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Short communication

Activation of the NMDA/glutamate receptor complex antagonizes the NMDA antagonist-induced antidepressant-like effects in the forced swim test

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Abstract:
The antidepressant activity of NMDA receptor antagonists has been demonstrated, and their mechanism of action was based on the assumption of their selectivity for the NMDA receptor only. However, no direct evidence for the NMDA receptor role in this activity was demonstrated.
Now, in order to prove the NMDA pathway of antidepressant-like action of the NMDA antagonists in the mouse forced swim test (FST) we examined if antidepressant activity of NMDA receptor antagonists is mediated by NMDA receptors and whether the activation of different modulatory sites of the NMDA receptor complex influence the action of the antagonists of different sites of NMDA receptor.
In our study, we used two NMDA ligands: competitive NMDA glutamate site antagonist CGP 37849, and glycine$_B$ antagonist L-701,324; both at doses found to be effective in the FST. The antidepressant-like activity of the compounds was abolished by the N-methyl-D-aspartic acid (NMDA) or by D-serine co-treatment. Ligands at the doses active in the FST did not alter locomotor activity.
The present study indicates the major role of the NMDA/glutamate pathway in the antidepressant-like activity of NMDA antagonists in the mouse FST.

Key words:
NMDA receptor, glutamate site, glycine$_B$ site, ligands, forced swim test, mice

Introduction

The NMDA receptor is an ionotropic glutamate receptor with the highest densities in the brain [5]. It is a receptor complex that consists of an integral ion channel with multiple, allosterically coupled recognition sites, including both a high affinity site for glutamate and a strychnine-insensitive glycine binding site, known as a $glycine_B$ receptor [33, 40]. The glycine binding site is activated by endogenous glycine, and glycine binding is an absolute requirement for NMDA receptor activation by glutamate [10] and, therefore, glycine acts as a co-agonist with glutamate [9].

The NMDA receptors participate in a wide range of both physiological and pathological processes of the central nervous system. A high density of NMDA receptors has been found in the cortico-limbic regions of the brain which have been postulated to play a role in emotional functions, anxiety and depression [39]. Extensive studies demonstrated antidepressant-like effects of various antagonists of the NMDA receptors. The antidepressant-like activity of competitive and non-competitive antagonists and inorganic inhibitors of NMDA receptor (zinc and magnesium) has been reported [6, 11, 12, 25–27, 30, 31, 35, 37, 38]. However, no direct evidence for the NMDA receptor role in this activity was demonstrated.

The aim of our study was to directly examine if the antidepressant activity of some NMDA receptor antagonists (CGP 37849 and L-701,324) is mediated by the NMDA receptor complex, plus to find out whether the activation of different regulatory domains of the NMDA complex affects the antidepressant action of NMDA receptor antagonists.

Materials and Methods

Animals

All procedures were approved by the Ethics Committee of the Medical University, Lublin and Collegium Medicum, Jagiellonian University, Kraków. The experiments were carried out on male Albino Swiss mice (25–30 g). The animals were kept under a natural day-night cycle with free access to food and water. Each experimental group consisted of 6–10 animals.

Drug administration

7-Chloro-4-hydroxy-3-(3-phenoxy)phenylquinolin-2-[1H]-one (L-701,324, 4 mg/kg, Sigma, USA) was suspended in a 1% aqueous solution of Tween 80 and administered *ip* 60 min before the test. N-methyl-D-aspartic acid (NMDA, 75 mg/kg, Sigma, USA), DL-/E/-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849, 0.625 mg/kg, Tocris, UK) were dissolved in 0.9% saline and administered *ip* 60 min before the test. D-serine (100 nmol/mouse, Sigma, USA) was also dissolved in 0.9% NaCl and administered intracerebroventricularly (*icv*) 15 min before the test. The *icv* administration was performed according to a modified method described by Lipman and Spencer [15]. The control animals received an *ip* or *icv* injection of saline (vehicle). The volume of vehicles or drug solutions for *ip* and *icv* administrations was 10 ml/kg and 5 μl per mouse, respectively.

Forced swim test

The studies were carried out on mice according to the method of Porsolt and co-workers [29]. Mice were propped individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water, maintained at 23–25°C. The animals were left in the cylinder for 6 min. After the first 2 min, the total duration of immobility was measured during a 4-min test. The mouse was judged to be immobile when it remained floating passively in the water.

Locomotor activity

The locomotor activity of the mice was measured with photoresistor actometers (circular cages, diameter 25 cm, two light beams). The animals were placed individually in an actometer for 10 min. Activity was then measured at 5-min intervals to characterize the dynamics of the changes. The number of crossings of the light beams by the mice was recorded as the locomotor activity.

Statistics

The obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by Bonferroni's Multiple Comparison Test, Dunnett's test or Student *t*-test, where appropriate. All the results are

presented as the means ± SEM; $p < 0.05$, which was considered to be statistically significant.

Results and Discussion

Functional antagonists of the NMDA receptor complex exhibit antidepressant-like activity in the rodent test and models of depression. Since 1990, when Trullas and Skolnick [38] demonstrated the antidepressant activity of AP-7, MK-801 and ACPC in the mouse forced swim test (FST) and tail suspension test (TST), the vast number of reports have both confirmed and extended this first achievement. The NMDA antagonists are active in the FST in mice [14, 16] and rats [18, 30] and tail suspension test in mice [14], and in learned helplessness [17], chronic unpredicted stress [23], chronic mild stress [24], and bulbectomy models [32]. Moreover, inorganic NMDA antagonists, zinc and magnesium, are also active in these rodent tests [11, 12, 25, 27]. However, the specificity of NMDA antagonist-induced antidepressant-like effects was not directly proven, but was based on the pharmacological data of the specific (*in vitro*) interaction of these ligands with a variety of receptors (e.g. [7]). NMDA antagonists demonstrate efficacy in clinical studies. Ketamine is effective in major depression [1, 41], although the clinical efficacy of memantine is not quite as obvious [8, 42]. Furthermore, the palliative effect of non-specific NMDA antagonist (amantadine and zinc) supplementation to antidepressant therapy was reported ([22, 34], our unpublished data). On the other hand, antidepressants induce adaptive changes in the NMDA receptor complex [36, 37]. These changes include the reduction of glycine affinity for glycine$_B$ sites as well as reduction in the ability of glycine to modulate glutamate sites [35]. These alterations demonstrated by radioligand binding techniques were confirmed using molecular biology, behavioral and electrophysiological methods [2, 3, 28]. Alterations in this receptor complex were demonstrated in the animal paradigm used for antidepressant screening (FST), in models of depression [20, 21] and suicide victims [19]. Thus, depression may be associated with enhanced NMDA signal transduction and the mechanism of antidepressant effect is related to reduction of this transmission.

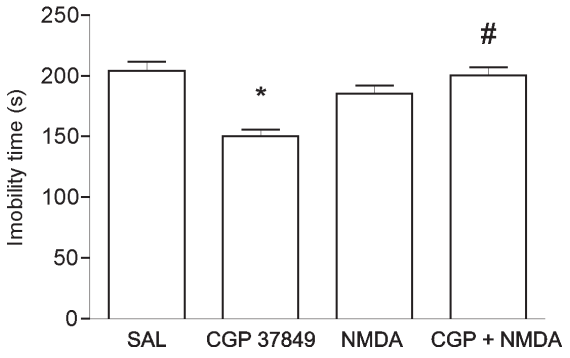

Fig. 1. Effect of joint administration of CGP 37849 and NMDA on immobility time in the FST. The values represent the means ± SEM (n = 8–10 mice per group). ANOVA: $F(3, 38) = 13.12$, $p < 0.0001$. * $p < 0.001$ *vs.* saline (SAL) group; # $p < 0.001$ *vs.* CGP group (Bonferroni's test)

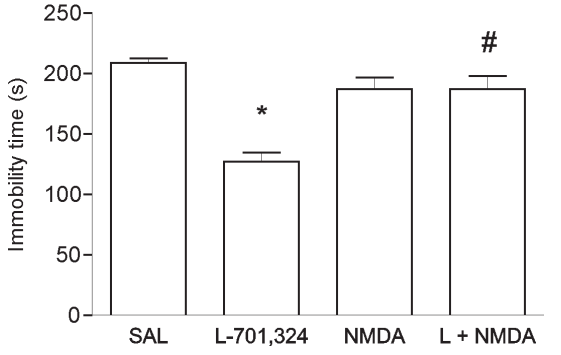

Fig. 2. Effect of joint administration of L-701,324 and NMDA on immobility time in the FST. The values represent the means ± SEM (n = 8–10 mice per group). ANOVA: $F(3, 33) = 14.43$, $p < 0.0001$. * $p < 0.001$, *vs.* saline (SAL) group; # $p < 0.001$ *vs.* L-701,324 group (Bonferroni's test)

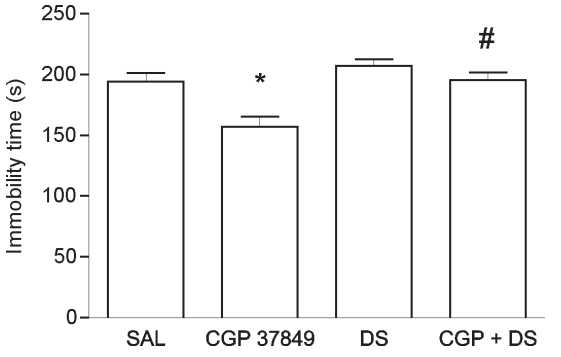

Fig. 3. Effect of joint administration of CGP 37849 and D-serine (DS) on immobility time in the FST. The values represent the means ± SEM (n = 8–10 mice per group). ANOVA: $F(3, 39) = 10.55$, $p < 0.0001$. * $p < 0.01$ *vs.* saline (SAL) group; # $p < 0.01$ *vs.* CGP 37849 group (Bonferroni's test)

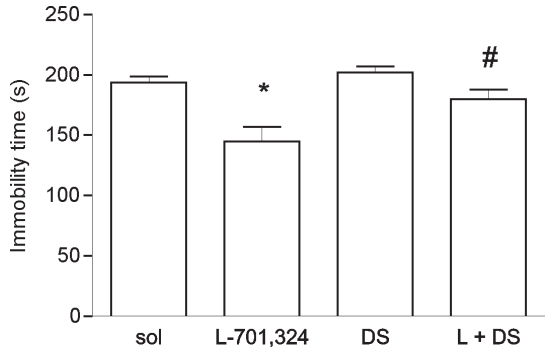

Fig. 4. Effect of joint administration of L-701,324 and D-serine (DS) on immobility time in the FST. The values represent the means ± SEM (n = 8–10 mice per group). ANOVA: $F(3, 41) = 10.26$, $p < 0.0001$. * $p < 0.01$ *vs.* saline (SAL) group, # $p < 0.05$ *vs.* L-701,324 group (Bonferroni's test)

Tab. 1. Effect of administration of NMDA receptor ligands on spontaneous locomotor activity in mice

Treatment	Dose	Activity counts	
		5 min	10 min
A	nmol/mouse (*icv*)/mg/kg (*ip*)		
Control	–	122.3 ± 15.7	172.3 ± 19.4
DS + L-701,324	100/4	89.2 ± 24.2	106.7 ± 23.8
DS + CGP 37849	100/0.625	108.0 ± 16.1	137.0 ± 18.2
B	mg/kg (*ip*)		
Control	–	133.0 ± 7.8	183.0 ± 14.1
L-701,324 + NMDA	4 + 75	107.6 ± 9.8	150.0 ± 11.2
C	mg/kg (*ip*)		
Control	–	109.4 ± 14.6	169.8 ± 23.5
CGP 37849 + NMDA	0.625 + 75	115.5 ± 13.8	138.5 ± 15.7

NMDA, L-701,324, CGP 37849 (*ip*) were administered 60 min before the tests, D-serine (DS) was administered *icv* 15 min before test. Control animals received either two *ip* injections or *ip* and *icv* injections given at respective times. The values represent the means ± SEM of 6–9 mice per group. The NMDA, D-serine, CGP 37849 and L-701,324 administered alone did not affect the locomotor activity (data were published in [26]). **A:** ANOVA: $F(2,18) = 0.6935$, $p = 0.5127$ for 5 min; $F(2,18) = 2.401$, $p = 0.1191$ for 10 min. **B:** Student's *t*-test: $t(15) = 1.292$, $p = 0.2158$ for 5 min; $t(15) = 1.852$, $p = 0.0838$ for 10 min. **C:** Student's *t*-test: $t(15) = 0.3051$, $p = 0.7645$ for 5 min; $t(15) = 1.075$ $p = 0.2993$ for 10 min

The present data confirmed data reported by other authors, namely that both CGP 37849 (a competitive NMDA receptor antagonist, [7]) and L-701,324 (glycine$_B$ receptor antagonist, [13]) exhibited antidepressant-like activity in rodent tests [13, 22]. CGP 37849 at a dose of 0.625 mg/kg (Fig. 1 and 3) and L-701,324 (4 mg/kg, Fig. 2 and 4) administered *ip* significantly reduced the immobility time in the FST in mice.

In the previous studies, the antidepressant effects seemed to be NMDA-specific, since the NMDA antagonists used are selective for specific sites of the NMDA receptor complex (e.g. [7]). However, the only direct evidence was demonstrated by Trullas and Skolnick, who showed that glycine co-administration had antagonistic effect on the antidepressant-like properties of ACPC (glycine$_B$ site partial agonist) [38]. The present data demonstrated that the antidepressant-like effect of CGP 37849 and L-701,324 was abolished by N-methyl-D-aspartate acid (NMDA) co-treatment. NMDA given alone at the dose of 75 mg/kg had no significant effect on the immobility time (Fig. 1 and 2). However, when NMDA was co-administered with CGP 37849 (0.625 mg/kg), it abolished the CGP 37849-induced antidepressant-like effect (Fig. 1). Also when NMDA was co-administered with L-701,324 (4 mg/kg), it inhibited the antidepressant-like effect of L-701,324 (Fig. 2).

We also demonstrated that D-serine, an agonist of the glycine$_B$ site, antagonized the antidepressant-like effects of the NMDA antagonists. D-serine given alone at a dose of 100 nmol/mouse had no effect on the immobility time (Fig. 3 and 4). When D-serine was combined with CGP 37849 (0.625 mg/kg), it abolished the CGP 37849-induced antidepressant-like effect (Fig. 3). Also, when D-serine was combined with L-701,324 (4 mg/kg), it reversed L-701,324-induced antidepressant-like effect (Fig. 4).

These data indicate that the stimulation of the glutamate site of the NMDA receptor complex antagonized the antidepressant-like activity of antagonists not only of glutamate, but also of the glycine$_B$ site. Similar results were obtained with the D-serine co-treatment. D-serine, a glycine$_B$ site agonist, abolished the antidepressant-like activity of the glycine$_B$ site antagonist L-701,324 as well as the glutamate site antagonist CGP 37849 (Fig. 3 and 4). Since the motor activity was not altered by all tested agents (Tab. 1), the effects in the FST were not related to psychostimulant activity. All present results indicate that the activation of the neurotransmitter glutamate site or the glycine$_B$ co-transmitter site of the NMDA receptor complex abolishes the antidepressant-like activity of antagonists of either site. These observations clearly

demonstrate that the antidepressant activity of NMDA antagonists is connected with reduction of NMDA receptor complex function.

In summary, this is the first direct demonstration that the activation of the NMDA receptor complex (by glutamate or the glycine$_B$ site) abolishes the antidepressant-like effect of antagonists of the NMDA receptor complex in the FST, further indicating the main role of the NMDA/glutamate pathway in the antidepressant activity of NMDA antagonists.

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