alone; up to ~120 min when given with MK-801). Water bath at 52 °C	(Larcher et al., 1998)
↑	Sprague–Dawley	0.15, 0.3 mg/kg MK-801, s.c., 40 min prior to morphine	7.5 mg/kg i.v.	(1) 2–3 s/10 s, (2) latencies in s	30 to 330, water bath at 52 °C	(Celerier et al., 1999)
↑	Sprague–Dawley	15 mg/kg dextromethorphan, p.o., simultaneously with morphine	15 mg/k, p.o.	(1) 3.5–4.5 s/8 s, (2) MPAE	30, 60, 90, 120, 150, 180, 210	(Mao et al., 1996)
↑ or −	Sprague–Dawley	0.1 or 0.05 mg/kg MK-801 i.p., immediately prior to morphine	1 or 5 mg/kg, i.p.	(1) 4.5–5 s/20 s, based on figure reading, (2) latencies in s	15, 30, 45, 60[f]	(Kest et al., 1992)
↑ or −	Albino Holtzman derived rats	15 mg/kg dextrorphan s.c., simultaneously with morphine	1.5, 3, 6 mg/kg s.c.	(1) NR/14 s, (2) latencies in s and AUC	30, 60, 90[g]	(Advokat and Rhein, 1995)
↑	Sprague–Dawley	10 μg MK-801 i.t., 10 min prior to morphine	ED_{50} studies, i.t.	(1) 3 s/10 s, (2) MPE	30, hot water tail immersion test at 50 °C	(Wong et al., 1996)
−	Sprague–Dawley	0.03, 0.1, 0.3, 3 mg/kg MK-801, i.p., 30 min prior to morphine	1 mg/kg, s.c.	(1) 3 s/10 s, (2) latencies in s	60	(Trujillo and Akil, 1991a)
−	Sprague–Dawley	10 μg i.t. or 10 μg i.c.v. MK-801, 5 min prior to morphine	1 μg i.t. or 4 μg i.c.v.	(1) NR/6 s (2) data expressed as AUC (MPE × min) and PEAK effect (MPE)	5, 15, 30, 60, 90, 120	(Luger et al., 1995)
↓	Sprague–Dawley	0.03, 0.3, 3 μg MK-801 into ventro-medial medulla	2.5 μg into periaqueductal grey matter	(1) 2.5–3 s/12 s, (2) latencies	30, 60, 90, 120[h]	(Spinella et al., 1996)

AUC, area under curve; MPAE, maximal possible antinociceptive effect; MPE, maximal percent effect; NR, not reported. ↑, potentiation; −, no effect; ↓, inhibition of opiate antinociceptive effect.

[a] Rats were trained on the hot plate for 4–5 days to obtain a stable pre-drug response latency.

[b] Dextromethorphan also potentiated the effect of morphine at doses that by themselves did not produce antinociception (1–2 mg/kg).

[c] Ketamine alone at 0.3–3 mg/kg had no effect in the hot-plate test. Antinociceptive effect of 5 mg/kg morphine was slightly enhanced by 1 mg/kg, but not 0.3 or 3 mg/kg of ketamine.

[d] Plesan et al. (1999), p. 55 reports that dextrorphan does not influence morphine-induced antinociception in Dark Agouti rats (Hoffmann et al., unpublished data).

[e] Dextromethorphan potentiated morphine-induced antinociception in Sprague–Dawley, SHR rats, to a lesser extent in Wistar-Kyoto and had no effect in Dark Agouti rats. Dose of 5 mg/kg dextromethorphan inhibited morphine-induced antinociception in SD rats; Doses 15 and 45 mg/kg potentiated and prolonged morphine effect in SD rats. Rats were trained on the hot plate for 4–5 days to obtain a stable pre-drug response latency.

[f] Potentiation of low (1 mg/kg) but no effect of high morphine dose (5 mg/kg).

[g] Potentiation of the low (1.5 mg/kg) but no effect of higher (3, 6 mg/kg) dose of morphine in spinalized rats.

[h] MK 801 at doses 0.03 and 3 μg, inhibited morphine-induced antinociception (dose of 3 μg entirely inhibited it), dose of 0.3 μg inhibited morphine-induced antinociception at 30 min "time-point post morphine".

"over the counter" antitussive medication (Tortella et al., 1989). Its main metabolite, dextrorphan, has also been reported to bind to the NMDA receptor complex with higher though still micromolar affinity (Chou et al., 1999). The recently synthesized MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexan HCl), which has a similar pharmacological profile in preclinical studies, is currently under intense investigation (Parsons et al., 1999a).

The antinociceptive action of μ-opioid receptor agonists can be examined in several ways, but the majority of data for rodents have been obtained with the use of the hot-plate (Woolfe and Macdonald, 1944) and the tail-flick (D'Amour and Smith, 1941) tests. Although in both the tail-flick and the hot-plate tests, the thermal stimuli affect the skin surface, the neurotransmission of nociceptive signals and antinociceptive responses are mediated by different neuronal pathways. It is believed that the tail-flick response represents mostly a spinal reflex, and that the hot-plate test response is mediated mainly by supraspinal pathways.

The modulatory effects of NMDA receptor antagonists on the antinociceptive action of μ-opioid receptor agonists were previously investigated in both tests. A search of literature revealed that in mice and rats, antagonists of the NMDA receptor complex potentiate, attenuate and/or have no effect on the antinociceptive properties of μ-opioid receptor agonists. A number of experimental variables, including the test type and its parameters, the site-specificity and the doses of NMDA receptor antagonists as well as the route of their administration (Table 1) make these experiments difficult to compare. Table 1 shows only the effects of uncompetitive NMDA receptor antagonists (NMDA channel blockers), since the effects of other antagonists of this receptor are even more diverse (Bespalov et al., 1998). In addition, Table 1 shows only the effects of NMDA receptor antagonists on morphine-induced antinociception in rats, because in the majority of studies, rats appear to respond to treatment with a potentiation of morphine antinociception, while in mice, an inhibition of morphine antinociception is noted frequently.

The present experiments were carried out in order to investigate the effects of dextromethorphan, memantine and MRZ 2/579 on morphine-induced antinociception, using an experimental setting in which the quality of the nociceptive stimulus is identical for the hind paw and for the tail (Hargreaves et al., 1988) and using morphine in doses producing a similar magnitude of antinociception recorded from the tail and the hind paw. To accomplish this, in a preliminary experiment, the effects of increasing doses of morphine on tail and hind paw antinociception were assessed by calculating the area under curves (AUC) for each morphine dose; AUC values were later used to calculate the antinociceptive ED_{50} by non-linear regression. Morphine-induced antinociception in rats pretreated with NMDA receptor antagonists was studied for the whole period of action of morphine. This procedure made it possible to determine the modulatory effects of clinically available and/or promising NMDA receptor antagonists on morphine antinociception under the same conditions for the hind paw and the tail.

2. Materials and methods

2.1. Animals

Male Wistar rats (~360 g weight at the beginning of the experiment) were housed under standard laboratory conditions for at least 2–3 weeks before the experiment started. During this phase, animals were frequently handled. Rats were kept in $58 \times 37 \times 19$-cm plastic cages, four rats per cage in an animal room with a controlled light–dark cycle (lights on at 0700; off: 1900) with food and water provided ad libitum.

2.2. Ethics

All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, revised 1985) and were approved by the internal Bioethics Commission.

2.3. Nociceptive test

2.3.1. Apparatus

An apparatus (custom made by Dr. G. Ozaki, University of California, USA) consisted of a glass surface upon which the rats were placed individually in Plexiglas cubicles ($9 \times 22 \times 25$ cm) (Hargreaves et al., 1988). The glass surface temperature was maintained at 30 ± 0.1 °C by a feedback-controlled, under-glass, and forced-air heating system. The thermal nociceptive stimulus originated from a focused projection bulb (8 V, 50 W) mounted in a stimulus branch manipulated manually. The stimulus was positioned under the tail or the foot pad. A trial was commenced by a switch, which activated the radiant heat source and started an electronic timer. A set of photodiode motion sensors (aimed at the aperture) detected light reflected from the tail or the paw and automatically turned off the lamp and the electronic clock when a tail or paw movement interrupted the reflected light. The withdrawal latency to the nearest 0.1 s was determined using an electronic clock circuit and a microcomputer. Both for the paw and the tail experiments, the stimulus current was set at 5–6 A and was monitored continuously by using the amperage delivered to the light source. The apparatus parameters were set to get ~10 s baseline latencies. The cut-off time, due to the construction of the apparatus, was 81.92 s.

2.3.2. Experimental procedure

Rats were transferred to an experimental room that was kept at a constant temperature (21 ± 2 °C). To assess

nociceptive responses to the thermal stimulus, rats were placed in plastic chambers on a glass floor and allowed to acclimate for 60 min before testing (first baseline). Thirty minutes later, the second baseline measurement was carried out. After the second baseline measurement, rats were administered: (a) placebo or morphine (s.c.) or NMDA receptor antagonist (i.p.) (single drug experiments) or, (b) NMDA receptor antagonist (i.p.) and 30 min later with morphine (s.c.) (double drug experiments). The nociceptive responses were determined for 210 min after the last injection at 30-min intervals.

During measurements, the radiant heat source was positioned under the glass floor directly beneath the tail or the hind paw. Nociceptive responses of the tails and the hind paws were measured in separate sets of rats. For each data point, two measurements (left and right hind paw, the tip [1/3 from the end] and the base [1/3 from the beginning] of the tail) were taken. The two latencies were averaged. All animals were tested only once.

Dextromethorphan was administered at doses of 5, 15, 30 and 2.5, 5, 15 and 30 mg/kg for tail and paw experiments, respectively. Memantine was administered at doses of 2.5, 5, 10 and 15 for tail and 2.5, 5 and 10 mg/kg for paw experiments, respectively. MRZ 2/579 was given at doses 1.25, 2.5, 5 and 1.25, 5 and 10 mg/kg for tail and paw experiments, respectively. Doses of morphine are expressed as the base and those of NMDA receptor antagonists as the respective salts.

Because NMDA receptor antagonists are able to potentiate the antinociceptive effects of low but not high doses of morphine (see Table 1) (Kest et al., 1992; Advokat and Rhein, 1995), we performed additional experiments estimating hind paw responses to morphine used at the dose of 4 mg/kg in rats pretreated with dextromethorphan

Table 2
Presented are mean ± S.E.M. Areas under curve (AUC) and PEAK values computed from the tail and hind paw withdrawal latency data

	AUC mean ± S.E.M.	PEAK mean ± S.E.M.
Tails		
Placebo (6)	2343 ± 395	15.4 ± 4.3
Morphine 2.5 only (6)	3908 ± 894	49.8 ± 12.8[c]
Dextromethorphan 30 only (6)	2186 ± 118	16.4 ± 3.3[c]
Dextromethorphan 5 + Morphine 2.5 (6)	5668 ± 487[a]	81.9 ± 0.0[b]
Dextromethorphan 15 + Morphine 2.5 (6)	7734 ± 598[c]	80.7 ± 1.2[c]
Dextromethorphan 30 + Morphine 2.5 (6)	8045 ± 560[c]	81.9 ± 0.0[b]
ANOVA $F(5,30)$ =	21.32, $P < 0.0001$	31.77, $P < 0.0001$
Memantine 10 only (5)	2438 ± 130	16.6 ± 2.3[b]
Memantine 2.5 + Morphine 2.5 (6)	5475 ± 529	65.1 ± 7.7
Memantine 5 + Morphine 2.5 (12)	5851 ± 505	70.7 ± 6.0
Memantine 10 + Morphine 2.5 (6)	8169 ± 1067[c]	78.5 ± 2.1[a]
Memantine 15 + Morphine 2.5 (6)	6608 ± 577[a]	75.8 ± 4.5
ANOVA $F(6,40)$ =	9.68, $P < 0.0001$	13.71, $P < 0.0001$
MRZ 2/579 10 only (6)	2734 ± 109	16.7 ± 1.7 [b]
MRZ 2/579 1.25 + Morphine 2.5 (6)	4345 ± 705	49.5 ± 8.6
MRZ 2/579 2.5 + Morphine 2.5 (6)	7517 ± 445[c]	75.1 ± 4.7[a]
MRZ 2/579 5 + Morphine 2.5 (6)	8451 ± 289[c]	81.9 ± 0.0[b]
ANOVA $F(5,30)$ =	21.99, $P < 0.0001$	16.69, $P < 0.0001$
Hind paws		
Placebo (6)	2667 ± 426	15.2 ± 2.8
Morphine 6 only (24)	3796 ± 424	31.5 ± 4.6
Dextromethorphan 30 only (6)	2396 ± 93	14.3 ± 1.1
Dextromethorphan 2.5 + Morphine 6 (6)	4686 ± 782	39.8 ± 9.3
Dextromethorphan 5 + Morphine 6 (11)	5934 ± 1025	51.4 ± 9.6
Dextromethorphan 15 + Morphine 6 (12)	4887 ± 491	53.3 ± 7.2
Dextromethorphan 30 + Morphine 6 (6)	6113 ± 748	53.6 ± 7.3
ANOVA $F(6,64)$ =	3.62, $P < 0.01$	4.46, $P < 0.001$
Memantine 10 only (5)	2824 ± 181	15.2 ± 1.0
Memantine 2.5 + Morphine 6 (6)	2833 ± 123	15.7 ± 0.9
Memantine 5 + Morphine 6 (6)	3294 ± 570	21.2 ± 5.5
Memantine 10 + Morphine 6 (12)	3782 ± 641	25.7 ± 5.8
ANOVA $F(5,35)$ =	0.77, $P > 0.05$	1.63, $P > 0.05$
MRZ 2/579 10 only (6)	2842 ± 223	15.9 ± 1.5
MRZ 2/579 1.25 + Morphine 6 (6)	3470 ± 276	26.9 ± 5.5
MRZ 2/579 5 + Morphine 6 (12)	3185 ± 304	23.6 ± 4.1
MRZ 2/579 10 + Morphine 6 (6)	3414 ± 295	26.5 ± 8.0
ANOVA $F(5,54)$ =	0.83, $P > 0.05$	1.32, $P > 0.05$

Statistically significant ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$) toward "Morphine only" treatment, Newman–Keul's test. The number of rats is given in the parentheses.

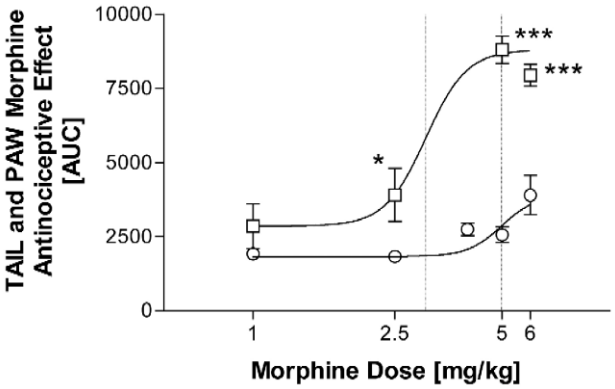

Fig. 1. Presented are mean ± S.E.M. Areas Under Curve (AUC) computed from tail (squares) and hind paw (circles) withdrawal latency data. AUC was calculated using trapezoid rule (Delta $X*(Y1+Y2)/2$) on a series of measurements from −30 to 210 min. ED_{50} was calculated with the use of GraphPad Prism 3.01 for Windows. In case of the hind paw measurements, doses higher than 6 mg/kg were not tested due to the risk of tissue damage. ED_{50} [mg/kg] for the tails and hind paws are indicated by the thin vertical lines and (with 95% Confidence Intervals) were: 3.05 (2.25–4.14) and 4.98 (4.29–5.79) mg/kg, respectively. For each dose, a different subset of rats ($N = 6–12$) was used. Asterisks indicate statistically significant difference from effects of morphine on hind paw antinociception $^{*}P < 0.05$; $^{***}P < 0.001$.

(30 mg/kg), memantine (10 mg/kg) and MRZ 2/579 (10 mg/kg) and tested as described earlier.

2.4. Drugs

Morphine HCl (Polfa, Kraków, Poland), memantine HCl and MRZ 2/579 HCl (Merz, Frankfurt/M., Germany) as well as dextromethorphan HBr (Sigma, USA) were dissolved in sterile physiological saline (placebo) and administered in a volume of 1 ml/kg. All NMDA receptor antagonists were administered intraperitoneally. Morphine was given subcutaneously.

2.5. Data analysis

AUC were calculated using trapezoid rule on a series of measurements from −30 to 210 min. PEAK indicates the time point corresponding to the longest tail or paw withdrawal latency. Statistical analysis was carried out using analysis of variance (ANOVA) performed on AUC and PEAK values, followed by Newman–Keul's test with the use of Statistica 5.0 for Windows. For certain comparisons shown in Fig. 1, the Student's *t*-test was used.

3. Results

3.1. The ED_{50} of morphine in tail and hind paw antinociceptive studies

The baseline responses for the nociceptive thermal stimuli for both tails and hind paws was about 9–13 s (data shown on the Figs. 2–4). The antinociceptive response recorded from the tails of drug-free rats was similar to that recorded from the hind paws (see AUC values in Table 2).

Preliminary experiments demonstrated that the antinociceptive morphine ED_{50} was 3 and 5 mg/kg for the tail and the paw, respectively (Fig. 1). In subsequent experiments, similar doses of morphine (2.5 and 6 mg/kg, respectively) were used, because these doses produced indistinguishable antinociception recorded from the tails and hind paws (3908 ± 894 and 3899 ± 663 units, respectively, Fig. 1).

3.2. The effects of NMDA receptor antagonists on morphine-induced antinociception recorded from the tail

None of the investigated NMDA receptor antagonists, dextromethorphan, memantine and MRZ 2/579, had an antinociceptive effect when given alone, but (based on AUC and PEAK calculations) all potentiated morphine (2.5 mg/kg)-induced antinociception recorded from the tail (Table 2, Figs. 2A–4A). Dextromethorphan potentiated significantly morphine antinociception recorded from the tail at all used doses (5, 15 and 30 mg/kg) (Table 2, Fig. 2A). Memantine potentiated tail morphine antinociception

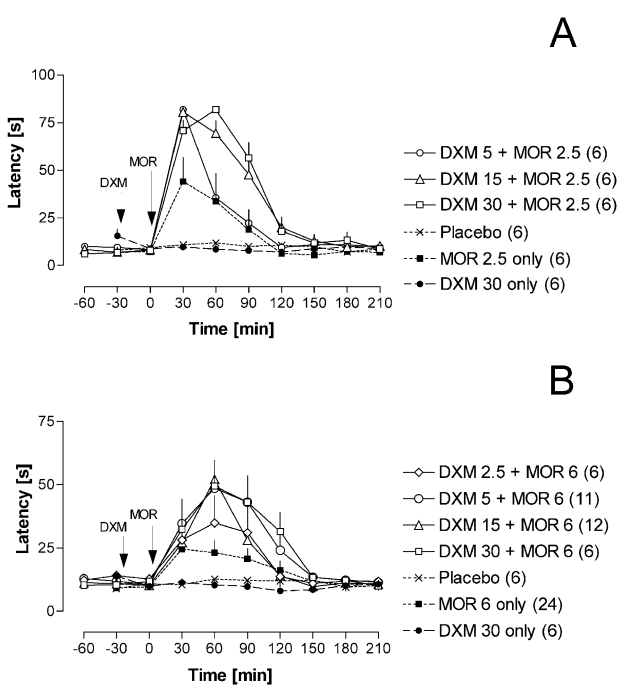

Fig. 2. Antinociceptive effects of morphine (MOR) on tail withdrawal (A) and hind paw withdrawal (B), and its modulation by various doses of dextromethorphan (DXM) in the radiant heat stimulator apparatus. In the "single drug experiment", DXM (30 mg/kg) or MOR (2.5 or 6 mg/kg for the tail or hind paw withdrawal experiments, respectively) was administered at time "0", immediately after the baseline test, and the measurements were continued at 30-min intervals up to 210 min. In the "double drug experiment", DXM and MOR were administered at 30-min intervals, 30 min after the initial baseline test at times indicated by arrows and the measurements were continued at 30-min intervals up to 210 min. Figure presents mean ± S.E.M. withdrawal latency [s]. The number of rats (N) are given in parentheses.

at doses of 10 and 15 mg/kg but not 2.5 and 5 mg/kg (Table 2, Fig. 3A). MRZ 2/579 was effective in augmenting morphine antinociception at doses of 2.5 and 5 mg/kg but not at 1.25 mg/kg (Table 2, Fig. 4A).

3.3. The effects of NMDA receptor antagonists on morphine-induced antinociception recorded from the paw

As was the case in the tail experiments, the NMDA receptor antagonists did not affect the paw nociceptive response when administered alone. However, in contrast to the tail, in the paw, these compounds did not significantly affect morphine antinociception, as based on AUC and PEAK calculations (Table 2, Figs. 2B–4B). Although one-way ANOVA performed on AUC or PEAK data reached statistical significance, none of the dextromethorphan doses (2.5, 5, 15 and 30 mg/kg) significantly affected morphine antinociception, as determined with post-hoc Newman–Keul's tests (Table 2, Fig. 2B). As determined by one-way ANOVA performed on AUC or PEAK data, neither memantine (2.5, 5 and 10 mg/kg; Table 2, Fig. 3B) nor MRZ 2/579 (1.25, 5 and 10 mg/kg; Table 2, Fig. 4B) affected hind paw morphine antinociception.

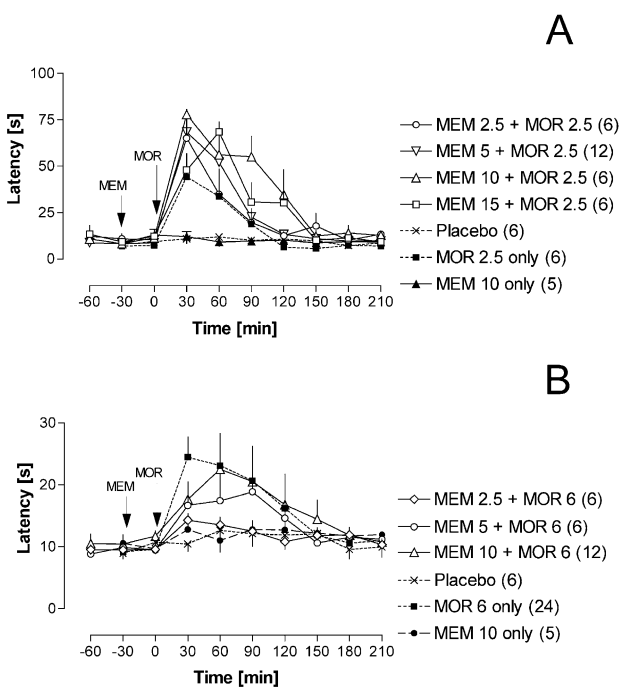

Fig. 3. Antinociceptive effects of morphine (MOR) on tail withdrawal (A) and hind paw withdrawal (B), and its modulation by various doses of memantine (MEM) in the radiant heat stimulator apparatus. In the "single drug experiment", MEM (10 mg/kg) or MOR (2.5 or 6 mg/kg for the tail or hind paw withdrawal experiments, respectively) was administered at time "0", immediately after the baseline test, and the measurements were continued at 30-min intervals up to 210 min. In the "double drug experiment", MEM and MOR were administered in 30-min intervals, 30 min after the initial baseline test at times indicated by arrows and the measurements were continued at 30-min intervals up to 210 min. Figure presents mean ± S.E.M. withdrawal latency [s]. The number of rats (N) are given in parentheses.

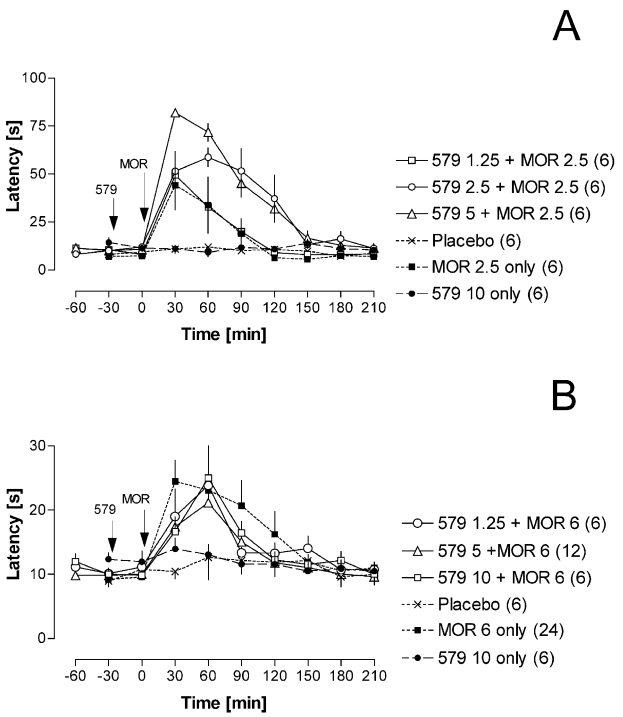

Fig. 4. Antinociceptive effects of morphine (MOR) on tail withdrawal (A) and hind paw withdrawal (B), and its modulation by various doses of MRZ 2/579 (579) in the radiant heat stimulator apparatus. In the "single drug experiment", MRZ 2/579 (10 mg/kg) or MOR (2.5 or 6 mg/kg for the tail or hind paw withdrawal experiments, respectively) was administered at time "0", immediately after the baseline test, and the measurements were continued at 30-min intervals up to 210 min. In the "double drug experiment", MRZ 2/579 and MOR were administered at 30-min intervals, 30 min after the initial baseline test at times indicated by arrows and the measurements were continued at 30-min intervals up to 210 min. Figure presents mean ± S.E.M. withdrawal latency [s]. The number of rats (N) are given in parentheses.

3.4. The effect of NMDA receptor antagonists on hind paw antinociception induced by low (4 mg/kg) dose of morphine

In preliminary experiments, 4 mg/kg of morphine did not produce significant antinociception in the hind paw (AUC: 2745 ± 216, $N = 6$, Fig. 1). Pretreatment with dextromethorphan (30 mg/kg), memantine (10 mg/kg) and MRZ 2/579 (10 mg/kg) did not influence the effect of morphine: the resulting AUC values were 3025 ± 130, 3164 ± 191 and 2409 ± 177, respectively ($N = 6$ for each treatment).

4. Discussion

The results of the present experiments indicate that morphine affects differently nociceptive responses to the same thermal stimuli recorded from the tail and the hind paw. Thus, while the baseline responses recorded from the tails and hind paws were the same (9–13 s), the maximum antinociceptive effect of morphine was greater, and the

morphine ED_{50} was lower, for the tails than for the hind paws. Morphine-induced antinociception recorded from the tails and the hind paws was also differently affected by the NMDA receptor antagonists. While dextromethorphan, memantine and MRZ 2/579 potentiated the antinociceptive activity of morphine recorded from the tail, these compounds did not change significantly morphine antinociception recorded from the hind paws.

Table 1 demonstrates that there are differences in the latency to withdraw the tails and paws from the influence of the painful stimuli. This is reflected by the fact that the "baseline" latencies were slightly different in the tail-flick and hot-plate tests (mostly 2–3 s and about 5 s, respectively), as were the "cut-off" times (about 10 s in the tail-flick and 30–60 s in the hot-plate test). This is likely due to the different quality of the nociceptive stimulus in the tail-flick and hot-plate tests.

Analysis of the data gained with different experimental set-ups (Table 1) does not suggest any particular difference in morphine potency for hind paw and tail antinociception. For example, used at doses of 1–15 mg/kg in different settings, morphine usually produces significant antinociception, and we failed to conclude that higher or lower doses of morphine are needed to produce significant tail as opposed to hind paw antinociception. To make things more complicated, researchers use different temperature settings of the hot-plate. The temperature of the hot plate is an important variable, because some of the analgesics may give "false negative" results if the temperature is set too high (Ankier, 1974). In the experiments concerning peripheral morphine administration presented in Table 1, dose–response curves were not investigated except in one study (Malec and Langwinski, 1981) where the parameters of the hot plate test were not reported. However, the morphine doses used in cited experiments were always sufficient to evoke significant antinociception under the given experimental conditions, though it is difficult to assess how closely these doses correspond to the antinociceptive ED_{50} in a given setting.

As revealed in the present experiments, the lack of differences in the baseline (drug-free) responses of tails and hind paws to the same painful stimulus may suggest that the neuronal pathways engaged in the processing of tail and hind paw nociceptive information do not differ in their sensitivity to noxious stimulation. However, the difference in the potency of morphine to inhibit responses to a painful stimulus affecting the tails and the paws may be due to the different neuropharmacology and neuroanatomy of the pathways mediating the antinociceptive effects of opioids in the paw and the tail.

The behavioral response to noxious stimuli originating from the tail (tail withdrawal) is known to be predominantly a spinal reflex (Irwin, 1950; Dewey and Harris, 1975). The nociceptive signals resulting from painful stimulation of the hind paw are under strong but not complete supraspinal control and probably involve neuronal pathways originating mainly in midbrain (periaqueductal grey matter) and medullary structures (the rostral ventromedial medulla, like nucleus raphe magnus and nucleus reticularis gigantocellularis) (Jensen and Yaksh, 1986). However, morphine also produces antinociception in the tail-flick test after intracerebroventricular administration and is active in the hot-plate test when given intrathecally (Luger et al., 1995). In the light of these observations, it seems that both spinal and supraspinal sites participate in antinociceptive effect of morphine, with the former, spinal component being dominant in the tail-flick test and the latter, supraspinal component being predominant in the hot-plate response.

Our working hypothesis to explain the potentiation of morphine antinociception by NMDA receptor antagonists involves the phenomenon known as "acute tolerance". The concept of acute tolerance was proposed to explain the fact that the antinociceptive effects of morphine last for a much shorter time than the serum and brain concentrations of this opiate are raised (Kissin et al., 1991). It has been proposed that processes involving pharmacodynamic interactions are likely responsible for the reduced antinociceptive potency of morphine observed even after a single dose. The attractiveness of the phenomenon of "acute tolerance" relies on the fact that, in some settings, NMDA receptor antagonists prevent the development of tolerance, which at the behavioral level is manifested as a potentiation of morphine antinociception (Table 1). Anatomically, μ-opioid and NMDA receptors are co-localized on synaptic membranes in the dorsal horn and periaqueductal grey matter (Commons et al., 1999). It cannot be excluded that both the density of opioid and/or NMDA receptors as well as the differences in the subunit composition of NMDA receptors in spinal and supraspinal structures may explain the difference in the modulatory effects of NMDA receptor antagonists on morphine antinociception. Of the studies concerning the influence of uncompetitive NMDA receptor antagonists on morphine-induced antinociception in rat tail-flick studies (Table 1), one (Spinella et al., 1996) demonstrates the inhibition of morphine antinociception by MK-801, which is in contrast to the majority of other studies in which potentiation or no effect was reported. In this study, an uncompetitive NMDA receptor antagonist was administered directly into ventromedial medulla (nucleus raphe magnus), which mediates supraspinal antinociception via, among others, glutamatergic pathways (Jensen and Yaksh, 1984; Satoh et al., 1983). The data reported by Spinella et al. resemble earlier findings of Jacquet (1988), who demonstrated the antinociceptive properties of NMDA administered into periaqueductal grey matter, as well as the potentiation of the action of morphine by NMDA and its inhibition by a competitive NMDA receptor antagonist (AP-7) at this brain site (Jacquet, 1988). Furthermore, it has been reported that peripheral administration of NMDA receptor antagonists produces a greater potentiation of morphine antinociception in spinalized rats

than in controls (Advokat and Rhein, 1995). Taken together, these data may suggest that inhibition of NMDA receptors at supraspinal sites may inhibit opioid antinociception while the inhibition of spinal NMDA receptors may potentiate it. Thus, the lack of potentiation of morphine antinociception by NMDA receptor antagonists in the hind paw reported in our study may be due to the inhibitory effects of these compounds at supraspinal sites. NMDA receptor antagonists administered intrathecally strongly potentiate the effects of morphine (Wong et al., 1996), suggesting facilitatory actions at the spinal level, which may explain our data demonstrating that NMDA receptor antagonists potentiate morphine antinociception recorded from the tail.

There is also a possibility that the doses of NMDA receptor antagonists used in our studies were sufficient to potentiate the effect of 2.5 mg/kg of morphine (tail-flick studies) but not the antinociceptive effect of 6 mg/kg of morphine (hind paw studies). This possibility implies also that the doses of NMDA receptor antagonists used in hind paw studies could be higher. However, the doses of dextromethorphan, memantine and MRZ 2/579 used in our study produce serum levels selective for inhibition of NMDA receptor activity, at least in respect to memantine and MRZ 2/579 (Parsons et al., 1999b). These doses appear to be devoid of the side effects (ataxia, myorelaxation, memory disturbance) reported in studies in which doses higher than 10 mg/kg of memantine (Parsons et al., 1999b) and 30 mg/kg of dextromethorphan (Dematteis et al., 1998) were used. Moreover, the side-effects are augmented when NMDA receptor antagonists are co-administered with opioids (Trujillo and Akil, 1991b). In our hands, 15 mg/kg of memantine administered with 2.5 mg/kg of morphine increased slightly but noticeably the activity of rats. It cannot be excluded that the lower potency of 15 mg/kg of memantine as compared with other doses of memantine (see Fig. 3) resulted from a slight hyperactivity of the rats rather than from modulation of antinociception induced by morphine.

With respect to dextromethorphan, we noted an increase in morphine hind paw antinociceptive activity; however, this effect was not statistically significant. It is possible that not only dextromethorphan but also its main metabolite, dextrorphan, participated in this effect. With memantine, we noted the opposite effect, a slight tendency to reduce morphine (6 mg/kg)-induced antinociception while almost equipotent doses of MRZ 2/579 appeared to have no influence. Interestingly, although these NMDA receptor antagonists appear to have relatively similar binding characteristics at the NMDA channel, kinetics and voltage dependency, they differ in selectivity for NMDA receptor subtypes. While MRZ 2/579 demonstrates no selectivity among NMDA receptor subtypes expressed in *Xenopus* oocytes, memantine is threefold more potent at NMDAR2C and NMDAR2D subunits than at NMDAR2A and, to a lesser extent, at NMDAR2B subtypes of NMDA receptors (Parsons et al., 1999a). Dextromethorphan shows fourfold higher potency for NR1/NR2A than NR1/NR2B receptors (Avenet et al., 1997). The various NMDAR2 subunits confer the functional, pharmacological and physiological variability of the NMDA receptors in different brain regions (Ishii et al., 1993; Porter and Greenamyre, 1995). These differences are probably responsible for the insignificant but observable dissimilar trends among the NMDA receptor antagonists used in their modulation of opioid activity in hot-plate test. It also cannot be excluded that these differences might also be responsible for the quite different effects on morphine antinociception in the tail and hind paw studies reported here.

Careful inspection of Table 1 suggests another possible explanation for the discrepancy between our results showing no potentiation of antinociceptive effect of morphine by uncompetitive NMDA receptor antagonists in the hind paw experiments, and its potentiation in the hot-plate test reported in other studies. Whereas we used Wistar rats, the potentiation of morphine antinociception reported by others was observed mainly with Sprague–Dawley rats. Although different strains of rats appear to respond differently to the combination of uncompetitive NMDA receptor antagonist and morphine (Plesan et al., 1999), our results are unlikely to be explained solely by strain differences. Thus, no potentiation of the antinociceptive effect of morphine by NMDA receptor antagonist has been observed also in Sprague–Dawley rats (Trujillo and Akil, 1991a), and studies in which the same nociceptive stimuli were applied to the paws and tails are lacking. To resolve the issue of strain differences and genetic make-up, our study ought to be repeated with other strains of rats.

It remains to be assessed whether the present findings in rats are relevant for therapy in humans. Preliminary clinical data demonstrating that NMDA receptor antagonists potentiate μ-opioid antinociception [the cases of Morphidex (Katz, 2000) and ketamine (Bell, 1999)] are encouraging.

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