Polish Journal of Pharmacology
Pol. J. Pharmacol., 2004, 56, 863–866
ISSN 1230-6002

PRELIMINARY COMMUNICATION

SELECTIVE mGlu5 RECEPTOR ANTAGONIST MTEP ATTENUATES NALOXONE-INDUCED MORPHINE WITHDRAWAL SYMPTOMS

Agnieszka Pałucha[1], Piotr Brański[1], Andrzej Pilc[1,2,#]

[1]Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków; [2]Institute of Public Health, Collegium Medicum, Jagiellonian University, Michałowskiego 12, PL 31-126, Kraków, Poland

Selective mGlu5 receptor antagonist MTEP attenuates naloxone-induced morphine withdrawal symptoms. A. PAŁUCHA, P. BRAŃSKI, A. PILC. Pol. J. Pharmacol., 2004, 56, 863–866.

Several lines of evidence suggest a crucial involvement of glutamate in the mechanism of drug addiction. The involvement of group I mGlu receptors in the mechanism of addiction has also been proposed. Given the recent discovery of selective and brain penetrable mGlu5 receptor antagonists, the effects of 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) were evaluated in the naloxone-precipitated morphine withdrawal model. Experiments were performed on male C57BL/6J (20–25 g) mice. Mice were rendered morphine-dependent and withdrawal was precipitated with naloxone. Two hours and 15 min after the last dose of morphine, mice were injected with a mGlu5 receptor antagonist. MTEP (1–10 mg/kg) in a dose-dependent manner inhibited the naloxone-induced symptoms of morphine withdrawal in morphine-dependent mice, remaining without any effect on the locomotor activity of mice. The data suggest that selective mGlu5 receptor antagonists may play a role in the therapy of drug-dependence states.

***Key words**: mGlu5 receptors, MTEP, naloxone, morphine withdrawal, dependence*

correspondence; e-mail: nfpilc@cyf-kr.edu.pl

Abbreviations: MTEP – *3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine*, NMDA – *N-methyl- D-aspartic acid*

INTRODUCTION

Glutamate acts by stimulating ionotropic and metabotropic glutamate receptors (mGluRs) [10], and seems to play a major role in both physiology and pathophysiology of the central nervous system (CNS). mGluRs are a family of eight G-protein coupled receptors, which are classified into three groups according to their sequence homology, effector coupling and pharmacology. Group I mGluRs (mGluR1 and mGluR5) are positively coupled to phospholipase C, while group II mGluRs (mGluR2 and mGluR3) and group III mGluRs (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to adenylate cyclase [2].

Converging lines of evidence indicate crucial involvement of glutamate receptors in the phenomena related to drug-seeking behavior [13]. The early data of Fundytus et al. [5, 6] demonstrated that chronic inhibition of group I mGluRs by intracerebroventricular administration of (S)-4-carboxyphenylglycine resulted in the diminished severity of naloxone-precipitated withdrawal symptoms in morphine-dependent rats. Up to recent years, studies concerning involvement of group I mGluRs in CNS functions were largely based on compounds which had only limited selectivity between mGluRl and mGluR5 subtypes and which did not penetrate into the brain. The discovery of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective and systemically active compound [17] which is a potent non-competitive mGluR5 antagonist, led to further experiments showing that MPEP ameliorated symptoms of morphine tolerance [9] and acquisition as well as expression of morphine-induced conditioned place preference [11] in rats. The present study was aimed to investigate whether 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP), which retains the *in vitro* potency of MPEP but has superior selectivity and reduced off-target liabilities [3], reduces the effect of naloxone-induced morphine withdrawal in mice.

MATERIALS and METHODS

All experiments were performed on male C57BL/6J (20–25 g) mice. The animals were kept on a natural day-night cycle at a room temperature of 19–21°C, with free access to food and tap water before the experiment. Each experimental group consisted of 8–10 animals/dose, the total number of animals was 80. All injections were made in a volume of 10 ml/kg. The experiments were performed by an observer unaware of the treatment. All experimental procedures were approved by Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. Mice were treated with morphine (30 mg/kg, *ip* twice daily) for 3 days, an additional dose was administered in the morning of the fourth test day. Two hours and 15 min after the last dose of morphine, the mice were injected with MTEP or saline. The animals were challenged with naloxone (4 mg/kg) 45 min after administration of MTEP and placed immediately in transparent glass beakers (volume of 10 l). The number of jumps was recorded during a 10-minute test period. The morphine-dependent mice treated with naloxone were used as a control group. Our previous studies [8] demonstrate that this schedule of injections provided the most optimal means for investigating morphine dependence in mice.

The spontaneous locomotor activity of mice was measured in photoresistor actometers (circular cages, 25 cm in diameter, 15 cm high, two light sources, two photoresistors), where the animals were placed individually 1 h after injection of MTEP solution. The number of crossings of light beams was measured within 30 min of experimental session. First measurement was performed 6 min after placing animals in actometers. The obtained data, presented as means ± SEM, were evaluated by a one-way analysis of variance, followed by Dunnett's multiple comparison test, where $p < 0.05$ was considered significant.

MTEP was suspended in a 1% aqueous solution of Tween 80. Morphine hydrochloride (Polfa, Kraków, Poland), naloxone (RBI, Natick, MA, USA) were dissolved in saline. All the compounds were administered *ip*.

RESULTS and DISCUSSION

The morphine-dependent mice treated with naloxone demonstrated a pronounced withdrawal syndrome, which was manifested by vertical jumping. Jumping was not observed in vehicle-treated mice challenged with naloxone or in morphine-

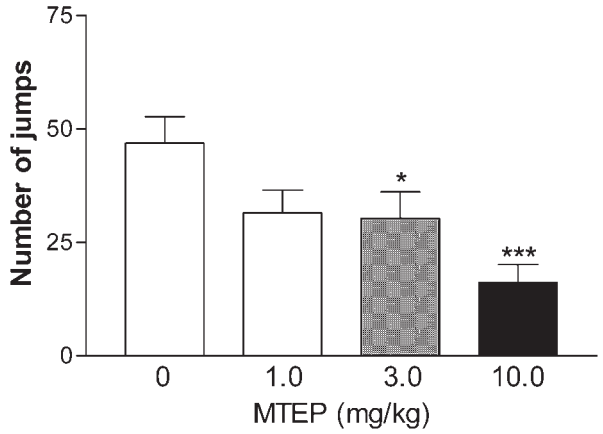

Fig. 1. Effect of MTEP on naloxone-induced jumping in 3 days morphine-dependent mice. Naloxone (4 mg/kg, *ip*) was administered 45 min after MTEP. Values represent the means ± SEM of the number of jumps during a 10 min experimental session, n = 6–10, * $p < 0.05$, *** $p < 0.01$ *vs.* control group

dependent mice challenged with vehicle (0 jumps per 10 min period). A novel mGluR5 antagonist, MTEP, produced a dose-dependent reduction in naloxone-induced jumping. The 10 mg/kg, *ip* dose of MTEP, decreased jumping by 60% ($F(3,44) = 6.754$, $p < 0.001$) (Fig. 1). At the same dose MTEP was without any effect on the locomotor activity of mice (Tab. 1).

Table 1. Effect of MTEP on the locomotor activity of mice

Compound	n	Dose (mg/kg)	Number of crossings	
			6 min	30 min
Vehicle	9	–	66 ± 11	162 ± 15
MTEP	8	1	73 ± 8	158 ± 18
	8	10	81 ± 6	183 ± 23

MTEP was administered *ip*, 1 h before the test. Values are expressed as means ± SEM, *n* is the number of mice per group

Our results extend the data which showed that MPEP blocked symptoms of morphine tolerance [9], demonstrating that treatment with MPEP leads to a decrease in symptoms of morphine withdrawal in mice. A number of hypotheses may explain a potential effect of MTEP on physical symptoms of morphine withdrawal. The majority of group I mGluRs are located postsynaptically [12], however MPEP has recently been reported to inhibit glutamate release by presynaptically located mGluR5 [15]. This mimics the function of group II mGluR, which are predominantly located presynaptically and in this way modulate glutamate release [12]. It has been shown previously that the agonist of group II of mGluR (LY354740) inhibited the naloxone/naltrexone-induced abstinence syndrome [8, 16], which suggests that a decrease in glutamate release, and in consequence blockade of glutamate-induced hyperexcitability may be involved in inhibition of morphine tolerance. Compounds, which reduce transmission at NMDA receptors, are known to attenuate symptoms of naloxone-precipitated opioid withdrawal [7]. Glutamatergic transmission, mediated by the stimulation of group I mGluRs, has also been shown to potentiate responses of ionotropic GluRs in various brain structures, including potentiation of NMDA currents [4]. On the contrary, several data indicate that mGluR5 antagonists reduce NMDA receptor activity in several brain areas [1, 14]. Therefore, the inhibition of group I mGluRs may lead to a decrease in NMDA-receptor-mediated neurotransmission, thus, contributing to the attenuation of morphine-withdrawal effects. It can be speculated that mGluR5 antagonists, which did not cause sedation or disturb the rota-rod performance, might play a role in the therapy of physical withdrawal symptoms, being free of the adverse effects produced by NMDA receptor antagonists.

Acknowledgment. Research was supported by Institute of Pharmacology, Polish Academy of Sciences, Kraków. MTEP was a generous gift from Dr. Nicolas Cosford (Merck, San Diego, USA).

REFERENCES

1. Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ: Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. J Neurosci, 2000, 20, 7871–7879.
2. Conn PJ, Pin JP: Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol, 1997, 37, 205–237.
3. Cosford NDP, Tehrani L, Roppe J, Schweiger E, Smith ND, Anderson J, Bristow L et al.: 3-[(2--Methyl-1,3-thiazol-4-yl)-ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. J Med Chem, 2003, 46, 204–206.
4. Fitzjohn SM, Irving AJ, Palmer MJ, Harvey J, Lodge D, Collingridge GL: Activation of group I mGluRs potentiates NMDA responses in rat hippocampal slices. Neurosci Lett, 1996, 203, 211–213.

5. Fundytus ME, Coderre TJ: Effect of activity at metabotropic, as well as ionotropic (NMDA), glutamate receptors on morphine dependence. Br J Pharmacol, 1994, 113, 1215–1220.
6. Fundytus ME, Ritchie J, Coderre TJ: Attenuation of morphine withdrawal symptoms by subtype-selective metabotropic glutamate receptor antagonists. Br J Pharmacol, 1997, 120, 1015–1020.
7. Higgins GA, Nguyen P, Sellers EM: The NMDA antagonist dizocilpine (MK-801) attenuates motivational as well as somatic aspects of naloxone-precipitated opioid withdrawal. Life Sci, 1992, 50, L167–L172.
8. Kłodzińska A, Chojnacka-Wójcik E, Pałucha A, Brański P, Popik P, Pilc A.: Potential anti-anxiety, anti-addictive effects of LY 354740, a selective group II glutamate metabotropic receptors agonist in animal models. Neuropharmacology, 1999, 38, 1831–1839.
9. Kozela E, Pilc A, Popik P: Inhibitory effects of MPEP, an mG1uR5 antagonist, and memantine, an N-methyl-D-aspartate receptor antagonist, on morphine antinociceptive tolerance in mice. Psychopharmacology, 2003, 165, 245–251.
10. Monaghan DT, Bridges RJ, Cotman CW: The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol, 1989, 29, 365–402.
11. Popik P, Wróbel M: Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. Neuropharmacology, 2002, 43, 1210–1217.
12. Schoepp DD: Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther, 2001, 299, 12–20.
13. Stephens DN: A glutamatergic hypothesis of drug dependence: extrapolations from benzodiazepine receptor ligands. Behav Pharmacol, 1995, 6, 425–446.
14. Thomas LS, Jane DE, Gasparini F, Croucher MJ: 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, 2001, 41, 523–527.
15. Thomas LS, Jane DE, Harris JR, Croucher MJ: Metabotropic glutamate autoreceptors of the mGlu(5) subtype positively modulate neuronal glutamate release in the rat forebrain *in vitro*. Neuropharmacology, 2000, 39, 1554–1566.
16. Vandergriff J, Rasmussen K: The selective mGlu2/3 receptor agonist LY354740 attenuates morphine-withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. Neuropharmacology, 1999, 38, 217–222.
17. Varney MA, Cosford NDP, Jachec C, Rao SP, Sacaan A, Lin FF, Bleicher L et al.: SIB-1757 and SIB-1893: selective, noncompetitive antagonists of metabotropic glutamate receptor type 5. J Pharmacol Exp Ther, 1999, 290, 170–181.

Received: September 7, 204; in revised form: December 1, 2004.