Inhibition of reinforcing effects of morphine and naloxone-precipitated opioid withdrawal by novel glycine site and uncompetitive NMDA receptor antagonists

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Accepted 14 May 1998

Abstract

The glycine site (MRZ 2/570 and L-701,324), and uncompetitive (MRZ 2/579) NMDA receptor antagonists inhibited morphine-produced behaviors related to drug-abuse. The expression of morphine dependence was blocked by pretreatment with all three compounds (3–7.5 mg/kg); the effects of glycine/NMDA antagonists were not dose-dependent. Mice which were morphine-free for 3 days still displayed a significant severity of the withdrawal syndrome when challenged again with naloxone. This extinction of a residual morphine dependence was markedly diminished by treatment with similar doses of NMDA receptor antagonists at the test following the wash-out period. The rewarding impact of morphine was investigated in rats using the place preference (CPP) paradigm. All NMDA receptor antagonists (2.5–10 mg/kg) inhibited both the acquisition and expression of morphine-induced CPP. Once established, morphine-induced CPP was observed until 2 weeks after conditioning. NMDA receptor antagonists given for 3 days after the end of conditioning did not influence the extinction of morphine-induced CPP. Microdialysis studies revealed that the behaviorally effective doses of MRZ 2/579 resulted in a brain concentration close to its in vitro potency as an NMDA receptor antagonist. These data suggest that novel glycine site and uncompetitive NMDA receptor antagonists may have therapeutic potential in the treatment of opioid abuse. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: NMDA/glycine antagonists; Morphine; Conditioned place preference; Naloxone-precipitated morphine withdrawal; Extinction; Microdialysis

1. Introduction

Physical dependence has traditionally been considered as a major factor contributing to the maintenance of compulsive use of opiates, and its withdrawal syndrome is a life-threatening clinical situation (Eddy et al., 1965). Contemporary theories emphasize the importance of the reinforcing (incentive) properties of abused substances in the development and maintenance of opioid addiction (Robinson and Berridge, 1993). Thus, it might be hypothesized that a potential ‘anti-addictive’ treatment should inhibit not only the expression of the opioid withdrawal syndrome, but also, the reinforcing impact of opioids. The ‘ideal’ anti-addictive treatment should, in addition, inhibit the ongoing process of addiction by eliminating its maintenance (facilitating the extinction process).

Converging lines of evidence indicate the essential involvement of N-methyl-D-aspartate (NMDA) receptors in phenomena related to the development, maintenance and expression of opioid addiction. The NMDA receptor complex is a ligand-gated cationic channel, most likely consisting of a pentameric assembly of subunits that contains several regulatory sites. Apart from a recognition site for the primary transmitter (glutamate) it includes sites sensitive to open channel blockers, glycine (which is obligatory for receptor activation), and polyamines (Kutsuwada et al., 1992; Danysz et al., 1995a). In preclinical settings, the function of the NMDA receptor may be modulated by specific antagonists, acting at each of these sites. How-
ever, clinical testing of some NMDA receptor channel blockers such as (+)MK-801, ((+)-5-methyl-10,11-di-
hydro-5H-dibenzocyclohepten-5,10-imine maleate], dizocilpine) (Troupin et al., 1986) and aiptiganel (CNS 1102, N-(1-naphthyl)-N(3-ethylphenyl)-N-methyl-
guanidine (Muir et al., 1994) revealed a number of undesired side-effects. These side-effects have been considered as important limiting factors for the development of NMDA receptor antagonists as therapeutic agents. To date, the low affinity, strongly voltage-de-
dependent uncompetitive NMDA receptor antagonists like dextromethorphan, amantadine and memantine, are the only NMDA receptor antagonists to have been successfully used in clinical practice (Ditzler, 1991; Rog-
awski, 1993; Danysz et al., 1995a).

Less explored and potentially more attractive develop-
mental drug candidates are antagonists of NMDA receptors acting at the strychnine-insensitive glycine recognition site (Johnson and Ascher, 1987; Danysz et al., 1995a). In contrast to the other NMDA receptor antagonists, glycine/NMDA antagonists fail to produce neurodegenerative changes in the cingulate/retrosplenial cortex (Chen et al., 1993; Berger et al., 1994), psychoto-
mimetic-like effects (Bristow et al., 1996a), and impair-
ment of learning at anticonvulsive doses (Chiamulera et al., 1990).

In preclinical studies, NMDA receptor antagonists reduce the physical (Trujillo and Akil, 1991; Higgins et al., 1992; Cappendijk et al., 1993; Popik et al., 1995; Popik and Skolnick, 1996) as well as motivational aspects (Higgins et al., 1992; Popik and Danysz, 1997) of the expression of morphine dependence, most often precipitated by the opiate antagonist, naloxone. In addition, NMDA receptor antagonists inhibit the develop-
ment (Trujillo and Akil, 1991) as well as the maintenance of morphine dependence (Popik and Skol-
nick, 1996). Similarly, the reinforcing actions of opiates could be reduced by NMDA receptor antagonists. Thus, Bесполов et al. (1994) demonstrated that the non-selective glutamate receptor antagonist, kynurenic acid, attenuates the acquisition and expression of conditioned place preference (CPP) induced by morphine. The same treatment inhibits morphine-induced facilitation of responding in the electrical intracranial self-
stimulation paradigm. Similar attenuation of the development of morphine-induced CPP has been recently shown for (+)MK-801 and DL-(E)-2- amino-4-
methyl-5-phosphono-3-pentanoic acid (CGP-37849), which are uncompetitive and competitive NMDA rece-
ptor antagonists, respectively (Tzschentke and Schmidt, 1995). It has been reported that ibogaine, also having antagonistic properties at NMDA receptors (Popik et al., 1995) inhibits morphine self-administra-
tion in rodents (Glick et al., 1991). ‘Anti-addictive’ effects of the NMDA receptor antagonist, dextromethorphan, have also been reported in initial clini-
cal trials (Koyuncuoglu and Saydam, 1990; Bisaga et al., 1997).

So far, the data on the effects of glycine/NMDA receptor antagonists on behaviors related to drug-abuse are very limited. Available studies addressed in particu-
lar the effects of glycine/NMDA receptor antagonists on the tolerance to the analgesic effects of opioids—
which seems unrelated to their potency to induce drug addiction. Thus, Kolesnikov et al. (1994) recently re-
ported that the partial glycine site agonist aminocyclo-
propane carboxylic acid (APC) prevented tolerance to a mu opioid agonist (morphine) and to a delta opioid agonist [d-Pen²,d-Pen⁵]enkephalin (DPDPE) when co-
administered with either of these opioids in CD-1 mice.

ACEA-1328 (5-nitro-6,7-dimethyl-1,4-dihydro-2,3-
quinoxalinedione) inhibited morphine tolerance in mice without affecting the basal nociceptive response.

NMDA antagonists used in the present study include MRZ 2/579 (1-amino-1.3,3,5,5-pentamethyl-cyclohexan hydrochloride), L-701,324 (7-chloro-4-hydroxy-3-(3-
phenoxy)-phenyl-2-(H)quinolone) and MRZ 2/570 (8-
bromo-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b]-
quinoline-5-oxide choline salt). MRZ 2/579 is a highly voltage dependent, moderate affinity NMDA channel blocker (IC₅₀ = 1.2 μM in patch clamp experiments) showing a favorable behavioral profile in animal models i.e. its potency in producing ataxia, myorelaxation, impairment of prepulse inhibition or stereotyped behav-
ior is rather low (Parsons et al., 1998). L-701,324 is a potent glycine/NMDA receptor antagonist blocking NMDA responses in cultured rat cortical neurons with a Kᵢ of 5.4 nM (Priestley et al., 1994). It penetrates well into the brain and at 3 mg/kg achieves a peak brain concentration of 0.69 μM (Bristow et al., 1996b). MRZ 2/570 is a selective NMDA receptor antagonist acting at the glycine site (IC₅₀ = 0.6 μM, patch clamp studies) (Parsons et al., 1997). It shows clear-cut central NMDA receptor antagonistic activity when injected systemically as demonstrated by an inhibition of responses to ion-tophoretically applied NMDA and antagonism of MES-induced convulsions (Parsons et al., 1997).

The objective of the present experiments was to
determine whether the glycine/NMDA receptor anti-
gonists, MRZ 2/570 and L-701,324 (Bristow et al., 1996b; Parsons et al., 1997) affect the severity of naloxone-pre-
cipitated morphine withdrawal and/or the reinforcing
impact of morphine. If positive, the data would be indicative of their potential usefulness in the treatment of opioid abuse. Additionally, since memantine has been previously demonstrated to be effective in these models (Popik and Danysz, 1997), we tested a new

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*Neuropharmacology 37 (1998) 1033–1042*
Morphine dependence in mice

2. Materials and methods

2.1. Subjects

Male Albino Swiss mice (~ 27.5 g) were housed in groups of eight in plastic cages (43 × 27 × 15 cm). Male Wistar rats (~ 300 g) were housed under standard laboratory conditions for at least 2 weeks before experiments begun. Animals were kept in plastic cages, four per cage (58 × 37 × 19 cm). Water and commercial food were available ad libitum and the animal room was maintained on a controlled light/dark cycle (lights on 07:00 h; off: 19:00 h). For microdialysis experiments male Sprague-Dawley rats (body weight 235–275 g, Charles River, Germany) were used. They were kept under standard laboratory conditions: 12/12h dark/light cycle, with free access to food and water.

2.2. Morphine dependence in mice

The procedure was similar to that described previously (Popik and Danysz, 1997). Briefly, four identical wooden boxes with the white and black chambers (30 × 20 × 25 cm each) were used. The chambers had distinct floor textures (plain wood in the white chamber and wire mesh in the black chamber). The gray central area (12 × 20 × 25 cm) constituted a ‘neutral’ chamber. The CPP procedure consisted of an adaptation, a pre-test, an acquisition of a conditioned response and the post-test. In some experiments, there was more than one post-test session. During the first 3 days of training (adaptation phase), the rats were placed individually in the apparatus and freely explored it for 10 min daily. These trials as well as our previous studies (Popik and Danysz, 1997) indicated that almost all subjects preferred the black chamber over the white one. On day 4, (pre-test), the time spent in the white chamber during a 10-min free exploration session was measured and recorded. This measure was used as an initial preference score for each subject.

To investigate the effects of NMDA receptor antagonists on the acquisition of morphine-induced CPP (experiment 4), on days 5, 7 and 9, rats were injected with various doses of NMDA receptor antagonists at 30 min, and placebo 15 min, respectively, before the conditioning trial in the black chamber of the apparatus. On days 6, 8, and 10, rats were injected with various doses of NMDA receptor antagonists at 30 min, and morphine (1 mg/kg, i.p.) at 15 min, respectively, before the conditioning trial in the white chamber of the apparatus. Conditioning trials (6 consecutive days) lasted for 30 min. Changes in the CPP scores were measured on day 11 (post-test) when rats were injected with vehicle 20 min before being placed in the apparatus. Again, the time spent in the white chamber was recorded during the session lasting 10 min. A similar procedure was used to measure the effects of NMDA receptor antagonists on the expression of morphine-induced CPP, with the exception that rats were injected only with the placebo (before being placed in the black chamber) or morphine (before being placed in the white chamber of the apparatus) during the acquisition trials. Twenty minutes before the post-test, rats were injected with vehicle or various doses of NMDA receptor antagonists.
In the separate experiment 5, we investigated the duration of the presence of morphine-induced CPP. Rats (\( N = 41 \)) were conditioned as described above to the effects of morphine. After conditioning, subjects were divided into three separate groups that differed in the way of assessing changes in the CPP. Thus, rats from group ‘A’ received post-test \( \# 1 \) on day 11 and additional post-tests \( \# 2 \) and \( \# 3 \) on days 18 and 25, respectively. Rats from group ‘B’ received two post-tests on days 18 and 25, while rats from group ‘C’ received only one post-test, on day 25.

The effects of NMDA receptor antagonists on extinction of morphine-induced CPP (experiment 6) were investigated by conditioning rats to the effects of morphine and measuring the initial shift of preference for the morphine-paired side (post-test \( \# 1 \)) on day 11 as described above. During the next 3 days, these subjects were divided into four groups that were treated with placebo, L-701,324, MRZ 2/570 or MRZ 2/579 twice a day. This 3-day period of injections was selected based on the similar, 3-day period of injections in experiment 3. In addition, the shortage of compounds did not allow use of a longer period of injections. On day 15, (\( \sim 18 \) h after the last injection), these subjects were again investigated for a possible change in their preference scores toward the morphine-paired side of the CPP apparatus.

2.4. Statistics

Data of experiments 1–3 were analyzed using a non-parametrical approach by Kruskal–Wallis ANOVA and Dunn’s multiple comparison or Mann–Whitney tests, where appropriate. In the CPP studies, the preference scores were expressed as a percent increase or decrease of time spent by a rat in a given chamber of the apparatus during pre-test and post-test (Popik and Danyss, 1997). Data were analyzed using ANOVA, followed by Newman–Keuls tests and, where appropriate, with Student’s \( t \)-test.

2.5. Brain concentration assessment of MRZ 2/579 with brain microdialysis

2.5.1. Surgery

For microdialysis probe implantation the animals were anesthetized with Hypnorm (1.0 ml/kg i.m., Janssen Pharmaceutica), placed in a stereotaxic frame and the skull was exposed. A small hole was drilled to allow the implantation of a microdialysis guide cannula (CMA/10, CMA/microdialysis, Sweden) in the anterior striatum relative to the bregma (AP: 1.0; L: 2.5, V: –3.0). A second hole was drilled, a screw was secured into the skull and the screw and guide cannula were cemented together onto the skull using dental cement (Paladur, Heraeus, Germany). A microdialysis probe (CMA/10, membrane length of 3.0 mm) was inserted into the guide cannula immediately after the surgery and the animals were allowed to recover for 22–26 h.

2.5.2. Experimental procedure

At the start of the experiment the inflow line was connected to a syringe pump (CMA/100) by means of a dual channel swivel (CMA) and the probe was perfused with artificial CSF (aCSF, composition in mM was: NaCl 145, KCl 0.6, MgCl\(_2\) 1.0, CaCl\(_2\) 1.2, ascorbic acid 0.2 in a 2 mM potassium phosphate buffer pH 7.4) at a flow rate of 3 \( \mu \)l/min. The outlet line was connected to a microfraction collector (CMA/140) and 10-min fractions were collected. After 2 h of equilibrium dialysis MRZ 2/579 was injected i.p. in a dose of 5 mg/kg. Microdialysate was collected for up to 140 min.

2.5.3. In vitro recovery experiment

The dialysate concentrations were corrected for the in vitro recovery of the probe. The probe was placed in an aCSF solution (37°C) with a known concentration of MRZ 2/579 (\( C_{\text{med}} = 1 \) \( \mu \)M) and dialysate samples were collected (\( C_{\text{out}} \)). Recovery was calculated as \( (C_{\text{med}}/C_{\text{out}}) \times 100\% \). The concentration of MRZ 2/579 in the dialysate was estimated using gas chromatography as described previously for memantine (Danyss et al., 1994).

2.6. Drugs

Morphine HCl (Polfa), naloxone HCl (Endo) and MRZ 2/579 HCl (Merz + Co) were dissolved in physiological saline; MRZ 2/570 choline (Merz + Co) in MilliQ water and L-701,324 (Tocris) in 5% methyl cellulose. Saline and 5% methyl cellulose were used as a placebo. The doses of morphine and naloxone correspond to the doses calculated as base, for all other agents as respective salts. For rats, all injections were given in a volume of 1 ml/kg, i.p. and in case of mice 10 ml/kg.

3. Results

3.1. Effects of the duration of treatment with morphine, morphine dose and naloxone dose on the intensity of morphine withdrawal syndrome in mice (experiment 1)

Although the schedule of injections to produce morphine dependence in mice was based on previous observations (see Section 4), we failed to find the relevant literature data demonstrating that the dose and duration of morphine treatment as well as the dose of naloxone challenge influences the severity of
Table 1
The effects of the duration of treatment with morphine, the morphine dose and naloxone dose on the intensity of the morphine withdrawal syndrome in mice

<table>
<thead>
<tr>
<th>Duration of morphine treatment (days)</th>
<th>Morphine dose (mg/kg)</th>
<th>Naloxone dose (mg/kg)</th>
<th>Jumps/10 min (N)</th>
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<tbody>
<tr>
<td>Placebo control</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<tr>
<td>Standard procedure</td>
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Kruskal–Wallis ANOVA:
KW = 39.57, P < 0.001

The data present mean ± S.E.M. of the number of jumps during the session lasting for 10 min.

* P < 0.01; † P < 0.001 versus number of jumps of placebo control mice.

* P < 0.01 versus number of jumps demonstrated by standard procedure mice, Dunn’s multiple comparison test.

* P < 0.05 versus standard procedure mice, Mann–Whitney test.

‘Placebo control’ mice were injected for 7 days with saline, and on the test day, with morphine (30 mg/kg) followed 2 h later by 4 mg/kg of naloxone.

‘Standard procedure’ mice were injected with 30 mg/kg of morphine for 3 days, and challenged with 4 mg/kg of naloxone.

morphine withdrawal in this species. Table 1 reveals no remarkable differences among groups, with the exception of the effects of the significantly lower dose of morphine (5 mg/kg) that resulted in a reduced severity of the withdrawal syndrome. Although the intensity of the withdrawal syndrome after 1 day of morphine injections did not appear different when Kruskal–Wallis one-way ANOVA was used, direct comparison of the number of jumps between groups treated with morphine for 1 day and 3 days using the Mann–Whitney test indicated that 1 day of morphine treatment resulted in a lower intensity of withdrawal signs (Table 1). Nonetheless, the experiments designed to evaluate the inhibitory effects of NMDA receptor antagonists on the expression of morphine withdrawal were carried out using the schedule of 3 days of 30 mg/kg of morphine injections and a 4 mg/kg naloxone challenge.

3.2. Effects of NMDA receptor antagonists on the expression of morphine withdrawal syndrome (experiment 2)

All three NMDA receptor antagonists inhibited the expression of morphine withdrawal, although in the case of glycine/NMDA antagonists, a U-shaped dose–response curve was seen, with medium doses being effective (Fig. 1). L-701,324 inhibited the expression of morphine withdrawal only at one dose of 3 mg/kg and MRZ 2/570 was effective only at a dose of 5 mg/kg (Fig. 1). MRZ 2/579 was effective at doses of 5 and 7.5 mg/kg.

3.3. Effects of NMDA receptor antagonists on the extinction of morphine dependence (experiment 3)

Mice (N = 71) were treated with morphine for 3 days, and on day 4, when challenged with naloxone, they jumped 11.0 ± 1.1 times during the first withdrawal test. These subjects were divided into four groups that were treated with placebo, MRZ 2/570, MRZ 2/579 or L-701,324 twice a day, for the subsequent 3 days until
Fig. 2. Effects of glycine/NMDA receptor antagonists MRZ 2/570 and L-701,324 and an uncompetitive NMDA receptor antagonist MRZ 2/579 on the extinction of morphine-dependence. The data represent the mean ± S.E.M. number of jumps recorded for 10 min. Mice were rendered dependent by administration of 30 mg/kg of morphine on days 1–3, and challenged with 4 mg/kg of naloxone on day 4 (horizontal line indicates mean, dashed lines indicate ± S.E.M.). On days 5–7, mice received NMDA receptor antagonist or placebo, and on day 8 were again challenged with 4 mg/kg of naloxone.

3.4. Effects of NMDA receptor antagonists on the acquisition and expression of morphine-induced CPP (experiment 4)

Rats injected with morphine in the white (initially non-preferred) chamber demonstrated a marked preference for this chamber during the drug-free post-test. All doses of all NMDA antagonists, with exception of the lower dose of MRZ 2/579 (1.25 mg/kg) inhibited the expression of morphine-induced CPP (Fig. 3).

To assess the effects on the acquisition of CPP, rats were injected with selected doses of NMDA receptor antagonists before each of the conditioning sessions. MRZ 2/570 (5 and 10 mg/kg), L-701,324 (3 mg/kg), as well as MRZ 2/579 (5 mg/kg) inhibited the acquisition of morphine-induced CPP ($P < 0.05–0.01$, Newman–Keuls test, after ANOVA [$F_{4,91} = 5.35$, $P < 0.001$]). The preference scores for the morphine paired chamber were respectively in %: 163.35 ± 23.94 ($N = 11$); 76.06 ± 12.69 ($N = 12$); 123.42 ± 29.30 ($N = 12$) and 210.8 ± 25.65 ($N = 10$) as compared to the group treated with morphine only (433.5 ± 61.73 [$N = 47$]).

An additional experiment was carried out to assess the reinforcing effect of MRZ compounds, as measured in the CPP procedure. Neither MRZ 2/570 nor MRZ 2/579 at doses of 5 mg/kg induced CPP. The preference ratios for MRZ 2/570 and MRZ 2/579 were 110.11 ± 13.62 ($N = 13$) and 151.31 ± 71.50 ($N = 13$), respectively. Both values were significantly lower as compared to morphine-induced CPP: $t = 2.82$ (df = 58), $P < 0.01$ and $t = 2.38$ (df = 58) $P < 0.05$, for MRZ 2/570 and MRZ 2/579, respectively.

3.5. Extinction of morphine-induced CPP (experiments 5 and 6)

Rats injected with morphine in the white (initially non-preferred) chamber demonstrated a marked preference for this chamber during the drug-free post-test. In group ‘A’, (post-tested on days 11, 18 and 25) this preference decreased about six times and this number was found to be significantly lower, compared to placebo group (Fig. 4). The horizontal line indicates mean preference of the control group, dashed lines indicate ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group; Newman–Keuls test. $N = 10–18$ for the treatments and 47 for the control group.
effect was seen on days 11 and 18 and absent on day 25. In group ‘B’, (post-tested on days 18 and 25) this effect was observed on both days 18 and 25. In group ‘C’, post-tested on day 25 only, a significant CPP was present (Table 2).

After 6 days of conditioning and the first post-test (day 11), rats were treated with NMDA receptor antagonists for 3 days, twice a day. On day 15 the second post-test was carried out. Table 3 demonstrates that there were no differences in conditioned place preference among groups.

3.6. Pharmacokinetics

Microdialysis studies indicate that MRZ 2/579 (5 mg/kg) reaches brain concentrations of 0.8 \( \mu \text{M} \) 60 min after administration and has a half-life of 3 h (Fig. 4). The estimates of brain concentrations were based on an in vitro recovery of 18%.

4. Discussion

The present data indicate that both the glycine/NMDA receptor antagonists as well as the new memantine-like uncompetitive NMDA receptor antagonist MRZ 2/579, (1) diminish the intensity of the naloxone-precipitated morphine withdrawal, (2) facilitate the extinction of morphine dependence, (3) attenuate both the acquisition as well as the expression of morphine-induced conditioned place preference, and (4) do not influence the extinction of this phenomenon.

In mice, a prominent feature of naloxone-precipitated morphine withdrawal is jumping behavior (Saelens et al., 1971; Popik and Skolnick, 1996). The data from experiment 1 (Table 1) demonstrate that the intensity of morphine withdrawal in mice depends predominantly on the dose of morphine (since the dose of 5 mg/kg produced significantly less jumps on the test day) rather than the dose of naloxone used to precipitate withdrawal. We also did not find dramatic differences in the response to naloxone after longer or shorter periods of morphine injections, compared to the ‘standard’, 3-day procedure. However, the fact that, (1) mice

![Fig. 4](image)

**Fig. 4.** Concentration of MRZ 2/579 in the brain as measured by brain microdialysis based on in vitro recovery of 18%. MRZ 2/579 was injected i.p. at the dose of 5 mg/kg. Values are mean ± S.E., \( N = 5 \).
treated for just 1 day with morphine (as compared to mice injected for 3 or 6 days) did not differ from mice treated with a placebo, but (2) differed from mice treated with morphine for 3 days, suggests some time dependency in the development of morphine dependence.

Several earlier observations (Trujillo and Akil, 1991) demonstrate that co-administration of NMDA receptor antagonists with morphine prevents the development of opioid dependence. Several classes of NMDA antagonists have been shown before to inhibit the physical as well as motivational aspects of the expression of morphine dependence, most often precipitated by naloxone (Trujillo and Akil, 1991; Higgins et al., 1992; Popik et al., 1995). The present findings extend these observations to the glycine/NMDA antagonists (experiment 2). Another approach, perhaps more adequately modeling clinical use (Koyuncuoglu and Saydam, 1990; Bisaga et al., 1997), may involve the application of NMDA receptor antagonists in attenuating the maintenance of opioid dependence. We have shown previously (Popik and Skolnick, 1996) that both the low affinity, uncompetitive NMDA receptor antagonist, memantine and the competitive NMDA antagonist, NPC 17742 (2R,4R,5S-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid) diminish the maintenance of ongoing morphine dependence. The data obtained in experiment 3 extend these observations to the glycine/NMDA receptor antagonists, and show that similar effects can be demonstrated for another uncompetitive NMDA receptor antagonist, MRZ 2/579. It seems therefore that the activity of NMDA receptors is necessary for the maintenance of morphine dependence. Thus NMDA receptor antagonists active in this clinically-relevant model of opiate dependence may have beneficial effects on ongoing drug dependence in humans.

Consistent with previous studies (for review, see Goudie (1991)), the present experiments revealed that morphine produced clear-cut conditioned place preference, reflecting its reinforcing (rewarding) potential. Data from experiment 4 demonstrate that both glycine site (MRZ 2/570 and L-701,324) as well as the uncompetitive (MRZ 2/579) NMDA receptor antagonists inhibited the acquisition and expression of morphine-induced CPP. The inhibition of morphine-induced CPP was found previously for the uncompetitive NMDA antagonists memantine (Popik and Danysz, 1997) and (+)MK-801 (Tzschentke and Schmidt, 1995), the competitive NMDA antagonist, CGP-37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid), (Tzschentke and Schmidt, 1995), and for the non-selective glutamate antagonist, kynurenic acid (Bespalov et al., 1994). Data from this laboratory demonstrated that the inhibition of morphine-induced CPP by memantine were neither related to nonspecific effects on learning and memory processes, nor to alteration of the morphine interoceptive cue (Popik and Danysz, 1997). It remains to be established whether the effects of the presently tested compounds on the acquisition and expression of morphine-induced CPP were as specific as those produced by memantine. The glycine antagonist MRZ 2/570 does not inhibit radial maze learning at the doses of 5 and 10 mg/kg, but L-701,324 impaired reference memory at 5 mg/kg (Danysz et al., 1998). Nevertheless, it seems unlikely that the possible effects of these NMDA receptor antagonists on learning and memory processes contribute to their inhibitory effects on the expression of morphine-induced CPP because in general, NMDA receptor antagonists do not affect memory retrieval (Danysz et al., 1995b).

The data from experiment 6 demonstrates that repeated (six doses over a 3-day period) treatment with NMDA receptor antagonists does not influence the extinction of morphine-induced CPP. Morphine-induced CPP is a long-lasting phenomenon, since both the Mucha and Iversen (1984) findings as well as the data of experiment 5 (Table 2) demonstrate its presence for up to 1 month or 2 weeks, respectively. In addition, our data suggest for the first time that the secondary reinforcing effects of morphine may undergo the process of extinction and thus, resemble learning phenomena. Thus, rats exposed to the testing environment three times, preferred the morphine-associated chamber after the first and second exposure, but not on the third exposure (Table 2). The lack of activity of NMDA receptor antagonists in facilitating the extinction of morphine-induced CPP may be related to several reasons. For example, the duration of treatment was too short to be effective. However, if indeed, the maintenance of CPP is governed by the same rules as other learning phenomena, the ineffectiveness of NMDA receptor antagonists is not surprising, since NMDA receptor antagonists do not influence memories that are well established (Danysz et al., 1995b).

Microdialysis experiments indicate that MRZ 2/579 injected at the dose of 5 mg/kg reaches an ECF concentration of 0.8 μM which is very close to its IC50 value at NMDA receptors as revealed by patch clamp experiments on cortical neurons (Parsons et al., 1998). While these data are based on in vitro recovery, our recent experiments with memantine (Hesselink et al., 1997) indicate that the in vivo recovery value (using the mass transfer method) is almost identical (18 versus 17%). Unfortunately, we do not have results of microdialysis studies assessing the brain concentration of MRZ 2/570 at the doses used in the present study. However, recent data indicate that its close derivative MRZ 2/576 (having chloride instead of bromide in the position 8) injected i.p. at the dose of 10 mg/kg produces a peak brain concentration of 0.9 μM, which is close to
its IC$_{50}$ for the glycine/NMDA site (Hesselink et al., 1997; Parsons et al., 1997). Thus, it can be suggested that at the behaviorally effective doses, brain levels were achieved which are in a range expected to affect NMDA receptors.

Preclinical studies with NMDA receptor antagonists acting at the glycine site that do penetrate into the brain, demonstrate that these compounds may have more favorable therapeutic and side-effect profiles compared to high affinity, uncompetitive NMDA receptor antagonists (see Section 1). Therefore, this group of agents has been suggested to be potentially attractive for drug development. Apart from NMDA receptor antagonists acting at the glycine site originating from Merz, few other agents of this kind are still under development, e.g. GV150526A (phase III in stroke) and ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydroxiquinoline-2,3 dione, phase III in stroke) (Kulagowski, 1996).

In toto, NMDA receptor antagonists acting at the glycine modulatory site have similar inhibitory effects on morphine-induced, drug-seeking-related phenomena as the agents acting at other recognition sites on this receptor complex. Since NMDA receptor antagonists acting at the glycine site appear to have a better side-effect profile, they seem to be an attractive group for development of putative anti-abuse substances. Further studies are required to elucidate the possible mechanism of these ‘anti-addictive’ effects.

Acknowledgements

The authors would like to thank B. Eilbacher for the excellent analysis of microdialysis samples. This study was supported by KBN grant no. 4. P05A.116.10.

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