Psychopharmacology (2003) 165:245–251
DOI 10.1007/s00213-002-1287-8

ORIGINAL INVESTIGATION

Ewa Kozela · Andrzej Pilc · Piotr Popik

Inhibitory effects of MPEP, an mGluR5 antagonist, and memantine, an *N*-methyl-D-aspartate receptor antagonist, on morphine antinociceptive tolerance in mice

Received: 22 April 2002 / Accepted: 11 September 2002 / Published online: 20 November 2002

Abstract *Rationale:* Inhibition of *N*-methyl-D-aspartate (NMDA) receptors by memantine, an NMDA-receptor antagonist, and other antagonists of ionotropic receptors for glutamate inhibit the development of opiate antinociceptive tolerance. The role of metabotropic receptors for glutamate (mGluR) in opiate tolerance is less known. *Objective:* In the present study, we examined the effect of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), the mGluR type-I (subtype mGluR5) antagonist, as well as the effect of co-administration of low doses of memantine and MPEP on morphine antinociceptive tolerance in mice. *Methods:* Morphine antinociceptive activity was tested twice, before and after chronic morphine administration, in the tail-flick test using a cumulative dose–response protocol. Tolerance was induced by six consecutive days of b.i.d. administration of morphine (10 mg/kg, s.c.). Saline, memantine (7.5 mg/kg and 2.5 mg/kg, s.c.), MPEP (30 mg/kg and 10 mg/kg, i.p.) and the combination of both antagonists at low doses was given 30 min prior to each morphine injection during its chronic administration. A separate experiment assessed the effects of memantine, MPEP and their combination on acute morphine antinociception using a tail-flick test. *Results:* MPEP (30 mg/kg but not 10 mg/kg) as well as memantine (7.5 mg/kg but not 2.5 mg/kg) attenuated the development of tolerance to morphine-induced antinociception. When given together, the low doses of MPEP (10 mg/kg) and memantine (2.5 mg/kg) also significantly attenuated opiate tolerance. None of the treatments with glutamate antagonists produced antinociceptive effects or significantly affected morphine-induced antinociception. *Conclusions:* The data suggest that both mGluR5 and NMDA receptors may be involved in the development of morphine antinociceptive tolerance.

E. Kozela (✉) · A. Pilc · P. Popik
Institute of Pharmacology, Polish Academy of Sciences,
12 Smetna Street, 31-343 Kraków, Poland
e-mail: kozela@if-pan.krakow.pl
Tel.: +48-12-6623374
Fax: +48-12-6374500

Keywords Antinociception · Mice · Morphine · Tail-flick test · Tolerance

Introduction

Glutamatergic neurotransmission plays a critical role in the physiology and the pathology of various central nervous system functions, including opiate dependence and antinociceptive tolerance. Compounds decreasing the activity of glutamatergic neurotransmission inhibit these and other phenomena produced by addictive substances (Bisaga and Popik 2000). In most studies, the effects of antagonists of glutamate receptor function [particularly *N*-methyl-D-aspartate (NMDA) glutamate receptors] were investigated. Some clinically available NMDA receptor antagonists, such as dextromethorphan and memantine, were even proposed as therapeutics in the treatment of opioid dependence (Bisaga and Popik 2000).

The role of metabotropic glutamate receptors (mGluRs) in the modulation of opioid-induced behaviors has not been broadly investigated, perhaps due to the lack of selective ligands acting on well-defined groups of mGluRs (Schoepp and Conn 1993; Pin and Duvoisin 1995). The recent availability of subtype-selective ligands allows for the investigation of the role of mGluRs in brain physiology and pathology. For example, the agonist of presynaptically located mGluR II (mGluR2 and 3) produced anticonvulsant and anxiolytic effects (Monn et al. 1997), and prevented the development of morphine dependence (Klodzinska et al. 1999; Vandergriff and Rasmussen 1999) and tolerance (Popik et al. 2000b) in mice.

During the past few years, the role of mGluR I (mGluR1 and mGluR5) in opioid-related behaviors has generated great interest, due to pioneering studies by Fundytus and Coderre demonstrating the attenuation of morphine withdrawal symptoms in rats by the relatively nonselective mGluR antagonists of the group-I and -II mGluRs (Fundytus and Coderre 1994; Fundytus et al. 1997). Metabotropic GluRs of group I are coupled

positively to the phosphoinositide (PI) system, and inhibition of this signaling system [by inhibition of protein kinase C (PKC) and Ca^{++} release] during prolonged morphine administration also reduced morphine withdrawal symptoms (Fundytus and Coderre 1996).

NMDA and mGluR5 receptor antagonists share a variety of in vivo effects. For instance, NMDA receptor antagonists produce neuroprotective (Seif el Nasr et al. 1990), anxiolytic (Trullas et al. 1989), antidepressant (Paul et al. 1994; Skolnick et al. 1996) and antiparkinsonian (Schmidt and Bubser 1989) actions. Neuroprotective (Rao et al. 2000), anxiolytic and antidepressant (Spooren et al. 2000; Tatarczynska et al. 2001) and antiparkinsonian (Ossowska et al. 2001) effects were also demonstrated for 6-methyl-2-(phenylethynyl)-pyridine (MPEP) – a potent (IC_{50}=36 nM), selective and systemically active mGluR5 noncompetitive antagonist (Gasparini et al. 1999). We therefore hypothesized that MPEP may affect the development of morphine tolerance, as memantine, the relatively low-affinity NMDA receptor antagonist, inhibited the development of morphine antinociceptive tolerance in mice (Popik et al. 2000a).

In addition, we investigated whether MPEP may affect the inhibitory effects of memantine on the development of morphine tolerance. We hypothesized that the combined administration of these antagonists at low doses would significantly inhibit the development of opiate tolerance. Such a hypothesis appeared plausible in light of data indicating an interaction between mGluR5 and NMDA receptors in ex vivo studies (see Discussion). In addition, we examined the effects of MPEP and the combination of MPEP with memantine on acute morphine-induced antinociception in the tail-flick test in mice.

Materials and methods

Animals

Male C57/BL mice (Breeding Facility of University Children Hospital, Krakow, Poland) weighing approximately 25 g at the beginning of experiments were housed in standard plastic cages (43×27×15 cm) with sawdust bedding in the animal room under a controlled light/dark cycle (lights on 0700 hours; off 1900 hours) with food and tap water provided ad libitum. Each experimental group consisted of 7–16 mice per dose. All mice were used only once. All experiments were carried out according to the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (1996) and were approved by the internal Bioethics Commission.

Test of morphine antinociception

Apparatus

A standardized tail-flick apparatus (Columbus Instruments, Ohio, USA) with radiant heat source was used to assess antinociceptive response. The intensity of the stimulus was adjusted so that the baseline latency was approximately 3 s. A maximum latency of 10 s (i.e., cut-off) was used to minimize damage to the tail. The tail withdrawal latency was measured from the start of heat stimulus until the mouse flicked its tail. Each response assessment consisted of two separate measurements taken at different portions of the tail (spaced by 0.5–1 cm) and separated by 10 s. The mean of these responses was used for subsequent comparisons.

Assessment of antinociceptive ED_{50} of morphine – test no. 1 and test no. 2

Morphine antinociceptive potency was investigated with the use of cumulative dose–response curves; the cumulative dose–response protocol allows for reducing the number of animals required to assess the development of morphine tolerance (Paronis and Holtzman 1991). After 1-h adaptation and baseline trials, each mouse was injected s.c. with a low dose of morphine (1 mg/kg). Thirty minutes after administration of this dose, the mouse was retested and immediately injected with the next dose of morphine that was increased by quarter of a log unit. Thus, because the initial dose of morphine was 1.0 mg/kg, the next dose was 1.78 mg/kg, for a cumulative dose of 2.8 mg/kg. This procedure with subsequent doses of morphine (every half an hour increased by quarter of a log unit) continued until either the mouse did not move his 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. When either one of these criteria for test ending was met, additional doses of morphine were administered if necessary, so that each animal received the same total dose of morphine during a given test. The assessments of antinociceptive ED_{50} of morphine (test no. 1 and test no. 2) were carried out without pretreatment with glutamate antagonists.

Induction of morphine antinociceptive tolerance

Experiment 1 was performed to evaluate the ability of MPEP to affect the development of opiate antinociceptive tolerance. Memantine was used as a reference compound, reported previously to inhibit the development of morphine antinociceptive tolerance in mice (Popik et al. 2000a). The antinociceptive measurements were performed in experimental room using the cumulative morphine dose–response protocol (see above) on experimental day 1 (test no. 1) and day 8 (test no. 2). During the 6 days between test no. 1 and test no. 2 (days 2–7), a test compound (MPEP or memantine) was administered to mice twice daily, followed 30 min later by placebo or 10 mg/kg morphine. Experimental groups were as follows: (a) placebo and placebo (n=9), (b) placebo and morphine (n=9), (c) memantine (2.5 mg/kg) and morphine (n=9), (d) memantine (7.5 mg/kg) and morphine (n=10), (e) MPEP (10 mg/kg) and morphine (n=9) and (f) MPEP (30 mg/kg) and morphine (n=9). All drug injections were given in the animal room. Cumulative dose–response curves generated in test no. 1 and test no. 2 were used to assess morphine antinociceptive tolerance, which was defined by the shift to the right of the dose–response curve from test no. 1 to test no. 2.

Experiment 2 was carried out to investigate the interaction between NMDA and mGluR5 receptors in the development of morphine tolerance. During the 6 days between test no. 1 and test no. 2, mice were treated with the combination of MPEP and memantine (administered immediately after MPEP), and 30 min later with placebo or morphine (10 mg/kg). Experimental groups were as follows: (a) placebo immediately followed by placebo and 30 min later by placebo (n=7), (b) placebo followed by placebo and 30 min later by morphine (n=6), (c) MPEP (10 mg/kg) followed by memantine (2.5 mg/kg) and 30 min later by morphine (n=10) and (d) MPEP (10 mg/kg) followed by memantine (7.5 mg/kg) and 30 min later by morphine (n=10). Because the data of "placebo" and "morphine" controls in experiments 1 and 2 were indistinguishable, the results of respective controls were pooled to constitute a common "placebo" control group (n=15) and a common "morphine" control group (n=16).

The effect of GluR antagonists on acute morphine antinociception

Experiment 3 evaluated the effects of MPEP, memantine, or MPEP with memantine on acute antinociception produced by morphine. MPEP (30 mg/kg), memantine (7.5 mg/kg), MPEP (10 mg/kg) with memantine (2.5 mg/kg) or placebo were given 30 min prior to 3 mg/kg morphine or placebo injections. The tail-flick test started immediately before the first administration of a compound(s) (baseline measurement). The following measurements continued in 30-min intervals up to 120 min after morphine administration. The dose of morphine (3 mg/kg) was similar to its antinociceptive ED_{50} dose in our setting (data not shown). This allowed for detecting potential inhibition or potentiation of morphine antinociception. The number of animals in groups was 7, 8, 8, 10, 10, 10 and 10 mice for placebo and placebo, memantine (7.5 mg/kg) and placebo, MPEP (30 mg/kg) and placebo, placebo and morphine, memantine (7.5 mg/kg) and morphine, MPEP (30 mg/kg) and morphine, and memantine (2.5 mg/kg) with MPEP (10 mg/kg) and morphine, respectively.

All tests were carried out during the light phase between 0900 hours and 1600 hours.

Drugs

Morphine HCl (Polfa, Kraków, Poland) and memantine HCl (Merz and Co, Frankfurt/M., Germany, generous gift from Professor Wojciech Danysz) were dissolved in sterile physiological saline (placebo) and administered subcutaneously. Placebo was also administered subcutaneously. MPEP (6-methyl-2-(phenylethynyl)-pyridine, Novartis, Switzerland) was suspended in 1% Tween 80 (Sigma-Aldrich, Poznan, Poland) by homogenization, neutralized with 1 N NaOH to pH 6 and administered intraperitoneally. All drugs were administered in the volume of 10 ml/kg.

Data analysis

In experiments 1 and 2, latencies (in seconds) of the tail-flick responses were converted to maximum possible effects (%MPEs) according to the formula: 100 × [(post-injectory latency–baseline latency)/(cut-off latency–baseline latency)] (Paronis and Holtzman 1991). %MPE values were used to construct morphine cumulative dose–response curves by non-linear regression; these curves were used to calculate antinociceptive ED_{50} values using GraphPad Prism version 3.00 software (GraphPad Software, Calif., USA). The ED_{50} values obtained on test no. 1 and test no. 2 were compared among groups, as were the fold shifts (determined by dividing individual test no. 2 ED_{50} values by the test no. 1 ED_{50} values).

In experiment 3, in which acute morphine antinociception was tested, the effects of GluR antagonists were compared with the use of area under curve (AUC) assessments calculated on %MPEs on a series of measurements from 0 min to 120 min (30-min intervals) using the trapezoidal rule (Delta X × (Y1+Y2)/2).

Statistical analyses [STATISTICA version 5.0 (StatSoft, Okla., USA)] involved one-way, between-subjects ANOVA. In experiment 3, mixed-design ANOVAs were performed on raw data (%MPE values) and one-way ANOVAs were performed on the AUC data. Direct comparison of data was always carried out using the post-hoc Newman-Keul's test. $P<0.05$ was considered significant.

Results

Effects of MPEP and memantine on morphine antinociceptive tolerance in mice (experiments 1 and 2)

As determined with one-way ANOVA, there were no differences in antinociceptive morphine ED_{50} values noted on test no. 1 among groups ($F_{7,80}=0.75$, $P>0.05$, Table 1). One-way ANOVAs performed on test no. 2 ED_{50} values and test no. 2/test no. 1 fold changes demonstrated significant differences among groups ($F_{7,80}=4.47$, $P<0.001$ and $F_{7,80}=4.57$, $P<0.001$, respectively). Mice treated with placebo + placebo between test no. 1 and test no. 2 demonstrated no change in antinociceptive morphine ED_{50} values (the resulting fold change of morphine antinociceptive potency was 1.07). This differed markedly from the treatment with morphine (10 mg/kg, b.i.d., 6 days) that produced a significant 2.59-fold decrease in the morphine antinociceptive potency.

In experiment 1, memantine pretreatment (7.5 mg/kg but not 2.5 mg/kg) inhibited the development of morphine-induced antinociceptive tolerance (fold changes in morphine potency 1.07 and 1.68, respectively). Similarly, pretreatment with MPEP (30 mg/kg but not 10 mg/kg) prevented the development of morphine tolerance (fold changes 1.04 and 1.92, respectively).

In experiment 2, the combination of low dose of MPEP (10 mg/kg) and memantine (2.5 mg/kg) administered

Table 1 Effects of 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and memantine on the development of tolerance to antinociceptive effects of morphine. Presented are mean±SEM antinociceptive morphine ED_{50} values (mg/kg) determined in test no. 1 and test no. 2 and resulting fold shifts of morphine antinociceptive potency (test no. 2/test no. 1). Morphine tolerance was induced by morphine administration (10 mg/kg) twice daily during 6 days between tests no. 1 and no. 2. Placebo, MPEP and memantine or combination of both drugs was administered 30 min prior to each of the morphine injections. *Asterisks* indicate a statistically significant difference toward "morphine control" group that received placebo + morphine during the development of morphine tolerance

Treatment	Test no. 1 ED_{50} (mg/kg)	Test no. 2 ED_{50} (mg/kg)	Test no. 2/test no. 1 fold change
Placebo + morphine	2.94±0.31	6.45±0.78	2.59±0.44
Placebo + placebo	3.86±0.56	3.65±0.58*	1.07±0.19**
MPEP 10 mg/kg + morphine	3.58±0.43	6.13±0.96	1.92±0.44
MPEP 30 mg/kg + morphine	2.98±0.25	3.15±0.5*	1.04±0.14**
Memantine 2.5 mg/kg + morphine	2.82±0.43	4.19±0.97	1.68±0.31
Memantine 7.5 mg/kg + morphine	3.47±0.59	3.14±0.41*	1.07±0.17**
Memantine 2.5 mg/kg, MPEP 10 mg/kg + morphine	3.66±0.58	3.24±0.44*	0.98±0.13**
Memantine 7.5 mg/kg, MPEP 10 mg/kg + morphine	3.09±0.36	2.79±0.60*	0.92±0.19**

*$P<0.05$
**$P<0.01$

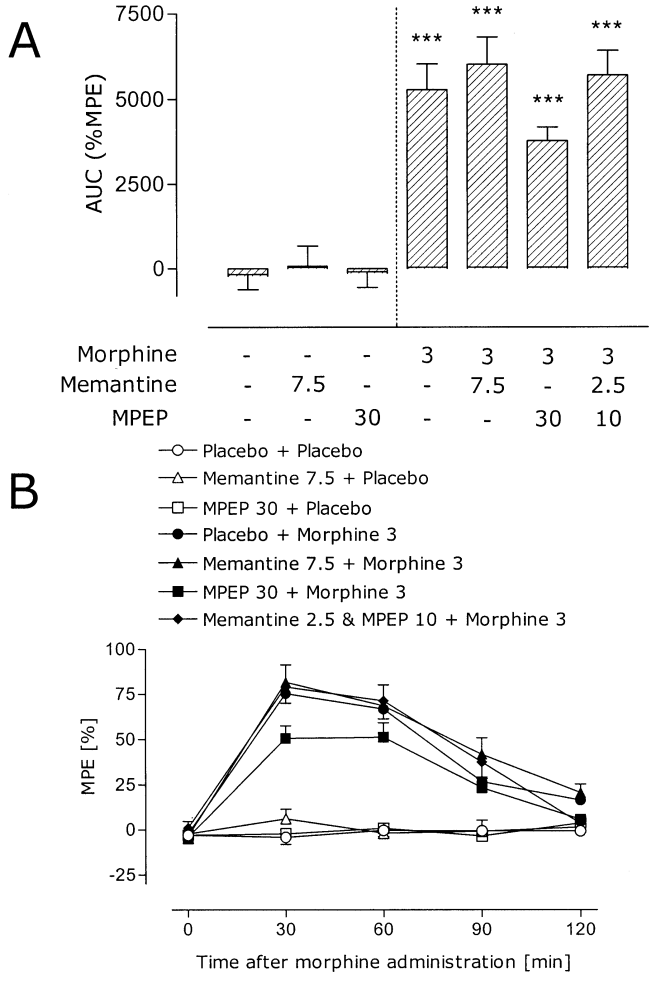

Fig. 1A, B Effects of 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and memantine on morphine-induced antinociception in the tail-flick test in mice. **A** Means+SEM for the area under curve (AUC) calculated from raw data expressed as maximal possible effect (%MPE) presented in **B**. ***P<0.001 vs placebo+placebo group

30 min before each dose of morphine was effective in preventing morphine antinociceptive tolerance (fold change 0.98).

The effects of MPEP and memantine on morphine-induced acute antinociception in tail-flick test in mice (experiment 3)

As shown in Fig. 1, neither memantine (7.5 mg/kg) nor MPEP (30 mg/kg) produced antinociceptive activity in the tail-flick test. Morphine (3 mg/kg)-induced antinociception was not changed by memantine (7.5 mg/kg), the combination of memantine (2.5 mg/kg) with MPEP (10 mg/kg) or MPEP (30 mg/kg) administration.

AUC data were analyzed with three separate one-way ANOVAs. ANOVA followed by post-hoc Newman-Keul's test performed on all data revealed that all morphine-treated groups differed significantly from groups not treated with morphine ($F_{6,56}$=19.96, P<0.001). However, ANOVAs performed on data of non-morphine (Fig. 1A, left three bars) and morphine-treated groups (Fig. 1A, right four bars) demonstrated no differences among treatments ($F_{2,20}$=0.10, P>0.05 and $F_{3,36}$=2.10, P>0.05, respectively).

Two-way, mixed-design ANOVAs on %MPE values with treatment as between-subject factor and time as a within-subject factor were performed separately for groups of mice treated and untreated with morphine during the test. Among morphine-untreated groups, ANOVA demonstrated no treatment ($F_{2,20}$=0.08, P>0.05) or time ($F_{4,80}$=0.70, P>0.05) effects; the interaction ($F_{8,80}$=0.76) was also not significant. In morphine-treated groups, there was a significant effect of time ($F_{3,108}$=86.22, P<0.001), no differences among treatments ($F_{3,36}$=2.10, P>0.05) and no significant interaction ($F_{9,108}$=1.16, P>0.05).

Discussion

The present study demonstrates that MPEP, the mGluR5 antagonist, attenuates the development of morphine-induced antinociceptive tolerance in mice. Results of experiment 1 demonstrate that the magnitude of inhibition of morphine tolerance by MPEP is similar to that produced by memantine (Table 1). The inhibitory effects of memantine on morphine tolerance were reported previously (Popik et al. 2000a) and will not be discussed here in detail. The joint administration of low doses of MPEP and memantine inhibited the development of morphine tolerance but did not affect acute morphine antinociception. At the highest dose (30 mg/kg), MPEP nonsignificantly diminished morphine-induced antinociception.

There are a number of hypotheses that could explain the inhibitory effect of MPEP on antinociceptive morphine tolerance. Activation of metabotropic receptors of group I (including mGluR5) produce PI hydrolysis, a metabolic process leading to mobilization of intracellular Ca^{++} and activation of PKC (Bordi and Ugolini 1999). Both PKC and Ca^{++} play crucial roles in opioid tolerance. PKC immunoreactivity was increased in dorsal horn neurons (Mao et al. 1995) and spinal and brain levels of Ca^{++} in synaptosomes were increased in morphine-tolerant mice (Welch and Bass 1995). Intracellular Ca^{++} initiates a number of second messenger-mediated intracellular signal transduction cascades (also by PKC activation) leading to, among other events, phosphorylation and consequent desensitization of mu-opioid receptors (Mestek et al. 1995; Wang et al. 1996). Moreover, inhibitors of PKC activation prevented increase in PKC immunoreactivity in dorsal horn neurons of morphine-tolerant rats and attenuated the development of tolerance to the antinociceptive effects of morphine (Mayer et al. 1995). All of these intracellular events provide room for MPEP, the mGluR5 antagonist that inhibits the PI pathway, to affect antinociceptive morphine tolerance.

Despite the fact that the sites that bind MPEP are located postsynaptically, this compound has recently been reported to inhibit glutamate release (Thomas et al. 2001) through presynaptically located mGluR5 receptors (Thomas et al. 2000). This mimics the function of group-II mGluRs, which are predominantly located presynaptically, and thus modulate glutamate release (Schoepp 2001). As we have shown previously, the agonist of group-II mGluRs (LY354740) inhibited the development of morphine antinociceptive tolerance (Popik et al. 2000b), which implicates a decrease in glutamate release in the inhibition of morphine tolerance.

Results of experiment 2 (Table 1) demonstrated that given together at low doses, MPEP and memantine inhibit the development of antinociceptive morphine tolerance. To our knowledge, the interaction between mGluR5 and NMDA receptor antagonists has not been described in the literature. Since the inhibitory role of NMDA receptor antagonists on opioid tolerance is well documented (Mao 1999), the possibility exists that MPEP may inhibit NMDA receptors directly. However, MPEP affects NMDA receptors at concentrations higher than 100 μM (Oleary et al. 2000; Gubellini et al. 2001; Spooren et al. 2001) but is able to inhibit mGluR5s with a much higher affinity (36 nM) (Gasparini et al. 1999), whereas the affinity of memantine to the NMDA channel is approximately 1 μM (Parsons et al. 1999). Thus, although in our experiment MPEP and memantine were given together at doses of 10 mg/kg and 2.5 mg/kg, respectively, the comparison of affinities of these compounds at the NMDA receptor suggests that a direct action of MPEP at NMDA receptors is unlikely.

MGluR5 antagonists reduced NMDA receptor activity in several brain areas (Doherty et al. 1997; Anwyl 1999; Awad et al. 2000; Attucci et al. 2001; Pisani et al. 2001). These data suggest functional interaction between these receptors, most likely mediated by PKC activation. The earliest direct evidence for such functional interaction was provided by Chen and Huang (1992) who demonstrated that intracellularly applied PKC potentiates NMDA-evoked responses in trigeminal neurons by increasing the probability of channel openings and by reducing the voltage-dependent Mg^{++} block of NMDA-receptor channels. At the behavioral level, these hypotheses should also be tested using behavioral measures other than the inhibition of morphine tolerance. This is due to the fact that, in tolerance studies, there is normally a low "ceiling" to "top" ratio as well as substantial variability that would prevent studying the precise nature of the interaction between NMDA and mGluR5 antagonists.

Previous reports indicate that neither NMDA (Olivar and Laird 1999) nor mGlu5 receptor antagonists (Walker et al. 2001) affect responses to acute noxious stimulations, although MPEP reduced inflammatory hyperalgesia in animal studies (Walker et al. 2001). Results of the present study confirm that neither memantine nor MPEP affected tail-flick response in otherwise drug-free mice (Fig. 1).

A separate issue studied in experiment 3 was the possibility that the inhibition of morphine antinociceptive tolerance by the GluR antagonists was due to an inhibition of opioid receptors. Such inhibition could diminish morphine antinociceptive activity, and via this mechanism inhibit the development of morphine tolerance. To consider this possibility we characterized the effects of memantine and MPEP (or their combination) on morphine-induced antinociception. In the case of memantine, we did not observe any modulation of morphine activity in the tail-flick test, but MPEP pretreatment diminished morphine antinociceptive action (Fig. 1). However, despite the use of various statistical approaches, this effect did not reach statistical significance. The lack of effect of memantine on acute morphine antinociception in the current study is in contrast with our previous results reported for Albino Swiss mice, where an inhibition of morphine antinociceptive effect was observed in the tail-flick test (Popik et al. 2000a). However, we also reported that, in Wistar rats, memantine potentiated morphine-induced antinociception recorded from the tail but did not affect morphine antinociception recorded from the paw (Kozela et al. 2001). Thus, the effects of memantine pretreatment on acute morphine antinociception appear to depend on the species and the strain of animals as well as the nociceptive test (Kozela and Popik 2002; Kozela et al. 2001). Nonetheless, memantine has repeatedly been reported to inhibit the development of morphine antinociceptive tolerance in various strains of mice (Belozertseva and Bespalov 1998; Popik et al. 2000a, 2000b). This suggests that the inhibitory effects of memantine and NMDA receptor antagonists in general on the development of morphine antinociceptive tolerance are independent of their acute effects on morphine antinociception (Mao 1999; Kozela and Popik 2002). To resolve whether this is also the case for mGluR5 antagonists, further studies with the use of other strains of mice and/or rats and other nociceptive tests are necessary.

Acknowledgements Authors want to acknowledge the supply of MPEP from Dr Rainer Kuhn, Pharma Novartis, Basel, Switzerland. This study was supported by KBN grant 4 P05A 025 19 to EK.

References

Anwyl R (1999) Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. Brain Res Brain Res Rev 29:83–120

Attucci S, Carla V, Mannaioni G, Moroni F (2001) Activation of type 5 metabotropic glutamate receptors enhances NMDA responses in mice cortical wedges. Br J Pharmacol 132:799–806

Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. J Neurosci 20:7871–7879

Belozertseva IV, Bespalov 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 Schmiedebergs Arch Pharmacol 358:270–274

Bisaga A, Popik P (2000) In search of a new pharmacological treatment of drug addiction: *N*-methyl-D-aspartate (NMDA) antagonists. Drug Alcohol Depend. 59:1–15

Bordi F, Ugolini A (1999) Group I metabotropic glutamate receptors: implications for brain diseases. Prog Neurobiol 59:55–79

Chen L, Huang LY (1992) Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation. Nature 356:521–523

Doherty AJ, Palmer MJ, Henley JM, Collingridge GL, Jane DE (1997) (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) activates mGlu5, but no mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. Neuropharmacology 36:265–267

Fundytus ME, Coderre TJ (1994) Effect of activity at metabotropic, as well as ionotropic (NMDA), glutamate receptors on morphine dependence. Br J Pharmacol 113:1215–1220

Fundytus ME, Coderre TJ (1996) Chronic inhibition of intracellular Ca^{2+} release or protein kinase C activation significantly reduces the development of morphine dependence. Eur J Pharmacol 300:173–181

Fundytus ME, Ritchie J, Coderre TJ (1997) Attenuation of morphine withdrawal symptoms by subtype- selective metabotropic glutamate receptor antagonists. Br J Pharmacol 120:1015–1020

Gasparini F, Lingenhohl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, Varney MA, Johnson EC, Hess SD, Rao SP, Sacaan AI, Santori EM, Velicelebi G, Kuhn R (1999) 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 38:1493–1503

Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, Conquet F, Calabresi P (2001) Selective involvement of mGlu1 receptors in corticostriatal LTD. Neuropharmacology 40:839–846

Klodzinska A, Chojnacka-Wojcik E, Palucha A, Branski P, Popik P, Pilc A (1999) Potential anti-anxiety and anti-addictive effects of LY 354740, a selective group II glutamate metabotropic receptors agonist in animal models. Neuropharmacology 38:1831–1839

Kozela E, Popik P (2002) The effects of NMDA receptor antagonists on acute morphine-antinociception in mice. Amino Acids 23:163–168

Kozela E, Danysz W, Popik P (2001) Uncompetitive NMDA receptor antagonists potentiate morphine antinociception recorded from the tail but not from the hind paw in rats. Eur J Pharmacol 423:17–26

Mao J (1999) NMDA and opioid receptors: their interactions in antinociception, tolerance and neuroplasticity. Brain Res Brain Res Rev 30:289–304

Mao J, Price DD, Phillips LL, Lu J, Mayer DJ (1995) Increases in protein kinase C gamma immunoreactivity in the spinal cord of rats associated with tolerance to the analgesic effects of morphine. Brain Res 677:257–267

Mayer DJ, Mao J, Price DD (1995) The development of morphine tolerance and dependence is associated with translocation of protein kinase C. Pain 61:365–374

Mestek A, Hurley JH, Bye LS, Campbell AD, Chen Y, Tian M, Liu J, Schulman H, Yu L (1995) The human mu opioid receptor: modulation of functional desensitization by calcium/calmodulin-dependent protein kinase and protein kinase C. J Neurosci 15:2396–2406

Monn JA, Valli MJ, Massey SM, Wright RA, Salhoff CR, Johnson BG, Howe T, Alt CA, Rhodes GA, Robey RL, Griffey KR, Tizzano JP, Kallman MJ, Helton DR, Schoepp DD (1997) Design, synthesis, and pharmacological characterization of (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY 354740): a potent, selective, and orally active group 2 metabotropic glutamate receptor agonist possessing anticonvulsant and anxiolytic properties. J Med Chem 40:528–537

Oleary DM, Movsesyan V, Vicini S, Faden AI (2000) Selective mGluR5 antagonists MPEP and SIB-1893 decrease NMDA or glutamate-mediated neuronal toxicity through actions that reflect NMDA receptor antagonism. Brit J Pharmacol 131:1429–1437

Olivar T, Laird JM (1999) Differential effects of *N*-methyl-D-aspartate receptor blockade on nociceptive somatic and visceral reflexes. Pain 79:67–73

Ossowska K, Konieczny J, Wolfarth S, Wieronska J, Pilc A (2001) Blockade of the metabotropic glutamate receptor subtype 5 (mGluR5) produces antiparkinsonian-like effects in rats. Neuropharmacology 41:413–420

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 (1999) Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist – a review of preclinical data. Neuropharmacology 38:735–767

Paul IA, Nowak G, Layer RT, Popik P, Skolnick P (1994) Adaptation of the *N*-methyl-D-aspartate receptor complex following chronic antidepressant treatments. J Pharmacol Exp Ther 269:95–102

Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34:1–26

Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P (2001) Metabotropic glutamate receptor 5 mediates the potentiation of *N*-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience 106:579–587

Popik P, Kozela E, Danysz W (2000a) Clinically available NMDA receptor antagonists memantine and dextromethorphan reverse existing tolerance to the antinociceptive effects of morphine in mice. Naunyn Schmiedebergs Arch Pharmacol 361:425–432

Popik P, Kozela E, Pilc A (2000b) Selective agonist of group II glutamate metabotropic receptors, LY354740, inhibits tolerance to analgesic effects of morphine in mice. Br J Pharmacol 130:1425–1431

Rao AM, Hatcher JF, Dempsey RJ (2000) Neuroprotection by group I metabotropic glutamate receptor antagonists in forebrain ischemia of gerbil. Neurosci Lett 293:1–4

Schmidt WJ, Bubser M (1989) Anticataleptic effects of the *N*-methyl-D-aspartate antagonist MK-801 in rats. Pharmacol Biochem Behav 32:621–623

Schoepp DD (2001) Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther 299:12–20

Schoepp DD, Conn PJ (1993) Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol Sci 14:13–20

Seif el Nasr M, Peruche B, Rossberg C, Mennel HD, Krieglstein J (1990) Neuroprotective effect of memantine demonstrated in vivo and in vitro. Eur J Pharmacol 185:19–24

Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R (1996) Adaptation of the *N*-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. Pharmacopsychiatry 29:23–26

Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S, Porsolt RD, Gentsch C (2000) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. J Pharmacol Exp Ther 295:1267–1275

Spooren WP, Gasparini F, Salt TE, Kuhn R (2001) Novel allosteric antagonists shed light on mGlu(5) receptors and CNS disorders. Trends Pharmacol Sci 22:331–337

Tatarczynska E, Klodzinska A, Chojnacka-Wojcik E, Palucha A, Gasparini F, Kuhn R, Pilc A (2001) Potential anxiolytic- and antidepressant-like effects of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist. Br J Pharmacol 132:1423–1430

Thomas LS, Jane DE, Harris JR, Croucher MJ (2000) Metabotropic glutamate autoreceptors of the mGlu(5) subtype positively

modulate neuronal glutamate release in the rat forebrain in vitro. Neuropharmacology 39:1554–1566

Thomas LS, Jane DE, Gasparini F, Croucher MJ (2001) Glutamate release inhibiting properties of the novel mGlu(5) receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP): complementary in vitro and in vivo evidence. Neuropharmacology 41:523–527

Trullas R, Jackson B, Skolnick P (1989) Anxiolytic properties of 1-aminocyclopropanecarboxylic acid, a ligand at strychnine-insensitive glycine receptors. Pharmacol Biochem Behav 34:313–316

Vandergriff J, Rasmussen K (1999) The selective mGlu2/3 receptor agonist LY354740 attenuates morphine-withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. Neuropharmacology 38:217–222

Walker K, Bowes M, Panesar M, Davis A, Gentry C, Kesingland A, Gasparini F, Spooren W, Stoehr N, Pagano A, Flor PJ, Vranesic I, Lingenhoehl K, Johnson EC, Varney M, Urban L, Kuhn R (2001) Metabotropic glutamate receptor subtype 5 (MGlu5) and nociceptive function I. Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain. Neuropharmacology 40:1–9

Wang ZJ, Arden J, Sadee W (1996) Basal phosphorylation of mu opioid receptor is agonist modulated and Ca2+-dependent. FEBS Lett 387:53–57

Welch SP, Bass PP (1995) Modulation of free intracellular calcium levels [(Ca++)i] in brain and spinal cord of morphine tolerant rats and mice. Pharmacol Biochem Behav 51:57–63