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Selective agonist of group II glutamate metabotropic receptors, LY354740, inhibits tolerance to analgesic effects of morphine in mice

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1 Antagonists of glutamate *N*-methyl-D-aspartate (NMDA) subtype receptor inhibit the development of tolerance to the antinociceptive effects of opioids. Another way to inhibit the function of glutamate receptors is the stimulation of presynaptic metabotropic group II (mGluRII) receptors. Because LY354740 ((+)-2-aminobicyclo [3,1,0] hexane-2,6-dicarboxylic acid) is the first systemically active agonist of group II mGlu receptors, we investigated if this compound might inhibit the development of tolerance to antinociceptive effects of morphine and fentanyl.

2 As assessed by cumulative dose-response approach in the tail-flick test, administration of 10 mg kg^{-1} morphine bid s.c. to male Albino Swiss mice for 6 days, right-shifted morphine dose-response curve by ~4 fold. In a separate group of mice, 12 injections of 0.04 mg kg^{-1} of fentanyl over 3 days, right-shifted fentanyl dose-response curve by ~3.3 fold.

3 In experiment 1, LY354740 (1 and 10, but not 0.1 mg kg^{-1}) as well as the reference compound, an uncompetitive NMDA receptor antagonist memantine (7.5 mg kg^{-1}) inhibited the development of morphine tolerance. Neither LY354740 (10 mg kg^{-1}) nor memantine (7.5 mg kg^{-1}) affected the development of tolerance to fentanyl. In experiment 2, neither LY354740 (1 and 10 mg kg^{-1}) nor memantine (7.5 mg kg^{-1}) affected the tail-flick antinociceptive response, or the acute antinociceptive effect of morphine.

4 The present results are the first to suggest that the development of antinociceptive morphine tolerance may be inhibited by metabotropic group II glutamate agonist.

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Abbreviations: ACEA-1328, 5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione; CGP 39551, DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic carboxyethylester; LY235959, (−)-3-SR,4a-RS,8a-SR-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid; LY274614, (±)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid; LY354740, (+)-2-aminobicyclo [3,1,0] hexane-2,6-dicarboxylic acid; mGluRII, metabotropic group II receptors; (+)MK-801, dizocilpine, (+)-5-methyl-10,11-dihydro-5H-dibenz(a,d)cycloheptene-5,10-imine hydrogen maleate; MPE, Maximum Percent Effect; NMDA, *N*-methyl-D-aspartate

Introduction

Glutamate, the major excitatory neurotransmitter of the brain, stimulates both ionotropic and metabotropic glutamate receptors (Monaghan *et al*., 1989; Conn & Pin, 1997), and plays a significant role both in physiology and pathophysiology of the central nervous system (Danysz *et al*., 1995a). Converging lines of evidence (reviewed by Bisaga & Popik, 2000) indicate substantial involvement of glutamate receptors in phenomena related to opioid tolerance and dependence. For example, antagonists of the *N*-methyl-D-aspartate (NMDA) receptor complex attenuate the development (Trujillo & Akil, 1991; Marek *et al*., 1991), expression (Cappendijk *et al*., 1993) and maintenance (Popik & Skolnick, 1996) of the ongoing morphine dependence. Recent work demonstrates similar effects of the agonist of metabotropic group II receptors (mGluRII), LY354740, that inhibits the expression of morphine dependence in mice (Klodzinska *et al*., 1999) and rats (Vandergriff & Rasmussen, 1999).

A phenomenon related to drug dependence is drug tolerance. Tolerance to the antinociceptive effects of μ-agonist morphine, the most widely used opioid analgesic, greatly limits its therapeutic efficacy and thus complicates the management of patients with chronic pain. 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 when a constant dose is repetitively administered (Foley, 1991). In laboratory animals, antinociceptive (analgesic) tolerance is usually assessed as a decrease in response in the presence of a constant nociceptive stimulus (Cochin & Kornetsky, 1964). The inhibition of glutamatergic transmission by means of the use of NMDA receptor antagonists is known to inhibit the development of morphine tolerance. 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 & Bespalov, 1998), memantine (Belozertseva & Bespalov, 1998; Popik & Kozela, 1999; Popik *et al*., 2000), dextromethorphan (Elliott *et al*., 1994b; Popik *et al*., 2000) and ketamine (Gonzalez *et al*., 1997), the glycine/NMDA receptor antagonist ACEA-1328 (Lutfy *et al*., 1995) as well as the competitive NMDA receptor antagonists, LY274614 (Elliott *et al*., 1994a), LY235959 (Bilsky *et al*., 1996) and CGP 39551 (Gonzalez *et al*., 1997). However, the effects of mGluRII agonists on morphine tolerance have not been investigated, perhaps due to the lack of selective pharmacological tools, which would penetrate into the brain.

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Metabotropic glutamate receptor subtypes (1–8) are classed in three groups based on sequence homology, signal transduction mechanism and pharmacology. The discovery of (+)-2-aminobicyclo [3,1,0] hexane-2,6-dicarboxylic acid (LY354740), which selectively and potently (EC_{50} 10–50 nM) stimulates human recombinant and native rat mGlu group II receptors, and is the first agonist of this class of receptors that penetrates into the brain (Monn *et al*., 1997; Bond *et al*., 1997), has offered a valuable tool to study its central effects following systemic administration to animals. The behavioural effects of LY354740 are characterized to some extent. This compound produces anxiolytic effects in the fear potentiated startle and elevated plus maze models of anxiety (Helton *et al*., 1998) and at doses of 0.5–1 mg kg^{-1} appears to produce anxiolytic effects in the Vogel test (Klodzinska *et al*., 1999). Similar anxiolytic effects (4–8 mg kg^{-1}) were shown in a four-plate test (Klodzinska *et al*., 1999). Administration of LY354740 at the dose of 8 mg kg^{-1} produced 55% reduction in the spontaneous locomotor activity in mice but did not disturb the locomotor coordination on the rota-rod test (Helton *et al*., 1998; Klodzinska *et al*., 1999). LY354740 did not show any 'antidepressive' effects in rodents, but at doses of 0.15–5 mg kg^{-1} inhibited naloxone-precipitated morphine withdrawal in morphine-dependent mice (Klodzinska *et al*., 1999).

The present experiments were carried out in order to investigate whether LY354740 may influence the development of morphine tolerance. Because of the report demonstrating that NMDA receptor antagonists inhibit antinociceptive tolerance produced by morphine but not selective μ- or δ-opioid agonists (Bilsky *et al*., 1996), the effects of LY354740 and memantine on antinociceptive tolerance produced by a selective μ-receptor agonist, fentanyl, were also studied.

Methods

Subjects

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 the standard laboratory cages and kept in a temperature-controlled colony room ($21 \pm 2^{\circ}C$) with a 12-h light/dark cycle (light on: 0700–1900 h). Commercial food and tap water were available *ad libitum*. Each experimental group consisted of 8–20 mice per dose. All mice were used only once.

Analgesia apparatus and testing

A standardized tail-flick apparatus (Analgesia Meter, Innovators in Instrumentation, NJ, U.S.A., model 33), set with adjusted sensitivity at six and break point at 84–85 with radiant heat source and connected to an automatic timer was used to assess antinociceptive response. The intensity of the heat stimulus was adjusted so that the baseline tail-flick 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 & Holtzman, 1991).

Morphine or fentanyl cumulative dose-response curves were used to reduce the number of animals required to assess the development of morphine tolerance (Paronis & Holtzman, 1991). After adaptation and baseline trials, each mouse was injected s.c. with a low dose of morphine (1 mg kg^{-1}). Thirty minutes later, the mouse was retested and injected with the next dose of morphine, such that the cumulative dose was increased by 0.25 log unit. Thus, because the initial dose of morphine was 1.0 mg kg^{-1}, the next dose was 1.78 mg kg^{-1}, for a cumulative dose of 2.8 mg kg^{-1}. This procedure continued until either the mouse did not move his tail within the cut-off time or until there was a plateauing of the dose-response curve, so that the latency did not increase from one dose to the next. Each analgesic responder was not subjected to further tail flick assessments but was injected with the subsequent dose of morphine so that each animal received the same total dose of morphine during a given test.

The construction of cumulative fentanyl dose-response curves was carried out similarly to the measurement of morphine antinociceptive effects with the exception that the doses of fentanyl started with 0.0022 mg kg^{-1}, increased by 0.5 log unit, and were performed in 15 min intervals (Paronis & Holtzman, 1991).

Comparison of morphine and fentanyl cumulative dose-response curves obtained on days 1 and 8 as well as days 1 and 5, respectively, were used to assess the extent of morphine tolerance.

Design of the experiments

Experiment 1 was designed to determine if LY354740 and memantine could inhibit the development of tolerance to the antinociceptive effects of morphine and fentanyl. On day 1, the first antinociceptive measurement was performed, followed by 6 days of twice daily morphine injections (10 mg kg^{-1}, s.c.) (Elliott *et al*., 1994a). The pretreatment with LY354740 (0.1, 1 or 10 mg kg^{-1}) and memantine (7.5 mg kg^{-1}) was given 30 min prior to each morphine dose on days 2–7 of this experiment.

Because of the report demonstrating that NMDA receptor antagonists inhibit antinociceptive tolerance produced by morphine but not selective μ- or δ-opioid agonists (Bilsky *et al*., 1996), in the parallel experiment, we investigated the effects of LY354740 (10 mg kg^{-1}) and memantine (7.5 mg kg^{-1}) pretreatment on antinociceptive tolerance produced by μ-agonist, fentanyl. The dose of 0.04 mg kg^{-1} of fentanyl was selected based on the preliminary experiment, which demonstrated that in our experimental setup fentanyl ED_{50} dose is ~0.02 mg kg^{-1}. The pretreatment with LY354740 and memantine was given 30 min before each of the fentanyl injections on days 2–4 of this experiment. Mice were treated four times per day, between 0800 and 2000 h, in 4 h intervals, because previous studies demonstrated that fentanyl has a shorter duration of action and more rapid elimination kinetics than morphine (Bilsky *et al*., 1996; Walker *et al*., 1997). As suggested (Walker *et al*., 1997), in order to keep the number of injections equal to the number of morphine injections, only 3 days of fentanyl treatment were given (i.e., 12 injections).

Experiment 2 was designed to determine whether LY354740 and memantine might produce their own antinociceptive effects and/or, change the antinociceptive effects of morphine. Morphine (5 mg kg^{-1}, s.c.) was administered 30 min after injection of LY354740 (1 or 10 mg kg^{-1}), memantine (7.5 mg kg^{-1}), or placebo.

Data presentation and statistics

Latencies (in sec) of the tail-flick responses were converted to MPEs [Maximum Percent Effects (Paronis & Holtzman, 1991)], according to the formula: 100 × [(post-injectory latency – baseline latency)/(cut-off latency – baseline latency)].

MPE values were used to construct morphine and fentanyl cumulative dose-response curves by non-linear regression; these curves were used to calculate antinociceptive ED_{50} values using GraphPad Prism ver. 3.00 (GraphPad Software, CA, U.S.A.) software. The ED_{50} values obtained on test #1 were compared among groups, as were the fold shifts (determined by dividing individual test #2 ED_{50} values by the test #1 ED_{50} values).

In the experiment 2, effects of compounds on tail flick responses were compared with the use of area under curve (AUC) assessments, which were calculated using trapezoid rule (Delta X * (Y1 + Y2)/2) on a series of measurements from 0–120 min. Statistical analyses involved one way between subjects ANOVA followed by Newman-Keul's test. $P<0.05$ was considered significant.

Drugs

Morphine HCl, fentanyl citrate (Polfa, Kraków, Poland) and memantine (generous gift of Dr W. Danysz, MERZ and Co, Frankfurt/M, Germany) were dissolved in sterile physiological saline. (+)-2-Aminobicyclo-[3,1]hexane-2,6-dicarboxylic acid (LY354740, kindly donated by Dr D.D. Schoepp, Eli-Lilly, Indianapolis IN, U.S.A.), was suspended in a 1% aqueous solution of Tween 80. Preliminary experiments demonstrated that mice administered with vehicles for memantine and LY354740 did not differ in terms of tail-flick response to the nociceptive stimulus, nor that these vehicles affect the antinociceptive response to morphine. Therefore, data obtained from these control groups were pooled and termed 'Placebo'. All injections were given s.c.

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 Institute of Pharmacology, Polish Academy of Sciences in Kraków Animal Care and Use Bioethics Commission.

Results

Effects of LY354740 and memantine on the development of tolerance to morphine and fentanyl (experiment 1)

As determined by one way ANOVA, there were no differences among groups in antinociceptive morphine ED_{50} values determined on test #1 $F(5,68)=0.696$, $P>0.05$, Table 1. Treatment with 10 mg kg^{-1} bid of morphine produced robust (4.18 fold) increase in the ED_{50} values as determined on test #2. In contrast, in mice that never received repeated morphine administration, but were injected with morphine only during tests #1 and #2 (placebo + placebo group), the fold shift was only 1.29. Pretreatment with LY354740 (1 and 10 mg kg^{-1} [but not 0.1 mg kg^{-1}]), given prior to each dose of morphine dose-dependently prevented the development of morphine tolerance. A similar inhibitory effect on morphine tolerance was observed in mice pretreated with memantine (7.5 mg kg^{-1}). This is evidenced by significant decrease in test #2/test #1 fold shifts of respective groups as compared toward control group treated chronically with morphine (Table 1).

One way ANOVA revealed no differences in antinociceptive fentanyl ED_{50} values on the test #1 among groups $F(3,35)=1.133$, $P>0.05$, Table 2. The treatment with fentanyl (0.04 mg kg^{-1} s.c. qid over 3 days) produced significant, 3.30 fold increase in the ED_{50} values as determined on test #2. In contrast, in mice that were treated with placebo between test #1 and test #2, the fold shift was ~ 1. Neither pretreatment with LY354740 (10 mg kg^{-1}), nor with memantine (7.5 mg kg^{-1}) affected the development of morphine tolerance, since the fold changes were 2.7 and 2.5, respectively (Table 2).

Table 1 LY354740 and memantine attenuate the development of morphine tolerance

Treatment, (n)	*Test #1 ED_{50}*	*Test #2 ED_{50}*	*T2/T1 fold change*
Placebo + Morphine (20)	5.53 ± 0.71	23.26 ± 4.73	4.18 ± 0.49
Placebo + Placebo (8)	6.71 ± 0.92	7.48 ± 0.85	1.29 ± 0.21**
Memantine 7.5 mg kg^{-1} + Morphine (8)	4.54 ± 0.49	9.59 ± 2.02	2.09 ± 0.39*
LY354740 0.1 mg kg^{-1} + Morphine (10)	5.29 ± 0.88	17.75 ± 3.50	3.90 ± 0.78
LY354740 1.0 mg kg^{-1} + Morphine (17)	4.57 ± 0.62	10.87 ± 1.74	2.46 ± 0.28*
LY354740 10.0 mg kg^{-1} + Morphine (11)	5.33 ± 1.25	9.99 ± 2.11	2.00 ± 0.26*
		ANOVA $F(5,68)=5.905$, $P<0.001$	

Presented are mean antinociceptive morphine ED_{50} ± s.e.mean values (mg kg^{-1}) determined during pre- and post-morphine-tolerance development tests as well as resulting fold changes. The tolerance was induced by 10 mg kg^{-1} s.c. bid morphine administration over 6 days. LY354740, memantine or placebo was given s.c. 30 min prior to each of the morphine injections. Asterisks indicate a statistically significant difference toward 'control' group that received placebo + morphine during the development of morphine tolerance (*$P<0.05$, **$P<0.01$, Newman-Keul's test).

Table 2 Effects of LY354740 and memantine on the development of tolerance to fentanyl

Treatment (n)	*Test #1 ED_{50}*	*Test #2 ED_{50}*	*T2/T1 fold change*
Placebo + Fentanyl (10)	23.93 ± 3.00	76.92 ± 16.6	3.30 ± 0.60**
Placebo + Placebo (10)	29.88 ± 2.85	28.93 ± 3.80	0.99 ± 0.12
Memantine 7.5 mg kg^{-1} + Fentanyl (9)	29.11 ± 4.30	63.23 ± 12.0	2.50 ± 0.50*
LY354740 10.0 mg kg^{-1} + Fentanyl (10)	22.59 ± 3.61	61.01 ± 15.9	2.71 ± 0.57*
		ANOVA $F(3,35)=4.223$, $P<0.025$	

Presented are mean antinociceptive fentanyl ED_{50} ± s.e.mean values (μg kg^{-1}) determined during pre- and post-fentanyl-tolerance development tests as well as resulting fold changes. The tolerance was induced by 0.04 mg kg^{-1} s.c. qid fentanyl administration over 3 days. LY354740, memantine or placebo was given s.c. 30 min prior to each of the fentanyl injections. Asterisks indicate a statistically significant difference toward 'control' group that received placebo + placebo during the development of fentanyl tolerance (*$P<0.05$, **$P<0.01$, Newman-Keul's test).

Effects of LY354740 and memantine on the tail-flick response and antinociceptive effects of morphine (experiment 2)

As the first injection, four groups of mice were treated with LY354740 (1 or 10 mg kg^{-1}), memantine (7.5 mg kg^{-1}) or placebo, and the one drug-free control group was treated with placebo. Tail-flick responses were determined 30 min after the first injection to determine whether LY354740 or memantine altered the tail flick response. Immediately later these mice were injected with 5 mg kg^{-1} of morphine and a separate drug-free group was injected with placebo (second injection).

With regard to the acute antinociceptive effects, there were no significant differences among groups (one way ANOVA: $F(4,40)=1.591$, $P>0.05$, (Figure 1, 'time 0'). The data gained at 30 min after the second injection were analysed with one way ANOVA which yielded significant differences among groups: $F(4,40)=16.53$, $P<0.0001$, (Figure 1, 'time 30'). The *post-hoc* analysis with the use of Newman-Keul's test revealed that compared to placebo + morphine group, memantine + morphine and placebo + placebo groups demonstrated significantly shorter tail-flick responses ($P<0.05$ and $P<0.001$, respectively). At 60 min after second injection: $F(4,40)=9.52$, $P<0.0001$, (Figure 1, 'time 60'), there was a difference ($P<0.01$) between placebo + morphine and placebo + placebo treated mice. This difference ($P<0.05$) persisted until 90 min after second injection $F(4,40)=5.365$, $P<0.01$, (Figure 1, 'time 90'), but was not apparent at 120 min after second injection.

The overall effects of various treatments on tail-flick responses assessed on AUC data demonstrate that the only placebo + placebo treatment differed significantly ($P<0.01$, Newman-Keul's test followed one way ANOVA $F(4,40)=9.31$, $P<0.0001$) from placebo + morphine treatment (Figure 1, inset).

Discussion

The present findings demonstrate that LY354740, the first systemically active agonist of group II mGlu receptors as well as memantine, a clinically available low affinity uncompetitive NMDA receptor antagonist, may inhibit the development of tolerance to antinociceptive effects of morphine but not of fentanyl. Neither LY354740 nor memantine affected the tail-flick antinociceptive response or the acute antinociceptive effect of morphine.

Group II mGlu receptors are localized presynaptically and to a lesser extent, postsynaptically (Petralia *et al.*, 1996; Shigemoto *et al.*, 1997) and are therefore regarded as autoreceptors (Ugolini & Bordi, 1995). It has been demonstrated that the activation of presynaptic mGlu receptors localized on glutamatergic nerve terminals causes a decrease in glutamate release both *in vitro* (Attwell *et al.*, 1995; 1998a,b) and *in vivo* (Battaglia *et al.*, 1997). Therefore, stimulation of presynaptic group II mGluR autoreceptors may produce the functional antagonism of the glutamatergic system (Lovinger & McCool, 1995) and thus, may inhibit glutamatergic excitatory transmission (for the reviews, see Glaum & Miller, 1994; Pin & Duvoisin, 1995).

The inhibitory effects of NMDA receptor antagonists on morphine tolerance in mice as shown in experiment 1 has been previously demonstrated by other investigators (Lutfy *et al.*, 1993; 1995; Elliott *et al.*, 1994a; Bilsky *et al.*, 1996; Gonzalez *et al.*, 1997; Belozertseva & Bespalov, 1998 as well as in our

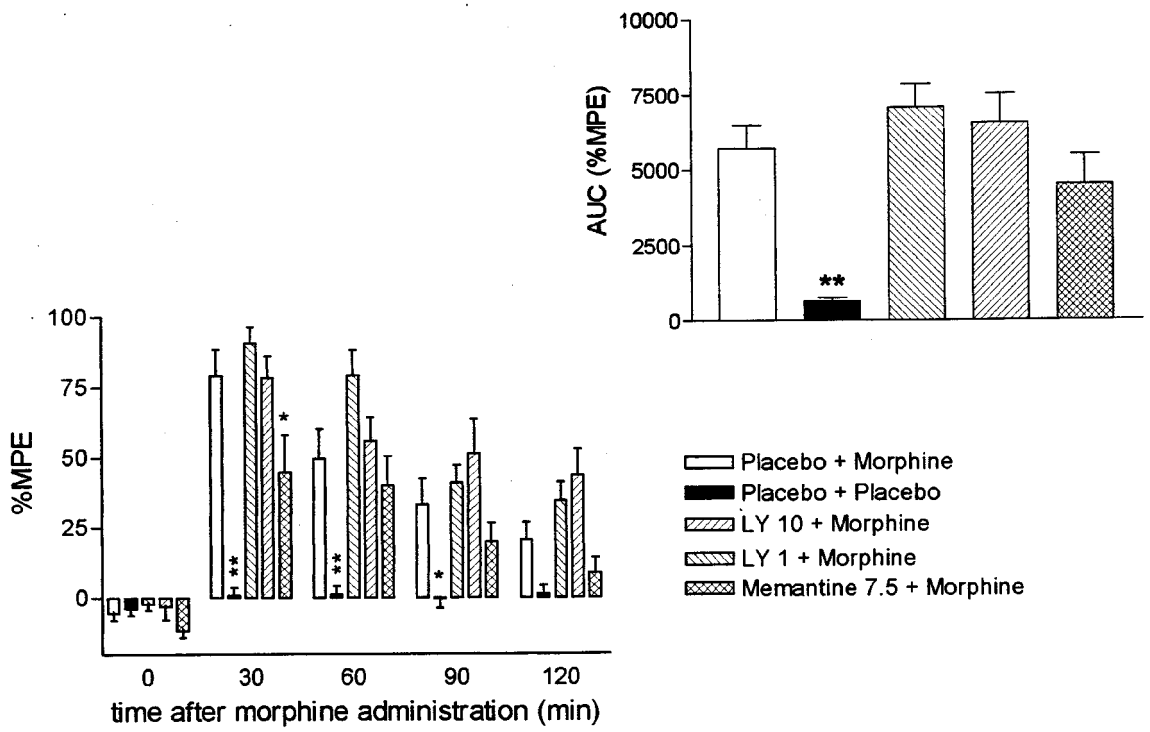

Figure 1 Time course of the tail-flick test responses of mice treated with a combination of LY354740 or memantine and morphine. Placebo or morphine (5 mg kg^{-1}) was administered s.c. 30 min after injection of LY354740, memantine or placebo. Data are expressed as means ± s.e.mean %MPE. $n=0$, 8, 9, 9, and 9 for placebo + morphine, placebo + placebo, 10 mg kg^{-1} of LY354740 + morphine, 1 mg kg^{-1} of LY354740 + morphine and 7.5 mg kg^{-1} of memantine + morphine, respectively. For other details, see text. Inset: Presented are mean ± s.e.mean Area Under Curve (AUC) values calculated on the same data. One way ANOVA $F(4,40)=9.31$, $P<0.0001$ and *post-hoc* Newman-Keuls test revealed that the treatment with placebo + morphine differed significantly (**, $P<0.01$) only towards placebo + placebo treatment.

laboratory (Popik & Kozela, 1999; Popik *et al*., 2000). However, the effects of mGlu II receptors agonist in this respect were unknown and deserve an explanation.

There are several hypotheses that may explain the inhibitory effects of LY354740 on morphine tolerance. First, the development of drug tolerance 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*., 1995b for the 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. This hypothesis is particularly attractive in light of the recent findings demonstrating that prolonged morphine administration may further increase the inhibitory effect of group II mGlu receptor agonists on glutamate release (Martin *et al*., 1999). Since, in addition, presynaptic mGlu receptor agonists are thought to inhibit NMDA receptor function (Glaum & Miller, 1994; Pin & Duvoisin, 1995), it can be hypothesized that the inhibitory effects of both LY354740 and memantine on morphine tolerance are related to an inhibition of neuronal, glutamate-dependent plasticity. However, the effects of memantine and LY354740 on the tolerance appear unrelated to their purported 'amnestic' effects. This is because LY354740 did not produce learning impairment (Helton *et al*., 1998), and the doses of memantine affecting learning processes are much above doses used in the present study. For example, while memantine significantly attenuated the development of morphine tolerance at a dose of 7.5 mg kg^{-1}, data from this laboratory indicate that the same dose did not impair the acquisition of spatial learning (Popik & Danysz, 1997). Similarly, in a continuous multiple-trial two-choice dark avoidance paradigm, memantine impaired learning at the dose of 20 mg kg^{-1} but not 10 mg kg^{-1} (Misztal & Danysz, 1995).

It is possible that an interaction between LY354740 and morphine occurred at the level of second messengers and/or receptors affected by both compounds. With regard to the additive action directly at the level of opioid receptors, we found no data in the literature concerning reasonable affinity of LY354740 at opioid receptors. Moreover, data from experiment 2 demonstrate the lack of antinociceptive effect of LY354740, suggesting that this compound is unlikely to act at the level of opioid receptors.

At the level of second messenger systems, like opiates (Duman *et al*., 1988), mGluRII agonists inhibit adenylate cyclase activity that produces an inhibition of cyclic AMP accumulation in the brain (Schoepp *et al*., 1992; Schaffhauser *et al*., 1997). However, the inhibitory effects of LY354740 on the development of morphine tolerance are unlikely explained by its effect on cyclic AMP system, because both LY354740 and morphine appear to affect the generation of this second messenger in the same direction. Moreover, if such a direct interaction would occur *in vivo*, LY354740 would potentiate the acute antinociceptive effects of morphine, which was not the case (experiment 2). It cannot be excluded however that the present experimental setting, which employed discrete, intermittent injections of morphine, in fact produced multiple withdrawal states resulting in an increase in the basal pain sensitivity and thus giving an impression of apparent tolerance (Laulin *et al*., 1999). It is known that prolonged inhibition of cyclic AMP system by opiates may led to an up-regulation of cyclic AMP system (Duman *et al*., 1988) which may form the basis of opiate dependence and tolerance (Nestler, 1993). It is also known that both LY354740 (Schoepp *et al*., 1992; Schaffhauser *et al*., 1997) and e.g., clonidine (Duman & Enna, 1986) inhibit adenylate cyclase activity and that both compounds attenuate symptoms of opiate withdrawal (Klodzinska *et al*., 1999; Vandergriff & Rasmussen, 1999; Gold *et al*., 1978). Therefore, it can be speculated that compounds that counteract an up-regulation of cyclic AMP system caused by chronic morphine administration and/or inhibit the cyclic AMP system despite its up-regulation, may attenuate morphine-induced tolerance produced by discrete, intermittent administration of opiates.

Finally, there is also a possibility that prolonged treatment with LY354740 might cause induction of liver enzymes, which in turn could increase the hepatic metabolism of morphine, giving an impression of morphine tolerance. Although without specific studies this hypothesis cannot be ruled out unequivocally, it appears unlikely in light of studies indicating that LY354740 is eliminated primarily by renal clearance in both rats and dogs (Johnson *et al*., 1997).

The failure of pretreatment with effective doses of LY354740 and memantine to inhibit the development of fentanyl tolerance confirms and extends previous findings demonstrating that uncompetitive and competitive NMDA receptor antagonists inhibit antinociceptive tolerance produced by morphine but not selective μ- or δ-opioid agonists (Bilsky *et al*., 1996). To explain the inhibitory effects of NMDA receptor antagonists on morphine tolerance and ineffectiveness of these compounds to affect fentanyl tolerance, (Bilsky *et al*., 1996) noted several differences regarding tolerance to morphine and more specific μ-opioid agonists. These include: (1) the fact that morphine is less selective at μ-opioid receptors than fentanyl; (2) the hypothesis implying different states or mechanisms of tolerance produced by morphine and specific μ-receptor agonists; (3) the notion that morphine is a partial agonist at the opioid μ-receptors, while fentanyl may have higher efficacy and, (4) differences in pharmacokinetics between morphine and fentanyl. Other hypotheses may include differences in internalization and phosphorylation of μ-receptors produced by morphine and more selective μ-opioid receptor agonists. Our data demonstrating that neither memantine nor LY354740 affected tolerance to the antinociceptive effects of fentanyl point to the conclusion that the inhibitory effect of mGluRII agonist on morphine tolerance is similar to the effect of NMDA receptor antagonist(s). It appears intriguing that despite profound differences in the molecular mechanism of action of LY354740 and memantine, these compounds seem to produce essentially the same effects on morphine and fentanyl antinociceptive tolerance. At present, the only hypothesis that may be proposed to explain this similarity would imply that mGluRII agonist inhibited morphine tolerance by decreasing the function of NMDA receptor *via* the inhibition of glutamate release (Glaum & Miller, 1994; Pin & Duvoisin, 1995).

In conclusion, the present study demonstrates that LY354740 and memantine inhibit the development of the tolerance to the analgesic effects of morphine without affecting nociceptive response *per se* or interfering with morphine analgesia. These findings indicate the therapeutic potential of metabotropic glutamate group II agonists in the management of chronic pain.

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