Potential antipsychotic effects of a selective non-competitive antagonist of metabotropic glutamate receptor 5 (mGluR5), 2-methyl-6-phenylethynylpyridine (MPEP), was examined in two commonly used screening tests: (1) the hyperactivity induced by an NMDA receptor antagonist phencyclidine (PCP), and (2) the hyperactivity induced by an indirect dopamine agonist, D-amphetamine. PCP was administered at a dose of 2.5 mg/kg s.c. and D-amphetamine was given at a dose of 1 mg/kg s.c. MPEP (5 mg/kg i.p.) significantly enhanced the locomotor activity increased by PCP, but inhibited amphetamine-induced hyperactivity. The opposite effect of MPEP in the two above-mentioned models questions significance of the blockade of mGluR5 receptors to antipsychotic effects.

Key words: locomotor activity, MPEP, D-amphetamine, phencyclidine, rats

INTRODUCTION

The overactivity of dopaminergic system in the limbic structures and the dysfunction of glutamatergic neurotransmission, mainly in the cortical regions, have been suggested to play a key role in the pathophysiology of schizophrenia (for review see 1, 2).

Glutamate regulates neuronal activity by acting on ionotropic and metabotropic G protein-coupled receptors (mGluRs). The mGluRs were assigned to three major groups. Stimulation of group I mGluRs (mGluR1 and mGluR5), which are mainly postsynaptic receptors, leads to an increase in phosphoinositide
hydrolysis, whereas activation of group II mGluRs (mGluR2 and mGluR3) or group III ones (mGluR4 and mGluR6-8) causes inhibition of adenyl cyclase and glutamate release (3).

The contribution of dysfunction of glutamatergic transmission to schizophrenia is supported by the fact that NMDA receptor blockers (e.g. phencyclidine (PCP)) elicit both positive and negative psychotic symptoms in humans (4). On this basis, it has been proposed that the hypofunction of NMDA receptors may be associated with psychotic states. In accordance with this view, drugs enhancing NMDA receptor functions via glycine sites potentiate the antipsychotic effects of neuroleptics by alleviating negative symptoms and improving cognitive functions (5).

Recently it has been reported that both phencyclidine and ketamine enhance glutamate release in the prefrontal cortex as an effect compensating for the blockade of NMDA receptors, which leads to the overstimulation of postsynaptic non-NMDA glutamatergic receptors (6, 7). Moreover, it has been proposed that the ligands of glutamatergic receptors that either diminish glutamate release or block postsynaptic non-NMDA receptors may also show antipsychotic properties. In support of this view, agonists of group II mGluRs and antagonists of AMPA/KA receptors have been shown to diminish the PCP/ketamine-induced deficits in working memory, hyperlocomotion and stereotypy, which are accepted as animal equivalents of psychotic symptoms (6, 7).

The aim of the present study was to determine whether the blockade of postsynaptic mGluR5 receptors is also involved in antipsychotic effects. To this end, we examined the influence of a selective non-competitive mGluR5 antagonist MPEP (2-methyl-6-phenylethynylpyridine) on the hyperlocomotion induced by phencyclidine and D-amphetamine (an indirect dopamine agonist), which are the models commonly used for screening potential antipsychotic compounds.

**MATERIALS AND METHODS**

**Animals**

The study was conducted on male Wistar rats weighing 220-270 g. They were kept under an artificial light/dark cycle (12/12 h; the light on from 6 a.m. to 6 p.m.) with free access to food and water.

The experiment was carried out in compliance with the Animal Protection Act of August 21, 1997 (published in Poland's Government Regulations and Gazette of Law [Dziennik Ustaw] no. 111/197, art. 724), and according to the NIH Guide for the Care and Use of Laboratory Animals.

**Locomotor activity**

Locomotor activity was measured in photoresistor actometers, L x W x H = 40 x 40 x 25 cm, with two light beams. The rats were acclimatized to the testing room 24 h prior to testing. 2-Methyl-6-phenylethynylpyridine (MPEP, Tocris, Cookson Ltd, 2.5 or 5 mg/kg i.p.), dissolved in redistilled...
water, was injected 10 min before phencyclidine HCl (RBI, 2.5 mg/kg s.c.), D-amphetamine sulfate (Sigma St. Louis, USA, 1 mg/kg s.c.) or physiological saline, s.c.. All the compounds were administered in a volume of 2 ml/kg. The animals were then placed individually in actometers, and their locomotor activity was measured for 140 min. Experimental groups consisted of 9-11 rats.

**Statistical analysis**

A one-way ANOVA, followed by an LSD (Least Significance Difference) test for post hoc comparisons were used for the analysis of data.

**RESULTS**

**The effect of MPEP on the phencyclidine-induced locomotor activity**

Phencyclidine (2.5 mg/kg) significantly increased the locomotor activity of rats (Table 1). That effect was not affected by pretreatment with MPEP at 2.5 mg/kg (Fig. 1). A one-way ANOVA showed a significant treatment effect \[F(3,35) = 12.73, \ P<0.001\], but a post-hoc comparison revealed only a significant difference

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**Fig. 1.** Influence of MPEP on the hyperlocomotion induced by phencyclidine (PCP) or amphetamine (AMPH). The mean values of locomotor activity induced by AMPH or PCP were accepted as 100%. The results were shown as a percentage (mean ± SEM) of those values. Statistical significance was estimated by one-way ANOVA and LSD tests vs respective AMPH- or PCP-treated rats. * - \(P<0.05\) vs AMPH, ** - \(P<0.01\) vs PCP.
between saline- and phencyclidine-treated rats (Table 1). MPEP administered alone at 2.5 mg/kg did not affect the locomotor activity of rats (Table 1).

In contrast, MPEP administered at 5 mg/kg significantly increased the phencyclidine-enhanced locomotor activity (Fig. 1). The one-way ANOVA showed a significant treatment effect \( F (3,35) = 25.64, P<0.001 \). A subsequent post-hoc analysis revealed a potent effect of phencyclidine in comparison with saline-treated animals \( P<0.01 \) (Table 1), and a significant difference between the animals treated with MPEP prior to phencyclidine and those receiving only phencyclidine \( P<0.01 \) (Fig. 1). MPEP administered alone at 5 mg/kg did not affect the locomotor activity of rats (Table 1).

### Table 1. The effect of phencyclidine (PCP), amphetamine (AMPH) and MPEP given alone on the locomotor activity of rats.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control Activity (activity counts, mean ± SEM)</th>
<th>PCP 2.5 mg/kg</th>
<th>AMPH 1 mg/kg</th>
<th>MPEP 2.5 mg/kg</th>
<th>MPEP 5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>153 ± 11</td>
<td>357 ± 59**a</td>
<td>-</td>
<td>222 ± 22 (NS)</td>
<td>-</td>
</tr>
<tr>
<td>Experiment II</td>
<td>169 ± 22</td>
<td>629 ± 78**b</td>
<td>-</td>
<td>-</td>
<td>220 ± 37 (NS)</td>
</tr>
<tr>
<td>Experiment III</td>
<td>113 ± 15</td>
<td>-</td>
<td>835 ± 129**c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Experiment IV</td>
<td>138 ± 14</td>
<td>-</td>
<td>804 ± 100**d</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a - a control group for combined treatment with PCP and MPEP at 2.5 mg/kg; b - a control group for combined treatment with PCP and MPEP at 5 mg/kg; c - a control group for combined treatment with AMPH and MPEP at 2.5 mg/kg; d - a control group for combined treatment with AMPH and MPEP at 5 mg/kg. ** P<0.01 vs. respective control (solvent-treated) rats.

The effect of MPEP on the D-amphetamine-induced locomotor activity

D-amphetamine significantly increased the locomotor activity of rats (Table 1). That effect was not affected by pretreatment with MPEP at 2.5 mg/kg (Fig. 1). A one way ANOVA showed a significant treatment effect \( F(2,25) = 11.09, P<0.001 \), but a post-hoc comparison revealed only a significant difference between saline- and amphetamine-treated rats (Table 1).

Pretreatment with MPEP at 5 mg/kg significantly decreased the locomotor stimulant effect of D-amphetamine (Fig. 1). A one-way ANOVA showed a significant treatment effect \( F(2,25) = 33.93, P<0.001 \). A subsequent post-hoc analysis revealed a significant effect of amphetamine in comparison with saline-treated animals \( P<0.01 \) (Table 1), and a significant difference between the animals treated with MPEP prior to amphetamine and those receiving only amphetamine \( P<0.05 \) (Fig. 1).
DISCUSSION

The present study shows that MPEP, a selective mGluR5 antagonist, evokes an opposite effect on the locomotor activity enhanced by an NMDA receptor antagonist phencyclidine and on hyperactivity produced by an indirect dopamine agonist amphetamine in rats. MPEP diminishes the amphetamine-induced locomotor stimulation, but potentiates phencyclidine-induced hyperlocomotion. This compound administered alone had no effect on locomotor activity, which is consistent with some earlier reports (8, 9).

The inhibition of the amphetamine-increased locomotion by MPEP in mice has only just been reported by McGeehan et al. (10), however, the mechanism responsible for this phenomenon is not clear. At least two possibilities should be considered. Firstly, the blockade of mGluR5 localized in the nucleus accumbens or the ventral tegmental area (11, 12) may inhibit mesolimbic dopaminergic neurons, thereby reversing the stimulatory effects of amphetamine on dopamine release in the nucleus accumbens (13) and on locomotor activity. In accordance with this assumption, stimulation of group I mGluRs enhanced the NMDA-induced oscillations of dopaminergic mesolimbic neurons (14) and activated the locomotion of rats (15). Moreover, despite the fact that no data on the effect of MPEP on dopamine level in the nucleus accumbens are available, our previous study showed that this compound at a dose of 5 mg/kg reduced the extracellular level of dopamine and inhibited the metamphetamine-induced dopamine release in the striatum (16). However, another mechanism of MPEP action also seems feasible. The amphetamine-induced increase in extracellular dopamine level results from the functional inhibition and reversal of the dopamine transporter due to its phosphorylation (17). Since the stimulation of mGluR5 also activates phosphorylation of dopamine transporter (18), it is supposed that the blockade of these receptors by MPEP may induce an opposite effect, thereby stabilizing the synaptic level of dopamine, and counteracting the amphetamine-induced behavioral effects.

In contrast to amphetamine, locomotor stimulation induced by the blockade of NMDA receptors by PCP or other antagonists seems to depend only partly on dopamine release in the nucleus accumbens (19), being mostly dopamine independent (20). Since MPEP inhibited the dopamine-dependent amphetamine-induced hypermotility, it seems that its augmenting effect on PCP-induced effects involves non-dopaminergic mechanisms. In line with this concept, several studies have reported positive interactions between NMDA and mGluR5 receptors. The activation of NMDA receptors enhances the mGluR5-mediated responses (21), whereas the activation of mGluR5 increases those mediated by NMDA (22, 23). Therefore, it may be speculated that the blockade of mGluR5 receptors by MPEP may potentiate the blockade of NMDA receptors induced by PCP and finally may increase the behavioral effect of the latter compound.
The hyperactivity induced by amphetamine or PCP is frequently used as a model for screening of potential antipsychotic compounds. However, since MPEP exerted opposite influence on these phenomena, it is difficult to estimate its putative role as an antipsychotic compound. The MPEP-induced suppression of the amphetamine effect may indicate its antipsychotic effect but the simultaneous potentiation of NMDA receptor hypofunction by this compound may contradict it. In agreement with the latter suggestion, MPEP has been found to augment also the phencyclidine-induced sensorimotor gating deficit, which is an animal equivalent of the disturbances observed in schizophrenia (24, 25). Similarly, mGluR5-deficient mice exhibited sensorimotor gating deficit (25).

Summing up, the present study apparently suggests that due to complex neuronal interactions, the blockade of mGluR5 receptors does not seem to be a promising approach to the treatment of psychotic states.

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