Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effect of glycine site/NMDA receptor antagonist MRZ2/576 on the conditioned place preference and locomotor activity induced by morphine in mice*

ZHU Yong-ping†[1], LONG Zai-hao[1,2], ZHENG Ming-lan[3], BINSACK Ralf[1]

([1]*Department of Toxicology, School of Medicine, Zhejiang University, Hangzhou 310006, China*)

([2]*Technology Center of Ningbo Entry-Exit Inspection and Quarantine Bureau, Ningbo 315012, China*)

([3]*Zhejiang Center for Disease Prevention and Control, Hangzhou 310009, China*)

†E-mail: zhuyp@zju.edu.cn

Received Mar. 16, 2006; revision accepted July 24, 2006

Abstract: Objective: To study the effect of glycine site/NMDA (N-methyl-D-aspartate) receptor antagonist MRZ2/576 on the conditioned place preference (CPP) and locomotor activity induced by morphine in mice. Methods: Different doses (1.25, 2.5 and 5 mg/kg, i.p.) of MRZ2/576 were used to evaluate the effect of MRZ2/576 on the acquisition and expression of CPP induced by morphine (5 mg/kg) in mice. In addition, we examined the locomotor activity of mice in conditioning and testing phase of CPP paradigm. Results: MRZ2/576 alone could not establish place preference, but a 5 mg/kg dose of MRZ2/576 could block both acquisition and expression of morphine-induced CPP. In testing phase of CPP, there was no statistical difference for locomotor activity between the groups; injection of MRZ2/576 showed a dose-dependent decrease of locomotor activity on both control and morphine-treated mice, especially 5 mg/kg of MRZ2/576 significantly suppressed the locomotor activity of mice. Conclusion: Based on the present results, we assume that MRZ2/576 can antagonize the rewarding effect of morphine, suggesting that this glycine site/NMDA receptor antagonist could be used to treat addictions due to its light side effect profile.

Key words: Morphine, MRZ2/576, NMDA receptor, Glycine site, Conditioned place
doi:10.1631/jzus.2006.B0998 **Document code:** A **CLC number:** Q42; R99

INTRODUCTION

Drug addiction is a long-term adaptation in brain elicited by repeated exposure to abusive drugs. However, so far there is no effective treatment of drug addiction. Substantial evidences suggest that the neurons of the mesolimbic dopamine system, which originate from ventral tegmental area (VTA) and project to nucleus accumbens (NAC) and other forebrain regions, are involved in mediating the rewarding effect of opioids. Other studies found that the glutamate receptors modulating the effect of opioids through their interaction with the dopamine receptor are involved in development of addiction (Bajo *et al*., 2006; Nestler, 2005; Franken *et al*., 2005).

Glutamate receptors belong to the glutamate receptor family and, depending on their ligand preference, can be divided into NMDA (N-methyl-D- aspartate) binding receptors and non-NMDA binding receptors. The NMDA receptor is an ionotropic (channel-containing) receptor and is allosterically regulated by Mg^{2+}, Zn^{2+}, polyamine, protons and redox potential. Activation of the NMDA receptor requires the simultaneous binding of two agonists: glutamate and glycine (Pláteník *et al*., 2000). Both competitive and uncompetitive NMDA receptor antagonists have been found to inhibit the conditioned place preference (CPP) induced by morphine, amphetamine and cocaine (Xi and Stein, 2002; Danysz *et al*., 2005; Makarska-Bialek *et al*., 2005; Bäckström and Hyytia,

* Project supported by the National Basic Research Program (973) of China (No. 2003CB515402), and the Science and Technology Council of Zhejiang Province (No. 2005C23G2010166), China

2006), suggesting a possible role in the treatment of drug addiction. But most of both classes of NMDA receptor antagonists have been reported to produce severe psycho-stimulant and psychotomimetic-like effects in animal and clinical experiments. Furthermore, conditioned place preference (CPP) experiments have established that both classes of NMDA antagonist have rewarding properties, implying that they would have abusive potential (Tzschentke, 2002). On the contrary, substantial studies showed that glycine-binding site NMDA receptor antagonists not only did not produce psychotomimetic effects, but also had no rewarding properties in CPP experiments (Papp *et al*., 2002; Danysz *et al*., 1998).

A potent antagonist for the glycine binding site of the NMDA receptor is MRZ2/576 (8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridaliono [4,5-b] quinoline-5-oxide choline salt), which was developed by MERZ Corporation (Germany). The component has a short half-life (about 20 min) and has a proven anticonvulsant and neuroprotective effect. In addition, MRZ2/576 cannot only prolong the suppression of morphine withdrawal by naltrexone in mice, but also retard the development of morphine tolerance in mice (Belozertseva *et al.*, 2000a; 2000b; Makarska-Bialek *et al.*, 2005; Bienkowski *et al*., 1999).

The current study was performed to evaluate the effect of MRZ2/576 on the acquisition and expression of morphine-induced CPP and concomitant locomotor activity in mice.

MATERIALS AND METHODS

Animals

Animal experiments were conducted in accordance with the NIH guide for care and use of laboratory animals and comply with the national laws on animal experiments. ICR male mice with body weight of 18~22 g where obtained from Zhejiang Medical Science College, China. The animals were grouped (4 mice per cage) and had free access to water and food. The cages were kept in a room with a 12 h:12 h light-dark cycle (lights on at 7:00 a.m.) and constant temperature of (22±2) °C.

Chemical reagents

Morphine hydrochloride (the First Pharmaceutical Company of Shenyang, China) and MRZ2/576 (MERZ Pharmaceutical GmbH, Germany) were dissolved in saline and distilled water, respectively. All drugs were administered i.p. in a volume of 10 ml/kg.

Apparatus

The CPP experiment was carried out in a special apparatus according to (Rezayof *et al*., 2003; del Rosario *et al*., 2002; Tzschentke, 1998) with minor modifications. Briefly, the apparatus consisted of four identical wooden shuttle boxes (30 cm×15 cm×15 cm), each divided into two chambers (15 cm×15 cm×15 cm) of equal size by a separator, one gray with smooth floor and the other black with wire mesh floor. A video camera was placed 70 cm above the floor and linked with a computer system. The mice behavior was recorded by the video camera and analyzed by MICETRACK software. The experiments were conducted under dim illumination (24~30 lx) and stable noise (37~40 dB), and each experiment had a conditioning phase (days 1~8) and a testing phase (day 9).

Procedures

1. CPP experiment

Conditioning phase: On days 1, 3, 5 and 7, the mice were put in the gray chamber for 50 min after injection of either MRZ2/576 or morphine. On days 2, 4, 6 and 8, the mice were placed in the black chamber for 50 min after saline injection.

Testing phase: On day 9, after the separator was raised 7 cm above the floor, the mice were placed in the shuttle box to freely access each chamber and mice moved in the shuttle boxes were captured by video.

Distances that mice moved both in the drug-paired conditioning and testing phases were measured to reflect the locomotor activity of the mice.

2. Effect of MRZ2/576 alone

To investigate whether MRZ2/576 (1.25, 2.5 and 5 mg/kg, i.p.) alone could establish CPP, MRZ2/576 was given to mice before the conditioning trial in the gray chamber, which was performed on days 1, 3, 5 and 7. On days 2, 4, 6 and 8, the mice were used for the conditioning trial in the black chamber after saline injection. Each conditioning trial (8 consecutive days) lasted for 50 min.

To investigate whether MRZ2/576 could block

the acquisition of CPP induced by morphine, the mice received various doses of MRZ2/576 (1.25, 2.5 and 5 mg/kg, i.p.) together with morphine (5 mg/kg, i.p.) right before the conditioning trial in the gray chamber (days 1, 3, 5 and 7). On days 2, 4, 6 and 8, the mice were given a saline injection and then used for the conditioning trial in the black chamber. Each conditioning trial (8 consecutive days) lasted for 50 min.

3. Effect of MRZ2/576 on the expression of morphine-induced CPP

A procedure similar to experiment 2 was used to measure the effects of MRZ2/576 on the expression of CPP by morphine, with the difference that the mice received an injection of either morphine (before being placed in the gray chamber) or saline (before being placed in the black chamber) during the acquisition trials. Before the CPP testing began (day 9), the mice were injected once with vehicle (saline) or various doses of MRZ2/576 (1.25, 2.5 and 5 mg/kg, i.p.).

4. Statistical analysis

All data were expressed as means±*SEM*. Both CPP and locomotion data were analyzed using one-way analysis of variance (ANOVA) followed by a post hoc LSD for multiple comparisons. Kendall's τ-b method was used for bivariate correlation test. Conditioning locomotion data were analyzed with two-factorial (treatment dose×day) repeated measures ANOVA.

RESULTS

The results obtained during the testing phase of experiment 1 (day 9; after 8 d of conditioning in which MRZ2/576 was applied alone) are shown in Table 1. Compared with the control, the used doses of MRZ2/576 (1.25, 2.5 and 5 mg/kg) neither prolonged nor reduced the time the mice spent in the gray side of the apparatus. Accordingly, MRZ2/576 did not produce place preference or place aversion [$F(3,28)=0.999$, $P=0.408$]. In addition, the statistical analysis did not reveal any significant differences between the saline-treated control group and the MRZ2/576-treated groups regarding the distance the animals moved [$F(3,28)=1.436$, $P=0.253$] on that day. Also, analysis comparing the time the mice spent in the gray side of apparatus with the distance the mice moved, showed no correlation ($r=-0.152$, $P=0.407$).

Table 2 shows the results of the testing day (day 9) of experiment 2, in which morphine was co-administered with various doses of MRZ2/576 during the conditioning phase. As shown in Table 2, morphine alone at a dose of 5 mg/kg prolonged the time the mice spent in the gray side of the apparatus when compared with the saline-treated control group ($P<0.01$), which proved that morphine could induce CPP in mice. The morphine-induced CPP effect was suppressed by MRZ2/576 at dose of 5 mg/kg ($P<0.05$), while other lower doses of MRZ2/576 did not produce such inhibitory effect (1.25 mg, $P=0.342$; 2.5 mg/kg, $P=0.051$). Regarding the locomotor activity of the mice on that day, there was no statistical difference between the groups in the distance covered according to ANOVA [$F(4,47)=1.474$, $P=0.225$]. Correlation analysis also showed that time spent in the gray side of apparatus had nothing to do with the distance the mice had moved ($r=0.099$, $P=0.486$).

As illustrated in Table 3, morphine (5 mg/kg) established CPP ($P<0.05$) when compared with saline control. MRZ2/576 showed a dose related decrease of time spent in the gray side of the apparatus, and especially a dose of 5 mg/kg showed a significant reduction of time the mice spent in the gray side (1.25 mg/kg, $P=0.08$; 2.5 mg/kg, $P=0.067$; 5 mg/kg, $P<0.001$). Accordingly, MRZ2/576 at a dose of 5 mg/kg reduced the distance mice moved, indicating a low locomotor activity of these mice. In addition, the time the mice spent in the gray side showed a strong correlation with the distance covered by the mice ($r=0.428$, $P=0.002$). Fig.1 shows the correlation between the time the mice spent in the drug paired side and the distance the mice treated with morphine plus 5 mg/kg MRZ2/576 covered.

Table 4 shows the data on the locomotor activity of the mice used in experiment 1, recorded during the conditioning phase after MRZ2/576 (or saline) was injected (days 1, 3, 5 and 7). MRZ2/576 induced a dose related decrease of locomotor activity of the mice, especially at a dose of 5 mg/kg, which showed significant differences. There were no differences of locomotor activity between the different conditioning phase [treatment effect: $F(3,84)=13.73$, $P<0.001$; day effect: $F(3,84)=0.813$, $P=0.49$; interaction effect: $F(9,84)=0.532$, $P=0.847$], so the impact of MRZ2/576 on locomotor activity could been seen

already immediately on day 1.

Table 5 shows data on the locomotor activity of the mice used in experiment 2, recorded during the conditioning phase after morphine alone (5 mg/kg) or together with various doses of MRZ2/576 were injected (days 1, 3, 5 and 7). All data reflected the distance that the mice moved in the grey chamber during the conditioning phase. Morphine at a dose of 5 mg/kg significantly increased the locomotor activity of the mice (P=0.006), but this effect could be inhibited by the co-administration of MRZ2/576 [(F(4,47) =7.115, P=0.000), especially at a dose of 5 mg/kg (P=0.000). Compared with day 1, MRZ2/576 at a dose of 5 mg/kg significantly lowered the morphine-increased locomotor activity of the mice on day 7 [treatment effect: F(4,141)=7.115, P<0.001; day effect: F(3,141)=5.692, P=0.001; interaction effect: F(12,141)=0.772, P=0.678].

Table 1 Effect of MRZ2/576 alone

MRZ2/576 (mg/kg)	n	Time spent in drug paired side (s)	Distance (m)
0.00	8	362.99±98.07	45.88±6.53
1.25	8	409.48±68.75	46.25±8.26
2.50	8	349.96±114.16	53.34±11.00
5.00	8	331.58±89.73	47.56±5.93

There were no statistical differences for time between groups as well as for distance

Table 2 Effect of MRZ2/576 on the acquisition of morphine-induced CPP

MRZ2/576 (mg/kg)	Morphine (mg/kg)	n	Time spent in drug paired side (s)	Distance (m)
0.00	0	8	387.99±124.63	44.58±11.67
0.00	5	8	501.64±81.09**	47.67±9.19
1.25	5	12	493.66±79.61**	47.72±8.91
2.50	5	12	481.52±96.28*	51.55±9.82
5.00	5	12	397.74±130.76	42.35±9.69

$^{**}P$<0.01, $^{*}P$<0.05 compared with saline control; There were no statistical differences for distance between groups [F(4,57)=1.071, P=0.379]

Table 3 Effect of MRZ2/576 on the expression of morphine-induced CPP

MRZ2/576 (mg/kg)	Morphine (mg/kg)	n	Time spent in drug paired side (s)	Distance (m)
0.00	0	8	370.90±79.58	48.41±8.76
0.00	5	8	503.84±101.28*	47.20±6.75
1.25	5	11	414.97±84.47	45.87±9.12
2.50	5	11	410.48±92.38	40.80±9.38
5.00	5	11	241.45±151.43$^{\#}$	27.70±10.38$^{\Delta}$

$^{*}P$<0.05 compared with saline control; $^{\#}P$<0.001 compared with morphine control; $^{\Delta}P$<0.001 compared with saline control

Table 4 Locomotor activity (m) during the conditioning phase in the drug paired side (experiment 1)

Treatment	Dose (mg/kg)	Day 1	Day 3	Day 5	Day 7
Saline	0.00	66.14±15.17	69.31±9.80	64.22±7.45	62.48±10.05
MRZ2/576	1.25	62.24±8.56	64.10±18.41	61.09±14.96	65.62±14.00
	2.50	51.38±23.47	60.12±12.02	59.74±16.66	58.55±10.98
	5.00	36.56±14.06*	38.19±9.10*	33.75±11.00**	36.79±12.53**

$^{**}P$<0.001, $^{*}P$<0.01 compared with saline group

Table 5 Locomotor activity (m) during the conditioning phase in the drug paired side (experiment 2)

Treatment	Dose	Day 1	Day 3	Day 5	Day 7
Saline	10 ml/kg	64.58±14.19	68.18±12.70	67.91±10.80	61.95±13.47
Morphine	5 mg/kg	96.68±8.37**	93.69±15.33**	89.38±13.20*	92.64±13.78**
Morphine+	(5+1.25) mg/kg	93.83±17.66**	96.63±18.08**	86.38±32.25*	81.86±23.14*
MRZ2/576	(5+2.5) mg/kg	87.99±22.56**	80.80±25.36*	88.36±28.96*	76.95±20.92$^{*\#}$
	(5+5) mg/kg	62.20±15.82	58.03±19.56	54.18±28.87	48.84±18.04$^{\#}$

$^{**}P$<0.001, $^{*}P$<0.01, compared with saline group; $^{\#}P$<0.01, compared with Day 1

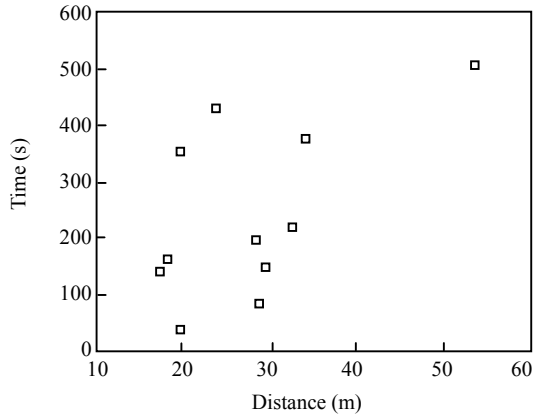

Fig.1 Correlation between time spent in the drug paired side and the distance of mice treated with morphine plus 5 mg/kg MRZ2/576 covered

DISCUSSION

In line with previous studies investigating the impact of other glycine site/NMDA receptor antagonists, our results showed that MRZ2/576, which alone could not establish conditioned place preference, could block the acquisition and expression of morphine-induced conditioned place preference.

Many investigations showed that the mesolimbic dopamine system is involved in the rewarding effect of opiate as well as other abusive substances, among which dopaminergic projection from VTA to NAC and other forebrain region is most important (Nestler, 2005; Pláteník *et al*., 2000). Past studies showed that morphine along with other abusive drugs can enhance dopamine release within the nucleus accumbens, and that dopamine receptor antagonists can block the acquisition of morphine induced conditioned place preference (Tzschentke, 1998; Schildein *et al*., 1998; Zarrindast *et al*., 2006). Moreover, transgenic mice lacking the dopamine D2 receptor do not have this capability (Xi and Stein, 2002). Although playing an important role in mediating the impact of morphine, the mesolimbic dopamine system alone seems not enough to explain and solve the actual problem in the treatment of addictions. Accordingly, the involvement of other central neural neurotransmitter systems in the rewarding effect of abusive drugs cannot be ruled out. Recently, investigations have given special attention to the glutamate receptor system, especially the NMDA receptor (Harris *et al*., 2004; Narita *et al*., 2005).

Intracranial mapping studies have identified the shell region of nucleus accumbens as a major site supporting drug-induced place preference conditioning. Within this region, there are extensive bi-directional interactions between DA (dopamine) input from the ventral tegmental area and glutamatergic inputs from the forebrain site, such as hippocampus and amygdala, these are known to be involved in place conditioning. These DA-NMDA receptor interactions within the accumbens shell are likely a candidate for a site where both DA and NMDA receptor antagonists block the acquisition of drug-induced conditioned place preference. However, in addition to direct DA-NMDA interaction, DA and glutamate synapses are co-localized on the dendritic spines of medium spiny neurons in the nucleus accumbens (Tzschentke, 2001).

Although behavioral experiments have shown that competitive NMDA antagonists (AP-5, CPP), uncompetitive NMDA antagonists (MK-801, ketamine, ifenprodil, memantine, etc.), and glycine site/NMDA antagonists (ACPC, l-701324, MRZ2/570, etc.) could block the development of morphine-induced conditioned place preference (Xi and Stein, 2002; Danysz *et al*., 2005; Makarska-Bialek *et al*., 2005; Bäckström and Hyytia, 2006; Quartaroli *et al*., 2001), the involved molecular mechanism is still unknown. To date, molecular and cloning studies revealed that the NMDA receptor is a channel-bearing protein complex which exists as a tetramer or pentamer. The complex is assembled from the NR1 with one or more type NR2 subunit (including NR2A, 2B, 2C and 2D). The glycine-binding site is localized exclusively with NR1, while glutamate is bound exclusively by the NR2 subunit (Pláteník *et al*., 2000). There are evidences that NR1 is involved in addiction including physical and psychological dependences. Injection of NMDA R1 antisense oligonucleotide can attenuate on the naloxone induced withdrawal signs from morphine (Zhu and Ho, 1998). Mice with reduced affinity of NMDA R1 glycine-binding site displayed decreased sensitivity to ethanol. The NR2B subunit is thought to play a more critical role in the rewarding effect of morphine than other NMDA subunits. Narita *et al*.(2000) found that the expression of NR2B subunit in limbic forebrain was significantly increased in morphine-treated

mice in comparison with control mice. Moreover, mice pretreated with an antibody specific to NR2B did not show morphine-induced conditioned place preference while antibodies against other NMDA receptor subunits could not block the impact of morphine. Further studies also indicated that especially the carboxyl terminal region of the NR2B subunit, which is directly phosphorylated by protein kinase Cγ isoform, is critical for the rewarding effects induced by morphine in the conditioned place preference paradigm (Kiefer *et al*., 2003; Narita *et al*., 2001).

It has been recognized that locomotor activity is another important behavioral aspect for most abusive drugs such as opiate and psychostimulant drugs. There are evidences that the underlying mechanisms for locomotor activity and for the rewarding effect are the same, i.e. the mesolimbic dopamine input from the ventral tegmental area to the nucleus accumbens also affects behavioral activity. A fundamental axiom of positive reinforcement with respect to motor activity is that motor activity will increase in scope and force if followed by an event that produces reward. Acute administrations of opiate and psychostimulant drugs can obviously enhance locomotor activity of experimental animals. Past studies showed that NMDA receptor activation by systemic administration of NMDA increased the level of locomotor activity and induced an increase in exploratory behavior in rats. NMDA produces also place preference as indexed by CPP paradigm (Rezayof *et al*., 2003; del Rosario *et al*., 2002; Panos *et al*., 1999; Kretschmer, 1998). So according to these facts, it can be concluded that a NMDA receptor antagonist should be able not only to block conditioned place preference, but also to lower locomotor activity. Our results showed that acute systemic administration effect of MRZ2/576 showed a dose-dependent decrease in locomotor activity, which accords with the above assumption. On the contrary, uncompetitive NMDA antagonists like PCP or MK-801 have been shown to increase the level of locomotor activity by themselves alone (Carlezon *et al*., 2000). Right now, the diverse effect of different NMDA receptor antagonists on locomotor activity remains unclear. Undoubtedly, locomotor activity is not only associated with the NMDA receptor, but also with the rewarding effect of a drug like morphine. But the interaction of these two areas is unclear and need further studies to elucidate it.

In addition, the results of our experiments provided evidence that locomotor activity does not relate with the expression of morphine-induced conditioned place preference, which is in line with previous studies (Narita *et al*., 2001).

Most experimental studies to date showed that NMDA antagonists produce incongruous tolerances in animals and human subjects, which limit their practical applications. There is a range both of binding/unbinding kinetics and of voltage dependence among the NMDA receptor antagonists. Those with slow unbinding kinetics and weak voltage dependence, such as MK-801, are associated with dysphoric side effects and are poorly tolerated in humans. In comparison, NMDA receptor antagonists with faster kinetic and stronger voltage dependence, such as memantine, are tolerated much better in humans and are already used to treat diseases (Parsons *et al*., 1999). Glycine site/NMDA receptor antagonist like MRZ2/576 has no intolerable side effects in animal experiments, but the underlying mechanism is different with the latter being achieved through regulating the function of Mg^{2+}. Mg^{2+} is endogenous NMDA receptor antagonist that blocks the NMDA receptor by binding to the channel pore with an apparent affinity that depends on the membrane potential. The uncompetitive NMDA receptor MK-801 binds to the inside of NMDA receptor channel pore and overlaps the Mg^{2+}-block site, which influences functions of Mg^{2+}. Conversely, glycine site/NMDA receptor binding site localizes on the surface of the NMDA receptor and binding with the antagonist should not interfere with the function of Mg^{2+}. Addition of glutamate and glycine decreases the binding of [3H] MK-801 to NMDA receptors in membrane preparations from rat brain, which is reversible by glutamate and glycine antagonist. Recently, a study found that NMDA and glycine increase the IC_{50} value of the Mg^{2+}-block site at pH 7.4 in NR1-1a/NR2a NMDA receptors expressed in xenopus oocytes. The increase of the IC_{50} value may correspond to a decrease in Mg^{2+}-block affinity. The effects result in an increased influx of Ca^{2+}, and this influx may constitute up to a third of the total Ca^{2+} influx induced by NMDA (Liu *et al*., 2001). Theses results suggest that glutamate and glycine regulate NMDA receptors not only by opening the channel gate, but also by affect-

ing the affinity of the Mg^{2+}-block site. Hence, this affinity-regulation of the Mg^{2+}-block site may provide a novel mechanism of glutamate and glycine to regulate the function of NMDA receptors, which may be the reason why glycine antagonists alleviate the negative side effect.

Our results showed that MRZ2/576 inhibited both acquisition and expression of morphine-induced conditioned place preference, which strongly suggests that the NMDA receptor is involved in the development of morphine addiction and that MRZ2/576 can induce a dose-dependent decrease in locomotor activity in both normal and morphine-treated mice.

ACKNOWLEDGEMENT

We thank Prof. W. Danysz, MERZ Pharmaceutical GmbH, Germany, who offered MRZ2/576 and gave valuable instructions on this experiment.

References

Bäckström, P., Hyytia, P., 2006. Ionotropic and metabotropic glutamate receptor antagonism attenuates cue-induced cocaine seeking. *Neuropsychopharmacology*, **31**(4): 778-786. [doi:10.1038/sj.npp.1300845]

Bajo, M., Crawford, E.F., Roberto, M., Madamba, S.G., Siggins, G.R., 2006. Chronic morphine treatment alters expression of N-methyl-D-aspartate receptor subunits in the extended amygdala. *J. Neurosci. Res.*, **83**(4):532-537. [doi:10.1002/jnr.20756]

Belozertseva, I.V., Danysz, W., Bespalov, A.Y., 2000a. Short-acting NMDA receptor antagonist MRZ2/576 produces prolonged suppression of morphine withdrawal in mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **361**(3):279-282. [doi:10.1007/s002109900179]

Belozertseva, I.V., Danysz, W., Bespalov, A.Y., 2000b. Effects of short-acting NMDA receptor antagonist MRZ2/576 on morphine tolerance development in mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **361**(6): 573-577. [doi:10.1007/s002109900207]

Bienkowski, P., Korso, E., Kostowski, W., Danysz, W., 1999. Effects of N-methyl-D-aspartate receptor antagonists on reinforced and nonreinforced responding for ethanol in rats. *Alcohol*, **18**(2-3):131-137. [doi:10.1016/S0741-8329(98)00075-5]

Carlezon, W.A.Jr, Kosten, T.A., Nestler, E.J., 2000. Behavioral interactions caused by combined administration of morphine and MK-801 in rats. *Psychopharmacology*, **151**(2-3):261-272. [doi:10.1007/s002130000462]

Danysz, W., Parsons, C.G., Karcz-Kubicha, M., Schwaier, A., Popik, P., Wedzony, K., Lazarewicz, J., Quack, G., 1998. GlycineB antagonist as potential therapeutic agents. Previous hopes and present reality. *Amino Acids*, **14**(1-3):235-239. [doi:10.1007/BF01345268]

Danysz, W., Kozelab, E., Parsons, C.G., Sladek, M., Bauer, T., Popik, P., 2005. Peripherally acting NMDA receptor/glycineB site receptor antagonists inhibit morphine tolerance. *Neuropharmacology*, **48**(3):360-371. [doi:10.1016/j.neuropharm.2004.11.005]

del Rosario, C.N., Pacchioni, A.M., Cancela, L.M., 2002. Influence of acute or repeated restraint stress on morphine-induced locomotion: involvement of dopamine, opioid and glutamate receptors. *Behav. Brain Res.*, **134**(1-2):229-238. [doi:10.1016/S0166-4328(02)00038-4]

Franken, I.H., Booij, J., van den Brink, W., 2005. The role of dopamine in human addiction: from reward to motivated attention. *Eur. J. Pharmacol.*, **526**(1-3):199-206. [doi:10.1016/j.ejphar.2005.09.025]

Harris, G.C., Wimmer, M., Byrne, R., Aston-Jones, G., 2004. Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. *Neuroscience*, **129**(3):841-847. [doi:10.1016/j.neuroscience.2004.09.018]

Kiefer, F., Jahn, H., Koester, A., Montkowski, A., Reinscheid, R.K., Wiedemann, K., 2003. Involvement of NMDA receptors in alcohol-mediated behavior: mice with reduced affinity of the NMDA R1 glycine binding site display an attenuated sensitivity to ethanol. *Biol. Psychiatry*, **53**(4): 345-351. [doi:10.1016/S0006-3223(02)01486-5]

Kretschmer, B.D., 1998. Ligand of the NMDA receptor-associated glycine recognition site and motor behavior. *Amino Acids*, **14**(1-3):227-234. [doi:10.1007/BF01345267]

Liu, Y., Hill, R.H., Arhem, P., von Euler, G., 2001. NMDA and glycine regulate the affinity of the Mg^{2+}-block site in NR1-1a/NR2A NMDA receptor channels expressed in Xenopus oocytes. *Life Sci.*, **68**(16):1817-1826. [doi:10.1016/S0024-3205(01)00975-4]

Makarska-Bialek, K., Kaminski, R.M., Czuczwar, S.J., 2005. Influence of the antagonist of the glycine site of NMDA receptors, MRZ2/576, on the anticonvulsant activity of conventional antiepileptic drugs in mice. *Pharmacol. Rep.*, **57**(4):458-466.

Narita, M., Aoki, T., Suzuki, T., 2000. Molecular evidence for the involvement of NR2B subunit containing N-methyl-D-aspartate receptors in the development of morphine-induced place preference. *Neuroscience*, **101**(3):601-606. [doi:10.1016/S0306-4522(00)00405-X]

Narita, M., Aoki, T., Ozaki, S., Yajima, Y., Suzuki, T., 2001. Involvement of protein kinase Cγ isoform in morphine induced reinforcing effects. *Neuroscience*, **103**(2): 309-314. [doi:10.1016/S0306-4522(00)00572-8]

Narita, M., Kato, H., Miyoshi, K., Aoki, T., Yajima, Y., Suzuki, T., 2005. Treatment for psychological dependence on morphine: usefulness of inhibiting NMDA receptor and its associated protein kinase in the nucleus accumbens. *Life Sci.*, **77**(18):2207-2220. [doi:10.1016/j.lfs.2005.04.015]

Nestler, E.J., 2005. Is there a common molecular pathway for addiction? *Nat. Neurosci.*, **8**(11):1445-1449. [doi:10.

1038/nn1578]

Panos, J.J., Rademacher, D.J., Renner, S.L., Steinpreis, R.E., 1999. The rewarding properties of NMDA and MK-801 (Dizocilpine) as indexed by the conditioned place preference paradigm. *Pharmacol. Biochem. Behav.*, **64**(3):591-595. [doi:10.1016/S0091-3057(99)00155-0]

Papp, M., Gruca, P., Willner, P., 2002. Selective blockade of drug-induced place preference conditioning by ACPC, a functional NDMA-receptor antagonist. *Neuropsychopharmacology*, **27**(5):727-744. [doi:10.1016/S0893-133X(02)00349-4]

Parsons, C.G., Danysz, W., Bartmann, A., Spielmanns, P., Frankiewicz, T., Hesselink, M., Eilbacher, B., Quack, G., 1999. Amino-alkyl-cyclohexanes are novel uncompetitive NMDA receptor antagonists with strong voltage-dependency and fast blocking kinetics: in vitro and in vivo characterization. *Neuropharmacology*, **38**(1):85-108. [doi:10.1016/S0028-3908(98)00161-0]

Pláteník, J., Kuramoto, N., Yoneda, Y., 2000. Molecular mechanisms associated with long-term consolidation of the NMDA signal. *Life Sci.*, **67**(4):335-364. [doi:10.1016/S0024-3205(00)00632-9]

Quartaroli, M., Fasdelli, N., Bettelini, L., Maraia, G., Corsi, M., 2001. GV196771A, an NMDA receptor/glycine site antagonist, attenuates mechanical allodynia in neuropathic rats and reduces tolerance induced by morphine in mice. *Eur. J. Pharmacol.*, **430**(2-3):219-227. [doi:10.1016/S0014-2999(01)01278-X]

Rezayof, A., Zarrindast, M.R., Sahraei, H., Haeri-Rohani, A., 2003. Involvement of dopamine receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats. *J. Psychopharmacol.*, **17**(4):415-423. [doi:10.1177/0269881103174005]

Schildein, S., Agmo, A., Huston, J.P., Schwarting, R.K., 1998. Intraacumbens injections of substance p, morphine, and amphetamine: effect on conditioned place preference and behavioral activity. *Brain Res.*, **790**(1-2):185-194. [doi:10.1016/S0006-8993(98)00062-6]

Tzschentke, T.M., 1998. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issue. *Prog. Neurobiol.*, **56**(6):613-672. [doi:10.1016/S0301-0082(98)00060-4]

Tzschentke, T.M., 2001. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. *Prog. Neurobiol.*, **63**(3):241-320. [doi:10.1016/S0301-0082(00)00033-2]

Tzschentke, T.M., 2002. Glutamatergic mechanisms in different disease state: overview and therpeutical implication—an introduction. *Amino Acids*, **23**(1-3):147-152. [doi:10.1007/s00726-001-0120-8]

Xi, Z.X., Stein, E.A., 2002. Blockade of ionotropic glutamatergic transmission in the ventral tegmental area reduces heroin reinforcement in rat. *Psychopharmacology*, **164**(2):144-150. [doi:10.1007/s00213-002-1190-3]

Zarrindast, M.R., Azami, B.N., Rostami, P., Rezayof, A., 2006. Repeated administration of dopaminergic agents in the nucleus accumbens and morphine-induced place preference. *Behav. Brain Res.*, **169**(2):248-255. [doi:10.1016/j.bbr.2006.01.011]

Zhu, H., Ho, I.K., 1998. NMDA-R1 antisense oligonucleotide attenuate withdrawal signs from morphine. *Eur. J. Pharmacol.*, **352**(2-3):151-156. [doi:10.1016/S0014-2999(98)00367-7]