

Piotr Popik · Ewa Kozela · Wojciech Danysz

## Clinically available NMDA receptor antagonists memantine and dextromethorphan reverse existing tolerance to the antinociceptive effects of morphine in mice

Received: 18 August 1999 / Accepted: 30 November 1999 / Published online: 02 February 2000

© Springer-Verlag 2000

**Abstract** The tail-flick test was used to investigate the effects of chronic administration of the *N*-methyl-D-aspartate (NMDA) receptor antagonists, dextromethorphan, memantine and MRZ 2/579, on the development and reversal of morphine tolerance in mice in three separate experiments. Experiment 1 investigated the effects of NMDA receptor antagonists on the development of tolerance. Morphine (10 mg/kg for 6 days, twice daily) produced a 5.9-fold rightward shift of the cumulative dose-response curves. Co-administration of dextromethorphan, memantine or MRZ 2/579 between tests 1 and 2 dose-dependently (5–10 mg/kg) inhibited the development of morphine tolerance. In experiment 2, in which the effects on the reversal were investigated, morphine-tolerant mice were treated b.i.d. for an additional 6 days (between tests 2 and 3) with vehicle+vehicle, NMDA receptor antagonist+vehicle, vehicle+morphine or NMDA receptor antagonist+morphine. Morphine-tolerant mice treated with vehicle+vehicle remained morphine tolerant, whereas this residual morphine tolerance was inhibited by administration of all three NMDA antagonists (each 10 mg/kg). Morphine-tolerant mice receiving vehicle+morphine injections demonstrated an unchanged degree of antinociceptive tolerance. In these mice, the co-administration of memantine and MRZ 2/579, but not dextromethorphan, resulted in the reversal of morphine tolerance. In experiment 3, memantine and MRZ 2/579 (10 mg/kg) inhibited the acute antinociceptive effect of morphine, but dextromethorphan did not. These data indicate that low-affinity, clinically available and/or therapeutically promising NMDA receptor antagonists may be used to inhibit ongoing morphine tolerance.

**Key words** NMDA antagonist · Memantine · Dextromethorphan · MRZ 2/579 · Analgesia · Antinociception · Tolerance · Morphine

### Introduction

Tolerance to the antinociceptive (analgesic) effects of the  $\mu$ -agonist morphine, the most widely used opioid analgesic, complicates the management of patients with chronic pain. Tolerance develops in patients receiving morphine for relief of e.g. cancer-related pain, and requires increments of morphine doses that, in turn, predispose patients to the side effects including sedation, constipation and, rarely, seizures and myoclonus (Foley 1991). The development of tolerance to opioid antinociception is manifested as a shift to the right of the dose-response curve or as a decrease in the intensity of the response on repetitive administration of a constant dose (Foley 1991). In laboratory animals, antinociceptive tolerance is usually assessed as a decrease in response in the presence of a constant nociceptive stimulus (Cochin and Kornetsky 1964).

Converging lines of evidence indicate that co-administration of either competitive or uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists attenuate the development of tolerance to the antinociceptive effects of morphine in rodents [for review see Herman and O'Brien (1997)]. In the mouse, such effects have been shown for the uncompetitive NMDA receptor antagonists (+)MK-801 (dizocilpine) (Lutfy et al. 1993; Elliott et al. 1994a; Bilsky et al. 1996; Gonzalez et al. 1997; Belozertseva and Beshpalov 1998) and ketamine (Gonzalez et al. 1997), the glycine/NMDA receptor antagonist ACEA-1328 (Lutfy et al. 1995) and the competitive NMDA receptor antagonists LY274614 (Elliott et al. 1994a), LY235959 (Bilsky et al. 1996) and CGP 39551 (Gonzalez et al. 1997). Moreover, antagonists of the NMDA receptor may inhibit also the maintenance of morphine tolerance, i.e. may inhibit the ongoing process of tolerance. For example, the competitive NMDA receptor antagonist LY274614 reverses morphine tolerance as measured in the hot-plate test in rats,

P. Popik (✉) · E. Kozela  
Institute of Pharmacology, Polish Academy of Sciences,  
12 Smętna Street, PL-31-343 Kraków, Poland  
e-mail: nfpopik@cyf-kr.edu.pl,  
Tel.: +48-12-6374630, Fax: +48-12-6374500

W. Danysz  
Department of Pharmacology, Merz and Co. GmbH and Co.,  
Eckenheimer Landstrasse 100, 60318 Frankfurt/M, Germany

i.e. rats rendered tolerant to morphine and then infused with LY274614 regain their antinociceptive sensitivity to morphine, compared with morphine-tolerant rats given saline infusion (Tiseo and Inturrisi 1993). This reversal of morphine tolerance is observed regardless of whether rats are given morphine together with the NMDA receptor antagonist or NMDA receptor antagonist alone during the "post-tolerance-development" period (Tiseo and Inturrisi 1993; Tiseo et al. 1994). Recently, Allen and Dykstra (1999) have repeated these findings in rats using the competitive NMDA receptor antagonist, LY235959 (the active isomer of LY274614), in the warm water tail-withdrawal procedure. In studies on mice, similar inhibitory effects were found for dextromethorphan (an uncompetitive NMDA receptor antagonist) (Elliott et al. 1994b) and ACPC (1-aminocyclopropane carboxylic acid, a partial agonist at glycine/NMDA receptors) (Kolesnikov et al. 1994) in the tail-flick test.

The aim of the present study was the systematic investigation of the effects of NMDA receptor antagonists on development and reversal of morphine tolerance, using clinically available and/or clinically promising low-affinity channel blockers.

Memantine (1-amino-3,5-dimethyladamantane), has been used clinically in the treatment of dementia and spasticity and has some efficacy in Parkinson's disease (Ambrozi and Danielczyk 1988; Parsons et al. 1999a). In vitro, memantine antagonizes NMDA-induced membrane currents and displaces [<sup>3</sup>H]MK-801 in frontal cortex membranes (Kornhuber et al. 1989) at concentrations likely to be achieved after peripheral administration of this compound (Danysz et al. 1997; Kornhuber et al. 1989). Memantine is neuroprotective in vitro and in vivo against NMDA-induced and/or ischaemic cell damage, and has anticonvulsant effects in rodents (Parsons et al. 1999a).

Although not investigated as intensively as memantine, MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexan hydrochloride) appears to have a quite similar pharmacological profile. MRZ 2/579 is a highly voltage dependent, moderate-affinity NMDA channel blocker (IC<sub>50</sub> 1.2 μM in patch clamp experiments) demonstrating a favourable behavioural profile in animal models with low potency for the production of ataxia, myorelaxation, impairment of prepulse inhibition or stereotype behaviour. This compound also demonstrates neuroprotective actions in vivo (Parsons et al. 1999b).

The third compound employed in the present study is dextromethorphan, which, like memantine, is clinically available and is also a low-affinity, use-dependent NMDA channel blocker (K<sub>i</sub> about 7 μM) (Chou et al. 1999). It is metabolized by cytochrome P450 2D6 to an active metabolite, dextrorphan (K<sub>i</sub> about 0.9 μM) (Chou et al. 1999). Dextromethorphan is a dextrorotatory opioid derivative that does not act at opioid receptors (Tortela et al. 1989) and has a well-established and favourable safety profile that permits its use in the general population as an over-the-counter medication (Bem and Peck 1992). It inhibits NMDA-induced currents (Netzer et al. 1993) and suppresses NMDA-induced seizures (Ferkany et al. 1988).

## Methods

**Animals.** Male Albino Swiss mice obtained from our Institute breeding stock and weighing 25–30 g at the start of the experiment were used. Mice were group-housed in standard laboratory cages and kept in a temperature-controlled colony room (21±2 °C) with a 12-h light/dark cycle (light on: 0700 hours, off: 1900 hours). Commercial food and tap water were available ad libitum.

**Apparatus.** A standardized tail-flick apparatus (Analgesia Meter, Innovators in Instrumentation, N.J., USA, model 33), set with adjusted sensitivity at 6 and break-point at 84–85 with a radiant heat source and connected to an automatic timer was used to assess the antinociceptive response. The intensity of the heat stimulus was adjusted so that the baseline latency was ~3.6 s. A maximum latency of 10 s (i.e. cut-off) was used to minimize damage to the tail. The tail-flick withdrawal latency was measured from the start of the heat stimulus applied to the distal 2 cm of the tail until the animal exhibited a flick of the tail. Two tail-flick responses were recorded for each mouse and each dose (Paronis and Holtzman 1991).

**Design of the experiments.** Experiment 1 was designed to determine whether dextromethorphan, memantine and MRZ 2/579 could inhibit the development of tolerance to the antinociceptive effects of morphine. On day 1, the first antinociception measurement was performed, followed by 6 days of twice daily morphine injections (10 mg/kg, s.c.) (Elliott et al. 1994a). The pretreatment with the NMDA receptor antagonists was given 30 min prior to each morphine dose on days 2–7 of this experiment. Comparison of morphine cumulative dose-response curves obtained on days 1 and 8 was used to assess the extent to morphine tolerance.

The aim of experiment 2 was to determine whether selected doses of NMDA receptor antagonists used in experiment 1 might reverse the already developed morphine tolerance. The schematic diagram of this experiment is shown in Fig. 1A. Briefly, on day 1, the first antinociception measurement (test 1) was performed, followed by 6 days of morphine injections (10 mg/kg, s.c. b.i.d.). Morphine tolerance was assessed on day 8 (test 2). Subsequently, on days 9–14, mice were treated with dextromethorphan, memantine or MRZ 2/579 (each 10 mg/kg b.i.d.), 30 min prior to vehicle administration. The separate groups were treated with the same NMDA receptor antagonists 30 min prior to 10 mg/kg s.c. morphine injections. The magnitude of the effects of NMDA receptor antagonists was determined on day 15 of experiment (test 3).

Experiment 3 was designed to determine whether selected doses of NMDA receptor antagonists used in experiments 1 and 2 might have innate antinociceptive properties and/or change the antinociceptive effects of morphine. The baseline tail-flick responses were examined immediately prior to administration of dextromethorphan, memantine and MRZ 2/579 (each 10 mg/kg) or vehicle. The tail-flick responses were then examined again 30 min later immediately prior to morphine (5 mg/kg, s.c.) injection. Assessment of tail-flick responses continued at intervals of 30 min up to 120 min after morphine injection. For comparison, the effects of the low-affinity NMDA channel blockers were additionally compared in this experiment with those produced by the competitive NMDA receptor antagonist NPC 17742 [2R,4R,5S-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid)] (6 mg/kg).

**Cumulative dose-response morphine antinociception measurement.** Cumulative dose-response morphine curves were used to reduce the number of animals required to assess the development of morphine tolerance (Paronis and Holtzman 1991). After adaptation and base-line trials, each mouse was injected with a low dose of morphine (1 mg/kg s.c.). Then, 30 min later, the mouse was re-tested and injected with the next dose of morphine, such that the cumulative dose was increased by 0.25 log units. Thus, with an initial dose of morphine of 1.0 mg/kg, the next dose was 1.78 mg/kg, for a cumulative dose of 2.8 mg/kg. This procedure continued until the mouse either did not move its tail within the cut-off time, or

until the dose-response curve reached a plateau, so that the latency did not increase from one dose to the next. Each antinociceptive responder was not subjected to further tail-flick tests but was injected with the subsequent doses of morphine so that all animals received the same total dose of morphine during a given test.

**Data presentation and statistics.** Latencies (in seconds) of the tail-flick response were converted to maximum percentage effects (MPE, Paronis and Holtzman 1991) according to the formula:

$$MPE(\%) = 100 \times \frac{(\text{postinjection latency} - \text{baseline latency})}{(\text{cut-off latency} - \text{baseline latency})}$$

**Table 1** Mean pre- and post-tolerance-development morphine antinociceptive ED<sub>50</sub> values from experiment 2. Values for mice at each testing condition are given for the prospective treatments between tests 2 and 3. Presented are mean±SEM antinociceptive morphine ED<sub>50</sub> values (mg/kg) determined before and after induction of morphine tolerance that was accomplished with the use of 10 mg/kg s.c. b.i.d. morphine injections over 6 days, between tests 1 and 2

Treatment between tests 2 and 3	n	Test 1 ED <sub>50</sub>	Test 2 ED <sub>50</sub>
Vehicle+vehicle	16	3.25±0.48	15.28±2.68
Dextromethorphan+vehicle	10	2.54±0.40	6.47±0.90
Memantine+vehicle	10	3.76±0.70	27.84±6.66*
MRZ 2/579+vehicle	8	5.36±0.68*	32.21±8.02*
ANOVA F <sub>3,43</sub>		3.62 (P<0.05)	5.63 (P<0.01)
Vehicle+morphine	8	4.46±0.81	22.52±4.19
Dextromethorphan+morphine	8	3.81±0.39	22.16±3.55
Memantine+morphine	7	3.98±0.71	16.09±3.59
MRZ 2/579+morphine	6	5.24±0.63	25.08±4.14
ANOVA F <sub>3,28</sub>		<1 (n.s.)	<1 (n.s.)

One way ANOVA revealed differences among groups. \*P<0.05, post hoc Newman Keuls test vs. respective control group

**Table 2** N-methyl-D-aspartate (NMDA) receptor antagonists dose-dependently attenuate the development of morphine tolerance. Mean±SEM antinociceptive morphine ED<sub>50</sub> values (mg/kg) determined during “pre-tolerance development” and “post-tolerance development” tests as well as resulting fold changes. Mor-

Treatment prior to morphine	Dose (mg/kg)	n	Test 1 ED <sub>50</sub>	Test 2 ED <sub>50</sub>	T2/T1 (-fold change)
Vehicle		17	3.11±0.48	14.70±2.58	5.85±1.14
Dextromethorphan	5	8	2.44±0.23	8.24±1.54	3.69±0.99
Dextromethorphan	10	6	3.73±0.57	4.86±1.75*	1.36±0.42*
Dextromethorphan	30	10	3.61±0.48	3.77±0.63**	1.17±0.24**
ANOVA F <sub>3,40</sub>			1.06 (n.s.)	5.53 (P<0.01)	5.13 (P<0.01)
Memantine	2.5	10	4.24±0.83	12.10±2.19	3.35±0.64
Memantine	5	9	5.71±0.72	4.74±1.02**	1.02±0.30**
Memantine	10	9	3.79±0.96	1.95±0.36**	0.86±0.27**
ANOVA F <sub>3,44</sub>			2.45 (n.s.)	7.30 (P<0.001)	7.112 (P<0.01)
MRZ 2/579	2.5	17	3.59±0.46	9.62±1.78	3.38±0.92
MRZ 2/579	5	13	3.60±0.69	5.09±0.62**	1.99±0.45**
MRZ 2/579	10	11	4.59±0.55	4.84±0.62**	1.15±0.16**
ANOVA F <sub>3,57</sub>			1.17 (n.s.)	6.15 (P<0.001)	5.327 (P<0.01)

\*P<0.05, \*\*P<0.01, Newman-Keuls test vs. vehicle group that received saline+morphine during the development of morphine tolerance

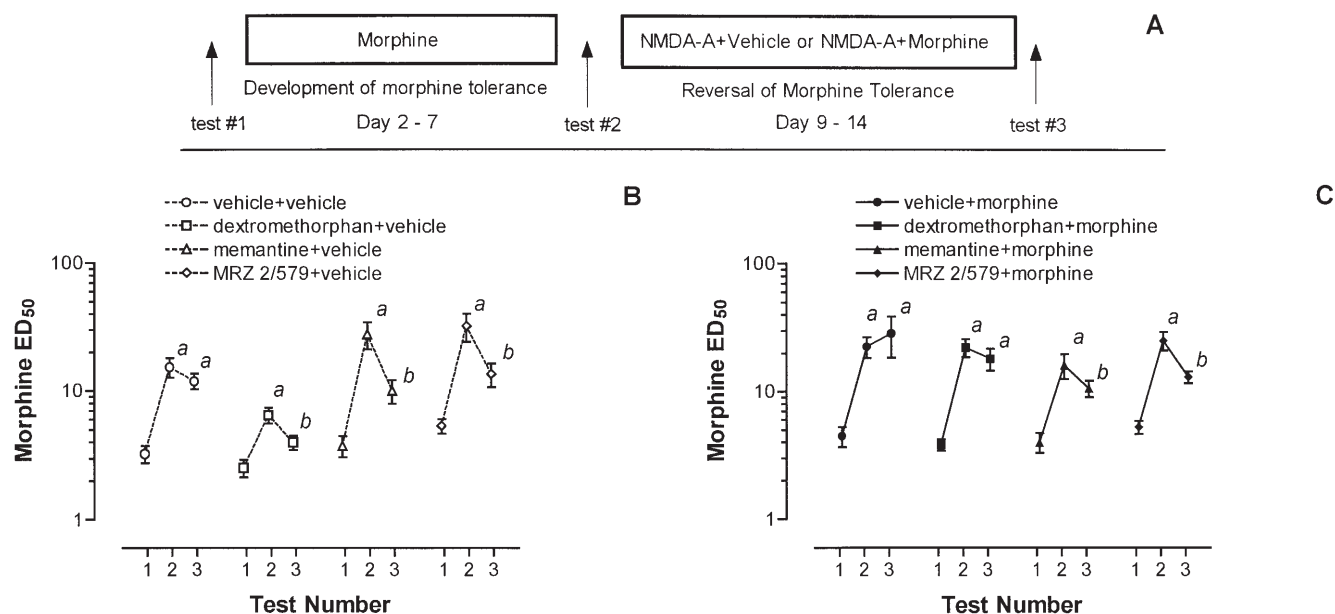
MPEs were used to construct morphine cumulative dose-response curves by non-linear regression; these curves were used to calculate antinociceptive morphine ED<sub>50</sub> using commercially available software (Prism v. 3.00, GraphPad Software, Calif., USA). The ED<sub>50</sub> values obtained in tests 1 and 2 were compared among groups, as were the fold shifts (determined by dividing individual test 2 ED<sub>50</sub> values by the test 1 ED<sub>50</sub> values).

Because experiment 2 was carried out over a period of several months, prior to a more detailed statistical analysis the baseline test 1 and the post-tolerance-development test 2 morphine antinociceptive ED<sub>50</sub> data were compared among groups as well as with respective controls (Table 1). These comparisons revealed that, in mice treated between tests 2 and 3 with NMDA receptor antagonist+vehicle, the test 1 ED<sub>50</sub> value differed significantly among groups and that the prospective MRZ 2/579 group test 1 ED<sub>50</sub> value was significantly higher than its vehicle control. In addition, test 2 ED<sub>50</sub> values were different among groups, and the prospective memantine and MRZ 2/579 groups test 2 ED<sub>50</sub> values were significantly higher than their vehicle controls. These results indicate that both the pre-treatment response to morphine and the magnitude of morphine tolerance was dissimilar among groups. These observations prompted the use of within-design statistical analysis (i.e. the comparisons of the magnitude of test 2/test 3 effect of NMDA receptor antagonist).

Statistical analyses involved one-way between-subject ANOVA followed by the Newman-Keuls test (experiment 1) and one-way repeated-measures ANOVA, one-way between-subject ANOVA followed by Newman-Keuls tests and three-way MANOVA with one repeated factor (experiment 2). Calculations were made using STATISTICA v. 5.0 (StatSoft, Okla., USA). For experiment 3, two-way repeated-measures ANOVA and one-way between-subject ANOVA followed by Newman-Keuls tests were calculated using SOLO (BMDP Statistical Software, Calif., USA).

**Drugs.** Morphine HCl (Polfa, Kraków, Poland), memantine HCl and MRZ 2/579 HCl (Merz and Co., Frankfurt/M, Germany), NPC 17742 (Nova Pharmaceutical, Md., USA) and dextromethorphan HBr (Sigma, St. Louis, Mo., USA) were dissolved in sterile physiological saline (vehicle) and administered in a volume of 10 ml/kg. The dosage of morphine hydrochloride is expressed as free base. All injections were given s.c., twice daily (at 0900 hours and 1630 hours), unless stated otherwise.

phine tolerance was induced by morphine administration (10 mg/kg s.c. b.i.d.) over 6 days. The NMDA receptor antagonist or vehicle was given s.c. 30 min prior to each of the morphine injections. T2/T1 is the ratio of the ED<sub>50</sub> values for tests 2 and 1 respectively



**Fig. 1A–C** The reversal of morphine tolerance by *N*-methyl-D-aspartate (NMDA) receptor antagonists (NMDA-A) in mice. **A** Mice were treated with morphine (10 mg/kg s.c. b.i.d.) over 6 days between tests 1 and 2 to induce morphine tolerance. The antinociceptive effects of morphine were determined in tail-flick tests 1–3 with the use of cumulative dose-response measurements. Between tests 2 and 3 one half the mice received b.i.d. injections of NMDA receptor antagonist 30 min prior to vehicle administration (**B**); the other half received b.i.d. NMDA receptor antagonist injection 30 min prior to 10 mg/kg morphine administration (**C**). Separate, repeated-measures one-way ANOVA performed on ED<sub>50</sub> values were used to determine the magnitude of morphine tolerance as well as the effect of the treatment with NMDA receptor antagonist on existing tolerance. Symbols indicate significant difference: *a*  $P < 0.05$  vs. respective test 1 measurement (indicating morphine tolerance); *b*  $P < 0.05$  vs. respective test 2 measurement (indicating a change in morphine tolerance) (post hoc Newman Keuls test). Due to the complexity of the figure, the precise magnitude of effects (*P*) is not shown. For *n* see legend to Table 1. Dextromethorphan, memantine and MRZ 2/579 were all used at the dose of 10 mg/kg. The keys in **B** and **C** indicate the treatment between tests 2 and 3

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.

## Results

### Effects of NMDA receptor antagonists on the development of morphine tolerance (experiment 1)

There were no differences in antinociceptive morphine ED<sub>50</sub> values in test 1 among groups (Table 2). Treatment with morphine (10 mg/kg, b.i.d.) produced a robust (5.9-fold) increase in the ED<sub>50</sub> values as determined in test 2. In contrast, pretreatment with dextromethorphan (10 or 30 mg/kg), memantine (5 or 10 mg/kg) and MRZ 2/579 (5 or 10 mg/kg), given prior to each dose of morphine com-

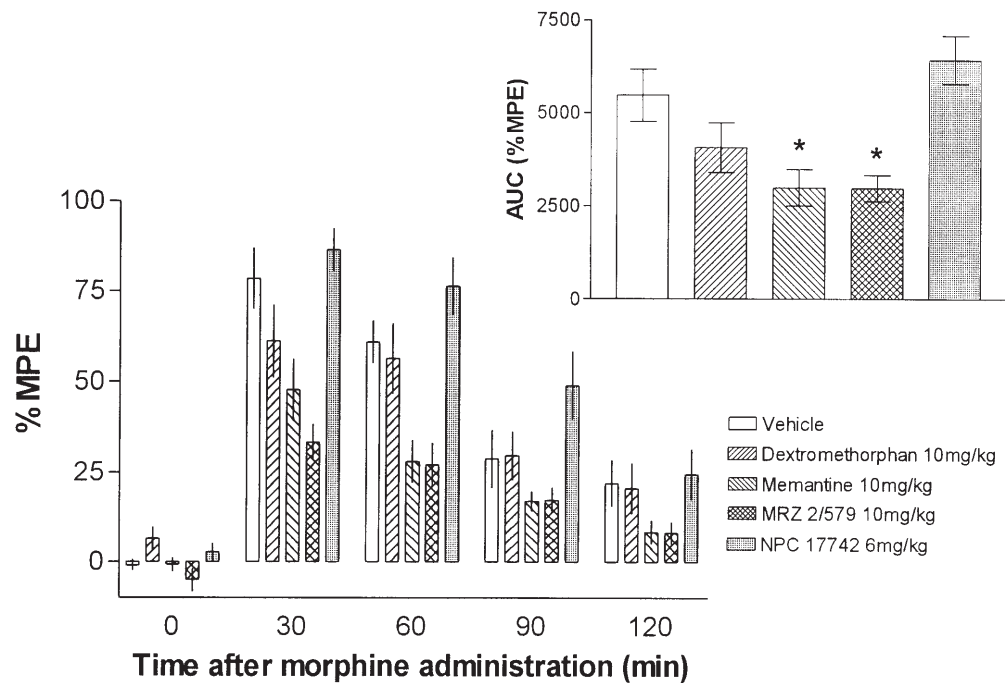
pletely prevented the development of morphine tolerance. This is evidenced by the significant decrease in antinociceptive morphine test 2 ED<sub>50</sub> values as well as test 2/test 1 fold changes of the respective groups compared with the controls using one-way ANOVA (Table 2).

### Effects of NMDA receptor antagonists on the reversal (maintenance) of morphine tolerance (experiment 2)

For the reasons explained in the Methods, to determine whether the treatment with NMDA receptor antagonists affected the maintenance of morphine tolerance, the separate three-way MANOVAs: *opiate* (morphine vs. vehicle) × *treatment* (NMDA antagonist vs. vehicle) × *test number* (test 2 vs. test 3), with *test number* as a repeated factor, were calculated. Using this approach, a statistically significant interaction between *treatment* and *test number* would indicate that the NMDA receptor antagonist affected the change in test 2/test 3 antinociceptive response. Such an interaction was not found for dextromethorphan ( $F_{1,38} 1.101, P > 0.05$ ). However, the treatment with memantine ( $F_{1,37} 5.617, P < 0.025$ ) and MRZ 2/579 ( $F_{1,34} 8.587, P < 0.01$ ) produced global inhibitory effects on the test 2/test 3 antinociceptive response.

Figure 1 shows the antinociceptive morphine ED<sub>50</sub> values determined in all three tests. Repeated-measures one-way ANOVAs were employed separately for each group to test the differences among tests 1, 2 and 3 antinociceptive morphine ED<sub>50</sub> values. Mice treated between tests 2 and 3 with vehicle+vehicle remained morphine tolerant, because both test 2 and 3 antinociceptive morphine ED<sub>50</sub> values differed significantly from the ED<sub>50</sub> values determined in test 1 ( $F_{2,30} 15.265, P < 0.001$ , Fig. 1B), whereas test 2 and 3 ED<sub>50</sub> values were not different from each other. Repeated-measures one-way ANOVAs also indicated differences among test 1, 2 and 3 morphine antinociceptive ED<sub>50</sub> values for dextromethorphan+vehicle ( $F_{2,18} 12.718$ ,

**Fig. 2** Time course of the antinociceptive (tail-flick test) responses of mice treated with a combination of various NMDA receptor antagonists and morphine. Morphine (5 mg/kg) was administered s.c. 30 min after injection of various NMDA receptor antagonists or vehicle. Data are expressed as a percentage of the maximal percentage effect (%MPE) (means $\pm$ SEM,  $n=6, 8, 9, 8$  and  $10$  for vehicle, dextromethorphan (10 mg/kg), memantine (10 mg/kg), MRZ 2/579 (10 mg/kg) and NPC 17742 (6 mg/kg), respectively. The inset shows mean $\pm$ SEM for the area under curve (AUC) values calculated from the same data. One-way ANOVA ( $F_{4,40}$  7.39,  $P<0.001$ ) and post-hoc Newman-Keuls test revealed that the pretreatment with memantine and MRZ 2/579 produced significant ( $P<0.05$ ) attenuation of morphine antinociceptive effect.



$P<0.001$ ), memantine+vehicle ( $F_{2,18}$  10.181,  $P<0.0025$ ) and MRZ 2/579+vehicle ( $F_{2,14}$  9.995,  $P<0.0025$ ). As shown in Fig. 1B, the residual morphine tolerance observed in vehicle+vehicle treated mice appeared to be inhibited by the treatment with all the NMDA receptor antagonists. This is because, in mice treated with dextromethorphan, memantine and MRZ 2/579, the test 3  $ED_{50}$  values differed significantly from the respective test 2  $ED_{50}$  values (such a difference was not observed in the control group).

The parallel experiment, in which mice were treated with NMDA receptor antagonist+morphine between tests 2 and 3 (Fig. 1C), yielded qualitatively similar results. The treatment with vehicle+morphine ( $F_{2,14}$  5.178,  $P<0.05$ ) did not affect already existing morphine tolerance since, although test 2 and 3 morphine antinociceptive  $ED_{50}$  values differed from test 1 values, they were not different from each other (Fig. 1C). Similarly, treatment with dextromethorphan+morphine ( $F_{2,14}$  11.389,  $P<0.0025$ ) did not change test 2/test 3 morphine antinociceptive  $ED_{50}$  values significantly. However, mice treated with memantine+morphine ( $F_{2,12}$  7.715,  $P<0.001$ ) or MRZ 2/579+morphine ( $F_{2,10}$  13.103,  $P<0.025$ ) demonstrated significantly reduced test 3  $ED_{50}$  values compared with test 2  $ED_{50}$  values.

#### Effects of NMDA receptor antagonists on the tail-flick response and antinociceptive effects of morphine (experiment 3)

The acute effects of NMDA receptor antagonists on tail-flick responses are shown in Fig. 2, (time "0"). Although one-way ANOVA revealed significant differences among groups ( $F_{4,40}$  2.864,  $P<0.05$ ), none of the NMDA receptor antagonists significantly affected tail-flick responses com-

pared with vehicle control. However, pretreatment with memantine and MRZ 2/579 resulted in significant inhibition of morphine antinociception. Thus, repeated-measures two-way ANOVA (*time* $\times$ *treatment*) to compare the effects of pretreatment with various NMDA receptor antagonists with vehicle pretreatment, revealed significant effect of *treatment* for memantine-treated mice ( $F_{1,59}$  12.37,  $P<0.01$ ) and MRZ 2/579-treated mice ( $F_{1,55}$  20.95,  $P<0.001$ ). Comparisons of the effects of vehicle pretreatment with the effects of pretreatment with other NMDA receptor antagonists revealed no significant effects. Similar conclusions were reached with the use of one-way ANOVA on the same data when expressed as the area under the curve (AUC; see legend to Fig. 2 for details).

#### Discussion

The present findings demonstrate that, in mice, the clinically available and/or promising, low-affinity, uncompetitive NMDA receptor antagonists dextromethorphan, memantine and MRZ 2/579 inhibit the development of antinociceptive morphine tolerance and reverse existing tolerance. In the latter respect, the effects of dextromethorphan were less pronounced, perhaps due to inadequate dosage. Memantine and MRZ 2/579, but not dextromethorphan or NPC 17742, inhibited the acute antinociceptive effects of morphine.

The attenuation of the development of morphine tolerance by NMDA receptor antagonists as shown in experiment 1 has been demonstrated previously (Lutfy et al. 1993; Elliott et al. 1994a; Bilsky et al. 1996; Gonzalez et al. 1997; Belozertseva and Bespalov 1998; Lutfy et al. 1995). The development of the drug tolerance phenome-

non represents a neuronal plastic change and thus resembles learning processes (Siegel 1976). Given the fact that NMDA receptor antagonists interfere with learning processes (see Danysz et al. 1995 for a review), it is possible that the compounds used in the present study might attenuate the development of morphine tolerance through the inhibition of neuronal plasticity. However, the purported inhibitory effects of memantine and dextromethorphan on the neuronal plasticity could not explain the reversal of morphine tolerance found in experiment 2, in which NMDA receptor antagonists were given to already tolerant mice. In fact, contrary to their inhibitory effects on the acquisition of new information, NMDA receptor antagonists do not affect the storage or recall of well-established associations (Danysz et al. 1995).

The present data agree with earlier reports demonstrating reversal of morphine tolerance in rodents treated between tests 2 and 3 with NMDA receptor antagonist in the absence of morphine [mice: dextromethorphan 30 mg/kg t.i.d., tail-flick test (Elliott et al. 1994b), rats: LY274614 delivered by minipumps at 24 mg/kg/24 h, hot-plate test (Tiseo and Inturrisi 1993)] and in the presence of continuous morphine administration [rats: LY274614 delivered by minipumps at 24 mg/kg/24 h, hot-plate test (Tiseo et al. 1994; Tiseo and Inturrisi 1993); rats: LY235959 1–10 mg/kg, hot water tail-withdrawal test (Allen and Dykstra 1999)]. These intriguing findings indicate that the ongoing activation of NMDA receptor is important not only for the development but also for the maintenance of morphine tolerance and dependence (Popik and Skolnick 1996; Popik et al. 1998). Thus, these observations point to a crucial difference between learning processes and plasticity related to chronic morphine administration.

An interesting, and perhaps confounding issue is whether the NMDA receptor antagonists possess innate antinociceptive activity and/or interfere with the antinociception produced by morphine. The data from experiment 3 demonstrate the lack of the antinociceptive effects of the compounds used in the present study, complementing earlier observations with memantine (Malec and Langwinski 1981) and dextromethorphan (Hoffmann and Wiesenfeld-Hallin 1996) (measured in the hot-plate test in rats).

However, we found that memantine and MRZ 2/579 reduced morphine antinociception, while dextromethorphan and NPC17742 did not affect it. The inhibitory effects of memantine and MRZ 2/579 on acute morphine antinociception in mice deserve a note. First, it is unlikely that these compounds blocked morphine actions at the level of the opiate receptors, since at concentrations up to 10  $\mu$ M they do not bind to the opioid  $\mu$  receptors (Danysz et al. 1997; Tortela et al. 1989). Next, as has been pointed out by Bespalov et al. (1998), reports on the effects of NMDA receptor antagonists on acute morphine antinociception are conflicting and depend on the site of action of a given compound at the NMDA receptor. To limit the present discussion to mice, (+)MK-801 either reduces morphine antinociception as measured in the hot-plate (Lipa and Kavaliers 1990; Saucier and Kavaliers 1994) and tail-flick (Lutfy et al. 1993) tests, or does not change

it in tail-flick tests (Elliott et al. 1994a; Bilsky et al. 1996). In the same species, competitive NMDA antagonists either increase morphine antinociception [NPC 12626, hot-plate test (Saucier and Kavaliers 1994), LY235959 in the tail-flick test (Bhargava 1997)] or do not change it in the tail-flick test [LY 274614 (Elliott et al. 1994a), LY235959 (Bilsky et al. 1996) and NPC 17742 (Kolesnikov et al. 1993)]. In light of these findings, our data appear to agree with lack of effects of uncompetitive NMDA receptor antagonists on morphine antinociception (dextromethorphan) and its reduction (memantine, MRZ 2/579). Our findings also seem to support the lack of effects of the competitive NMDA receptor antagonist, NPC 17742, on morphine antinociception in mice.

Dextromethorphan at 10 mg/kg did not affect reversal of morphine tolerance (in mice treated with dextromethorphan+morphine in experiment 2) and did not inhibit the acute antinociceptive effect of morphine (experiment 3). The most likely explanation is that the dose of dextromethorphan was too low. For example, dextromethorphan's inhibitory effects on the maintenance of morphine antinociception were reported for the dose of 30 mg/kg t.i.d. (Elliott et al. 1994b). However, the choice of 10 mg/kg was based on the inhibitory effects of dextromethorphan on development of morphine tolerance (experiment 1). It thus cannot be excluded that the inhibition of acute morphine antinociceptive effects by NMDA receptor antagonists is in some way related to their ability to reverse morphine tolerance, both of which seem unrelated to the effects on the development of morphine tolerance. However, the other explanations also cannot be excluded. First, there are differences in pharmacological profiles between memantine and MRZ 2/579 and dextromethorphan. Dextromethorphan produces several effects on the other channels and receptors (Church et al. 1991; Netzer et al. 1993), and a comparison of doses needed to inhibit e.g. NMDA-induced convulsions (100 mg/kg, Ferkany et al. 1988) suggests that only a weak effect on NMDA receptors can be expected for 10 mg/kg. Finally, as pointed by Elliott et al. (1994b), dextromethorphan's binding sites are different from the known distribution of NMDA receptors.

The present findings showing inhibition of morphine tolerance by NMDA receptor antagonists cannot be explained by a pharmacokinetic interaction between these compounds and morphine. First, both the present as well as earlier work (Lutfy et al. 1993; Elliott et al. 1994a; Bilsky et al. 1996; Gonzalez et al. 1997; Belozertseva and Bespalov 1998; Lutfy et al. 1995) demonstrate similar, inhibitory effects on morphine tolerance produced by a number of structurally dissimilar NMDA receptor antagonists. In addition, the findings of Bhargava and Matwyshyn (1993) demonstrating that (+)MK-801 inhibits the tolerance to the antinociceptive but not to the hyperthermic effect of morphine, suggest that pharmacokinetic interaction between an NMDA receptor antagonist and morphine is unlikely.

Chronic treatment with morphine produces tolerance to a variety of its effects. For example, tolerance to the re-

warding effects of opiates has been observed both in humans (Martin and Sloan 1977) and rats (Shippenberg et al. 1988). An important insight provided by the present findings is that, if NMDA receptor antagonist treatment were to reverse similarly existing tolerance to the other effects of opiates, (particularly reward, euphoria and dependence), such a compound would have excellent therapeutic applications. Thus, it can be hypothesized that these compounds would decrease the daily dose and/or number of injections, that would lead to the harm reduction produced by opiates. Data from this laboratory indicate that low-affinity uncompetitive NMDA receptor antagonists may block both the conditioned reward produced by morphine (Popik and Danysz 1997; Popik et al. 1998) and, in particular, reverse the maintenance of morphine dependence (Popik and Skolnick 1996; Popik et al. 1998). It remains to be established whether the tolerance to effects related to the addictive potential of opiates may be similarly reverted by low-affinity, uncompetitive NMDA antagonists.

In conclusion, the present study demonstrates that the clinically available NMDA receptor antagonists memantine and dextromethorphan as well as the novel uncompetitive NMDA receptor antagonist MRZ 2/579 with a similar, favourable preclinical pharmacological profile, inhibit the development of the tolerance to the antinociceptive effects of morphine and reverse it in tolerant mice. The possibility of reversal of existing morphine tolerance would be very useful from the clinical perspective because this would not impose necessity of interruption of treatment with morphine in order to restore its full antinociceptive activity. These findings indicate the therapeutic potential of NMDA receptor antagonists in the management of chronic pain in combination with opiates, and, perhaps, in the treatment of opiate addiction.

**Acknowledgement** This study was supported by KBN grant 4 P05A 042 17.

## References

- Allen RM, Dykstra LA (1999) The competitive NMDA receptor antagonist LY235959 modulates the progression of morphine tolerance in rats. *Psychopharmacology* 142:209–214
- Ambrozi L, Danielczyk W (1988) Treatment of impaired cerebral function in psychogeriatric patients with memantine – results of a phase II double-blind study. *Pharmacopsychiatry* 21:144–146
- Belozertseva IV, Beshpalov AY (1998) Effects of NMDA receptor channel blockers, dizocilpine and memantine, on the development of opiate analgesic tolerance induced by repeated morphine exposures or social defeats in mice. *Naunyn-Schmiedeberg's Arch Pharmacol* 358:270–274
- Bem JL, Peck R (1992) Dextromethorphan. An overview of safety issues. *Drug Saf* 7:190–199
- Beshpalov A, Kudryashova M, Zvartau E (1998) Prolongation of morphine analgesia by competitive NMDA receptor antagonist d-CPPene (SDZ EAA 494) in rats. *Eur J Pharmacol* 351:299–305
- Bhargava HN (1997) Enhancement of morphine actions in morphine-naive and morphine-tolerant mice by LY 235959, a competitive antagonist of the NMDA receptor. *Gen Pharmacol* 28:61–64
- Bhargava HN, Matwyshyn GA (1993) Dizocilpine (MK-801) blocks tolerance to the analgesic but not to the hyperthermic effect of morphine in the rat. *Pharmacology* 47:344–350
- Bilsky EJ, Inturrisi CE, Sadee W, Hruby VJ, Porreca F (1996) Competitive and non-competitive NMDA antagonists block the development of antinociceptive tolerance to morphine, but not to selective mu or delta opioid agonists in mice. *Pain* 68:229–237
- Chou YC, Liao JF, Chang WY, Lin MF, Chen CF (1999) Binding of dimemorfan to sigma-1 receptor and its anticonvulsant and locomotor effects in mice, compared with dextromethorphan and dextrorphan. *Brain Res* 821:516–519
- Church J, Shacklock JA, Baimbridge KG (1991) Dextromethorphan and phencyclidine receptor ligands: differential effects on K(+)- and NMDA-evoked increases in cytosolic free Ca<sup>2+</sup> concentration. *Neurosci Lett* 124:232–234
- Cochin J, Kornetsky C (1964) Development and loss of tolerance to morphine in the rat after single and multiple injections. *J Pharmacol Exp Ther* 145:1–10
- Danysz W, Zajackowski W, Parsons CG (1995) Modulation of learning processes by ionotropic glutamate receptor ligands. *Behav Pharmacol* 6:455–474
- Danysz W, Parsons CG, Kornhuber J, Schmidt WJ, Quack G (1997) Aminoadamantanes as NMDA receptor antagonists and antiparkinsonian agents – preclinical studies. *Neurosci Biobehav Rev* 21:455–468
- Elliott K, Minami N, Kolesnikov YA, Pasternak GW, Inturrisi CE (1994a) The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, N<sup>ω</sup>-nitro-L-arginine, attenuate analgesic tolerance to the mu-opioid morphine but not to kappa opioids. *Pain* 56:69–75
- Elliott K, Hynansky A, Inturrisi CE (1994b) Dextromethorphan attenuates and reverses analgesic tolerance to morphine. *Pain* 59:361–368
- Ferkany JW, Borosky SA, Clissold DB, Pontecorvo MJ (1988) Dextromethorphan inhibits NMDA-induced convulsions. *Eur J Pharmacol* 151:151–154
- Foley KM (1991) Clinical tolerance to opioids. In: Basbaum AI, Besson JM (eds) *Towards a new pharmacotherapy of pain: report of the Dahlem workshop: Beyond morphine*. Wiley, New York, pp 181–204
- Gonzalez P, Cabello P, Germany A, Norris B, Contreras E (1997) Decrease of tolerance to, and physical dependence on morphine by, glutamate receptor antagonists. *Eur J Pharmacol* 332:257–262
- Herman BH, O'Brien CP (1997) Clinical medications development for opiate addiction: focus on nonopioids and opioid antagonists for the amelioration of opiate withdrawal symptoms and relapse prevention. *Semin Neurosci* 9:158–172
- Hoffmann O, Wiesenfeld-Hallin Z (1996) Dextromethorphan potentiates morphine antinociception, but does not reverse tolerance in rats. *Neuroreport* 7:838–840
- Kolesnikov YA, Ferkany J, Pasternak GW (1993) Blockade of mu and kappa1 opioid analgesic tolerance by NCP17742, a novel NMDA antagonist. *Life Sci* 53:1489–1494
- Kolesnikov YA, Maccacchini M-L, Pasternak GW (1994) 1-Aminocyclopropanecarboxylic acid (ACPC) prevents mu and delta opioid tolerance. *Life Sci* 55:1393–1398
- Kornhuber J, Bormann J, Retz W, Hubers M, Riederer P (1989) Memantine displaces [<sup>3</sup>H]MK-801 at therapeutic concentrations in postmortem human frontal cortex. *Eur J Pharmacol* 166:589–590
- Lipa SM, Kavaliers M (1990) Sex differences in the inhibitory effects of the NMDA antagonist, MK-801, on morphine and stress-induced analgesia. *Brain Res Bull* 24:627–630
- Lutfy K, Hurlbut DE, Weber E (1993) Blockade of morphine-induced analgesia and tolerance in mice by MK-801. *Brain Res* 616:83–88
- Lutfy K, Shen KZ, Kwon IS, Cai SX, Woodward RM, Keana JFW, Weber E (1995) Blockade of morphine tolerance by ACEA-1328, a novel NMDA receptor glycine site antagonist. *Eur J Pharmacol* 273:187–189

- Malec D, Langwinski R (1981) Central action of narcotic analgesics. VIII. The effect of dopaminergic stimulants on the action of analgesics in rats. *Pol J Pharmacol Pharm* 33:273–282
- Martin WR, Sloan JW (1977) Neuropharmacology and neurochemistry of subjective effects, analgesia, tolerance and dependence produced by narcotic analgesics. In: Martin WR (ed) *Drug addiction*. Springer, Berlin Heidelberg New York, pp 43–158
- Netzer R, Pflimlin P, Trube G (1993) Dextromethorphan blocks N-methyl-D-aspartate-induced currents and voltage-operated inward currents in cultured cortical neurons. *Eur J Pharmacol* 238:209–216
- Paronis CA, Holtzman SG (1991) Increased analgesic potency of mu agonists after continuous naloxone infusion in rats. *J Pharmacol Exp Ther* 259:582–589
- Parsons CG, Danysz W, Quack G (1999a) Memantine is a clinically well tolerated NMDA receptor antagonist – a review of preclinical data. *Neuropharmacology* 38:735–767
- Parsons CG, Danysz W, Bartmann A, Spielmanns P, Frankiewicz T, Hesselink M, Eilbacher B, Quack G (1999b) Amino-alkyl-cyclohexanes are novel uncompetitive NMDA receptor antagonists with strong voltage-dependency and fast blocking kinetics: in vitro and in vivo characterization. *Neuropharmacology* 38:85–108
- Popik P, Danysz W (1997) Inhibition of reinforcing effects of morphine and motivational aspects of naloxone-precipitated opioid withdrawal by NMDA receptor antagonist, memantine. *J Pharmacol Exp Ther* 280:854–865
- Popik P, Skolnick P (1996) The NMDA antagonist memantine blocks the expression and maintenance of morphine dependence. *Pharmacol Biochem Behav* 53:791–798
- Popik P, Mamczarz J, Fraczek M, Widla M, Hesselink M, Danysz W (1998) Inhibition of reinforcing effects of morphine and naloxone – precipitated opioid withdrawal by novel glycine site and uncompetitive NMDA receptor antagonists. *Neuropharmacology* 37:1033–1042
- Saucier DM, Kavaliers M (1994) Antagonistic effects of the selective, competitive N-methyl-D-aspartate (NMDA) receptor antagonist, NPC 12626, on kappa opiate-induced analgesia in male deer mice. *Brain Res* 637:292–296
- Shippenberg TS, Emmett Oglesby MW, Ayesta FJ, Herz A (1988) Tolerance and selective cross-tolerance to the motivational effects of opioids. *Psychopharmacology* 96:110–115
- Siegel S (1976) Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. *Science* 193:323–325
- Tiseo PJ, Inturrisi CE (1993) Attenuation and reversal of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist, LY274614. *J Pharmacol Exp Ther* 264:1090–1096
- Tiseo PJ, Cheng J, Pasternak GW, Inturrisi CE (1994) Modulation of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist LY274614: assessment of opioid receptor changes. *J Pharmacol Exp Ther* 268:195–201
- Tortela FC, Pellicano M, Bowery NG (1989) Dextromethorphan and neuromodulation: old drug coughs up new activities. *Trends Pharmacol Sci* 10:501–507