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Antidepressant-like effect of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist in the olfactory bulbectomized rats

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Summary. Using the olfactory bulbectomy model of depression, we examined the antidepressant-like activity of 2-methyl-6-(phenylethynyl)-pyridine (MPEP) in rats. Bulbectomized rats required a significantly greater number of trials to acquire the response similar to sham-operated controls in the passive avoidance model. Both the prolonged (but not acute) treatment with MPEP and with antidepressant drug-desipramine restored the learning deficit. The results indicate that the prolonged blockade of mGlu5 receptors exerts antidepressant-like effects in rats.

Keywords: Excitatory amino acids – Metabotropic glutamate receptors – mGlu5 receptors – MPEP – Antidepressant effects – Olfactory bulbectomy – Animal models of depression

Depression is a psychiatric disorder with high morbidity and mortality. The WHO estimates that at present depression is the fourth cause of human disability-adjusted life years and predicts that it can be the second by the year 2020 (Murray and Lopez, 1997). Although antidepressant drugs influencing monoaminergic systems in the brain were discovered almost 50 years ago, the mechanism of their therapeutic action remains unknown and the monoaminergic theory of depression no longer provides explanation of the mode of action of all antidepressant drugs.

Glutamate, which is abundant in the brain (Mc Geer et al., 1987), plays a major role in both the physiology and pathophysiology of the central nervous system. Glutamate exerts its effects by stimulation of ionotropic and metabotropic glutamate receptors (Monaghan et al., 1989; Pin and Duvoisin, 1995). The recent data show the adaptation of ionotropic glutamate receptors of N-methyl-D-aspartic acid (NMDA) receptor complex after antidepressant treatment (Skolnick et al., 1996). That, together with findings that functional NMDA receptor antagonists possess antidepressant-like actions (Layer et al., 1995; Skolnick et al., 1996), indicates the possible involvement of glutamatergic system in the etiology of depression. More recently discovered metabotropic glutamate receptors 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 mGlu receptors (mGlu1 and mGlu5) are positively coupled to phospholipase C; group II mGlu receptors (mGlu2 and mGlu3) and group III mGlu receptors (mGlu4, mGlu6, mGlu7 and mGlu8) are negatively coupled to adenylate cyclase (Conn and Pin, 1997).

It has been proposed an involvement of group I mGlu receptors in psychiatric disorders such as depression and anxiety. It has been shown that antagonists of group I mGlu receptors exert anxiolytic-like effects after intrahippocampal injection in rats (Chojnacka-Wojcik et al., 1997), and that chronic antidepressant treatment influences expression of group I mGlu receptors in the hippocampus (Bajkowska et al., 1999; Pilc et al., 1998; Zahorodna and Bijak, 1999). Up to now studies concerning involvement of mGlu5 receptors in CNS functions were largely based on compounds which had only limited selectivity between mGlu1 and mGlu5 receptor subtypes (Nicoletti et al., 1996) and which do not penetrate through the blood-brain barier. Only recently novel, selective and systemically active compounds have been described (Gasparini et al., 1999; Varney et al., 1999). The most potent of this series is 2-methyl-6-(phenylethynyl)-

pyridine (MPEP), a noncompetitive antagonist with an IC_{50} of 36 nM at the human mGlu5a receptor in the PI hydrolysis assay but no significant effect at other metabotropic or ionotropic glutamate receptors (Gasparini et al., 1999). To evaluate whether MPEP has antidepressant-like effects, we studied its effects in the olfactory bulbectomy model of depression in rats.

Bilateral olfactory bulbectomy in the rat is associated with neurochemical, physiological and behavioural changes which parallel some of the symptoms observed in depressed patients (Jesberger and Richardson 1985). This symptoms are reversed by chronic, but not acute, treatment with antidepressant drugs (Cairncross et al., 1979; Jancsar and Leonard, 1984; Leonard and Tuite, 1981; Lloyd et al., 1983). Other classes of psychotropic drugs, such as anxiolytics and antipsychotics, do not reverse bulbectomy-induced behavioural deficits (van Riezen et al., 1977). Hence our earlier studies have shown that MPEP exerts antidepressant-like effect in the tail suspension test, but not in the forced swimming test [(Tatarczynska et al., 2001) 2544/id], we decided to investigate if MPEP produces antidepressant-like effect in the olfactory bulbectomy model of depression.

Methods

The experiments were performed on male Wistar rats (200–250 g). The animals were kept on a natural day-night cycle at a room temperature of 19–21°C, with free access to food and water. Each experimental group consisted of 8 naive animals. The experiments were performed by an observer blind 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.

Olfactory bulbectomy; surgical procedure

Two weeks after arrival in the laboratory, bilateral olfactory bulbectomy was performed under equisetine anesthesia. Following exposure of the skull, burr hole were drilled 7 mm anterior to the bregma and 2 mm either side of the middle line at a point corresponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction and the burr holes were filled with haemostatic sponge. The skin was closed. Sham-operated animals were treated in the same way but the olfactory bulbs were left intact. The animals were allowed to recover for 14 days following surgery, they were handled daily by the experimenter throughout the recovery period to eliminate any aggressiveness that would otherwise arise.

Passive avoidance experiments

The apparatus consisted of an open topped box with black walls, 55 cm square with a stainless steel grid floor. The rods were 1, 2 cm apart and connected to the terminals of a stimulator delivering square wave pulses at a constant voltage. The shock delivered was constant at 0.75 mA and lasted 1 s. In the center there was a wooden platform 12 × 12 × 4 cm. Each rat was placed on this platform and when it has left the platform with all four paws it received an electric shock. The animal was immediately removed from the experimental cage and transferred to its home cage. After 30 sec the next trial was initiated. The training of the rat was stopped if the rat learned to stay still on the platform for one minute or if fifteen trials were given.

Drug treatment

Two weeks following surgery chronic (14 days) drug treatment began. 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and desipramine (Sigma) were dissolved in sterile saline and administered intraperitoneally (i.p.). The last dose was given 45 min before the passive avoidance test. All drugs were given in an injection volume of 1 ml/kg at a dose of 10 mg/kg. Control animals received injections of destilled water in volume of 1 ml/kg.

Results

Antidepressant-like effect of MPEP

As shown in Table 1, four weeks after surgery (2 weeks after chronic saline treatment) sham operated animals learned in the passive avoidance procedure in approx. 4 trials. Rats with bilateral olfactory bulb ablation needed an average of 9 trials to reach this criterion. Desipramine, given once daily in dose of 10 mg/kg, restored the learning deficit of bulbectomized rats after 14 days of treatment (Table 1), remaining without any effect in the sham-operated animals. MPEP, an antagonist of mGluR5 receptors diminished the effect of bulbectomy and had no influence on behavior of sham-operated animals (Table 1). In the case of single drug administration neither desipramine nor MPEP or citalopram were effective in restoring the learning deficits (results not shown).

Table 1. Effect of olfactory bulbectomy, MPEP, desipramine on passive avoidance learning

Treatment	dose (mg/kg)	number of trials	N
Sham-op	–	4.375 ± 0.98	8
OB.	–	9.125 ± 1.48**	8
Sham + DMI	10	3.69 ± 0.98	8
OB. + DMI	10	5.82 ± 0.42[a]	8
Sham + MPEP	10	5.26 ± 0.644	8
OB. + MPEP	10	5.25 ± 0.98[a]	8

MPEP or desipramine (*DMI*) were administered two weeks after olfactory bulbectomy (*OB*) for 14 days, i.p. The test was performed 45 min after last dose. Values are the mean ± S.E.M, n = 8, * $P < 0.01$vs sham operated animals, [a] $P < 0.01$ vs OB animals.

Conclusions

Different animal models have been developed both to detect antidepressant activity and to attempt to simulate certain aspects of the disease. The behavioural despair test (Porsolt et al., 1978), and the tail suspension test (Steru et al., 1985) are often used to predict the antidepressant-like effects of drugs. The earlier studies have shown that MPEP did shorten the immobility time in a tail suspension test, while it was inactive in the behavioral despair test. The tail suspension, comparing to the behavioral despair test, has a higher predictive validity for identifying potentially useful pharmacotherapies for depression, e.g. it detects the antidepressant effects of specific serotonin reuptake inhibitors (Ali-Kodja et al., 1986). Olfactory bulbectomy model of depression detects the antidepressant-like activity of several classes of antidepressant drugs, including substances which do not act on the monoaminergic systems in brain (Lloyd et al., 1983). Other classes of psychotropic drugs such as anxiolytics and antipsychotics, do not reverse bulbectomy-induced behavioural deficits (van Riezen et al., 1977).

MPEP restored the learning deficit in the olfactory bulbectomy model of depression, in a manner similar to classical antidepressant drug-desipramine, used as a positive control. The effect of MPEP was unrelated to its purported "amnestic" effect, because MPEP do not produce learning impairments (Spooren, personal communication). The substance also does not change exploratory activity so sedation motor impairments possibly were not responsible for the effect of MPEP (Tatarczynska et al., 2001). Earlier data indicate that the excitatory effect of an agonist of the group I mGlu receptor system is influenced by prolonged treatment with an antidepressant drug imipramine or by chronic electroconvulsive (ECS) treatment (Pilc et al., 1998). In those experiments performed in the CA1 area of the hippocampus, the (R,S)-3,5-dihydroxyphenylglycine-mediated increase of the population spike, was attenuated both by chronic imipramine and ECS (Pilc et al., 1998). It can be speculated therefore, that the inhibition of group I mGlu receptor mediated neurotransmission can contribute to the antidepressant-like effect of MPEP.

The preclinical data indicate that compounds, which reduce transmission at NMDA receptors, display antidepressant-like activity (Skolnick 1999). Glutamatergic transmission via stimulation of group I mGlu receptors has also been shown to potentiate the ionotropic glutamate responses in various preparations (Glaum and Miller, 1993, 1994), including potentiation of NMDA currents (Fitzjohn et al., 1996; Ugolini et al., 1999). The blockade of group I mGlu receptors by MPEP may therefore lead to a decrease in NMDA-receptor-mediated neurotransmission and might contribute to the antidepressant-like effect of MPEP. It can be speculated that MPEP, which neither causes sedation nor disturbs the rota-rod performance, might not produce side effects typical for other antagonists of NMDA receptors.

In conclusion, MPEP is a selective, systematically active antagonist of mGlu5 receptors. It produced antidepressant-like effect in the olfactory bulbectomy model of depression. The above results indicate that antagonists of mGlu5 receptors may play a role in the therapy of anxiety and/or depression. Identification of the sites of action of MPEP and of the mechanism of these effects still requires further studies.

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