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# Effects of ifenprodil on the antidepressant-like activity of NMDA ligands in the forced swim test in mice

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ABSTRACT

Multiple pre-clinical and clinical studies clearly displayed implication of the NMDA receptors in development of depressive disorders since a variety of NMDA receptor antagonists exhibit an antidepressant-like effect. The main aim of our study was to assess the influence of ifenprodil — an allosteric modulator selectively binding at the NR2B subunit on the performance in the forced swim test in mice of various NMDA receptor ligands interacting with distinct components of the NMDA receptor complex. Ifenprodil at a dose of 10 mg/kg enhanced the antidepressant-like effect of CGP 37849 (a competitive NMDA receptor antagonist, 0.312 mg/kg), L-701,324 (an antagonist at glycine site, 1 mg/kg), MK-801 (a non-competitive antagonist, 0.05 mg/kg) and D-cycloserine (a partial agonist of a glycine site, 2.5 mg/kg) but it did not shorten the immobility time of animals which concurrently received an inorganic modulator of the NMDA receptor complex, such as $Zn^{2+}$ (2.5 mg/kg) or $Mg^{2+}$ (10 mg/kg). On the other hand, the antidepressant-like effect of ifenprodil (20 mg/kg) was reversed by *N*-methyl-D-aspartic acid (an agonist at the glutamate site, 75 mg/kg) or D-serine (an agonist at the glycine site, 100 nmol/mouse).
In conclusion, the antidepressant-like potential of ifenprodil given concomitantly with NMDA ligands was either reinforced (in the case of both partial agonist and antagonists, except for magnesium and zinc) or diminished (in the case of conventional full agonists).



## 1. Introduction

Depression is one of the most common recurring psychiatric diseases widespread all over the world, which prevalence is still increasing. Development of depressive disorders is preliminarily associated with disturbances in noradrenergic and serotonergic transmission in brain (Willner et al., 2012). However, multiple pre-clinical and clinical studies clearly displayed implication of the ionotropic NMDA receptors, as well. Alterations of the glutamate levels and NMDA receptor abnormalities were observed in depressive patients participating in clinical trials (Hashimoto, 2009; Law and Deakin, 2001; Nowak et al., 2003; Sanacora et al., 2004). Moreover, the involvement of the NMDA neurotransmission in the antidepressant-like effects of paroxetine and tianeptine has been reported (Ghasemi et al., 2009; Wlaź et al., 2011). Similar interactions between the NMDA receptor ligands and imipramine, fluoxetine and reboxetine were noticed by Poleszak et al. (2011).

The NMDA receptor heteromeric complex is considered as a tetramer comprising two glycine-binding NR1 subunits with two glutamate-binding NR2 subunits or, less frequently two glycine-binding NR3 subunits. NR1 subunit, ubiquitously distributed in central nervous system, is encoded by a single gene but occurs as eight distinct isoforms. NR2 subunits (NR2A, NR2B, NR2C and NR2D) are encoded by four different genes. Their distribution in the central nervous system is not uniform — NR2A subunits are commonly found in the brain whereas NR2B expression is restricted primarily to the forebrain; NR2C subunits are predominantly localised in the cerebellum. The type of NR2 subunit is thought to determine functional and pharmacological properties of the whole NMDA receptor complex (Bhatt et al., 2013; Traynelis et al., 2010; Williams, 2009). There are numerous binding sites within the NMDA receptor complex, i.e. a binding site for glycine or D-serine, a glutamic acid binding site, the binding sites for zinc, magnesium, polyamines, redox agents and others (Monaghan and Jane, 2009).

*Abbreviations:* CGP 37849, DL-/E/-amino-4-methyl-5-phosphono-3-pentenoic acid; DCS, D-cycloserine (D-4-amino-3-isoxazolidone); DS, D-serine; FST, forced swim test; i.c.v, intracerebroventricularly; i.p, intraperitoneally; L-701,324, 7-chloro-4-hydroxy-3-(3-phenoxy)phenylquinolin-2[1H]-one; MK-801, dizocilpine ((5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate); NMDA, *N*-methyl-D-aspartate; TST, tail suspension test.

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A variety of NMDA receptor antagonists exhibited an antidepressant-like effect, measured by the forced swim test (FST) and/or tail suspension test (TST) in animal models (Maj et al., 1992b, 1992c; Poleszak et al., 2007a; Rosa et al., 2003). Their activity was comparable to that observed for antidepressant used in clinical practise (Decollogne et al., 1997; Lopes et al., 1997; Wędzony et al., 1995). Yet, high-affinity NMDA receptor blockers like MK-801 and phencyclidine, acting within the ion channel or CGP 37849, an inhibitor of the glutamate site, exert severe side effects (like ataxia, memory loss, increased locomotion) that disqualify them from clinical utility (Farlow, 2004; Tricklebank et al., 1989; Willetts et al., 1990). However, ketamine, traxoprodil and memantine were successfully used in depressive patients (Berman et al., 2000; Preskorn et al., 2008; Zarate et al., 2006a, 2006b). Ifenprodil and its analogues devoid of many adverse reactions, seem to be more promising, better tolerated agents. According to Kew et al. (1996), ifenprodil effectively inhibits the NMDA receptors activated by high concentrations of glutamate; though this inhibition is not thorough — the basal level of glutamate neurotransmission remains. In contrast, the first-generation NMDA receptor antagonists cause the generalised blockage of receptor activity. Such dissimilarity in drug action may be responsible for the differences in their safety profiles (Bhatt et al., 2013; Mony et al., 2011).

As the conventional antidepressant therapy is not sufficient, due to the frequent lack of clinical efficacy along with the undesirable side effects, scientists still search for better alternatives (i.e. new compounds or safe combinations of the well-known agents) (Willner et al., 2012). The main objection of our study was to assess the influence of ifenprodil — an allosteric modulator selectively binding at the NR2B subunit, on the performance in the FST in mice of various NMDA receptor ligands interacting with distinct components of the NMDA receptor complex. The FST, besides TST, is the most popular validated model evaluating an antidepressant activity of substances (Petit-Demouliere et al., 2005).

## 2. Methods

### 2.1. Animals

Experiments were conducted on naïve adult male Albino Swiss mice (25–30 g). The animals were housed in groups of 10 in standard cages, in the environmentally controlled rooms, under a 12:12 h light/dark cycle. They had free access to food and water except for the short time when they were removed from their home cages for testing. The experiments began after at least 1-week acclimation period in the laboratory conditions. Each experimental group consisted of 8–11 randomly assigned animals. Each mouse was tested only once. Separate groups of animals were used in the FST and in the locomotor studies. All experimental procedures involving animals were conducted in accordance with European Union and Polish legislation acts concerning animal experimentation. They were approved by the Local Ethics Committee at the Medical University of Lublin. All efforts were made to minimize animal suffering and to reduce the number of mice used in the experiments.

### 2.2. Drugs

The following agents were used: ifenprodil (10 mg/kg or 20 mg/kg, Sigma), NMDA (*N*-methyl-D-aspartic acid, 75 mg/kg, Sigma), CGP 37849 (dl-(E)-amino-4-methyl-5-phosphono-3-pentenoic acid, 0.3 mg/kg, Abcam Biochemicals), D-cycloserine (d-4-amino-3-isoxazolidone, 2.5 mg/kg, Sigma), L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy) phenylquinolin-2[1H]-one, 1 mg/kg, Sigma), MK-801 (dizocilpine, (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine hydrogen maleate, 0.05 mg/kg, Sigma), D-serine (100 nmol/mouse, Sigma), magnesium hydroaspartate (Farmapol, Poznań, Poland) and zinc hydroaspartate (Farmapol, Poznań, Poland). Dosages of magnesium and zinc referred to pure magnesium and zinc ions — 10 mg/kg and 2.5 mg/kg, respectively. All substances (except for L-701,324) were dissolved in physiological saline. L-701,324 was suspended in a 1% aqueous solution of Tween 80 (POCH). All solutions were prepared immediately prior to the experiment. The solutions were administered intraperitoneally (i.p.), except for D-serine which was administered intracerebroventricularly (i.c.v.) according to a modified method described by Lipman and Spencer (1980). Ifenprodil, NMDA, CGP 37849, D-cycloserine, L-701,324, MK-801 and zinc hydroaspartate were given 60 min before behavioural testing while magnesium hydroaspartate and D-serine were injected 30 min and 15 min before the experiment, respectively. The active and ineffective doses of drugs were selected on the basis of the results of previous experiments (Poleszak et al., 2007a, 2011; Szewczyk et al., 2009). The active and sub-effective doses of ifenprodil have been selected on the basis of the unpublished outcomes of previous tests performed in our laboratory. Animals from the control groups received i.p. or i.c.v. injections of saline (vehicle), depending on the tested group. In order to avoid the risk of obtaining the false results caused by an additional activation of glutamatergic system after i.c.v. administration, each animal in the experiments with D-serine was given an i.c.v. injection — either D-serine or vehicle. The volume of vehicle or drug solutions for i.p. administration was 10 ml/kg and for i.c.v. administration was 5 μl per mouse.

### 2.3. Forced swim test

Forced swim test was performed according to the method described by Porsolt et al. (1977). Mice were individually placed into the glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C. The animals were left in the cylinders for 6 min. The total duration of immobility was recorded during the last 4 min of the 6-min testing period. The mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only the movements necessary to keep their heads above the water level.

### 2.4. Spontaneous locomotor activity

In order to ensure that the changes in motor activity of mice did not disturb the interpretation of the results obtained in the FST, the spontaneous locomotor activity was measured using an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, Columbus, OH, USA). This automatic device consists of four transparent cages with a lid, set of four infrared emitters (each emitter has 16 laser beams) and four detectors monitoring animal movements. Mice were placed individually in the cages for 30 min. Activity was evaluated between the 2nd and the 6th minute, which corresponds with the time interval analysed in the FST. The spontaneous locomotor activity was measured by determining the amount of distance travelled in centimetres.

### 2.5. Statistical analysis

The obtained data were assessed by the one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls *post hoc* test. All results were presented as the means ± standard error of the mean (SEM). $p < 0.05$ was considered as a statistically significant difference.

## 3. Results

### 3.1. Effect of the sub-effective dose of ifenprodil in combination with the sub-effective dose of CGP 37849 on the immobility time in the FST in mice

Fig. 1 shows that the joint administration of ifenprodil and CGP 37849 produced a significant reduction in the immobility time of animals in the FST, though neither of the substances at the used doses exerted the effect by itself. However, this anti-immobility effect was only observed in comparison to the CGP 37849 group. One-way