Pharmacological Reports
2013, 65, 1124–1131
ISSN 1734-1140

NMDA receptor activation antagonizes the NMDA antagonist-induced antianxiety effect in the elevated plus-maze test in mice

Ewa Poleszak[1], Anna Serefko[1], Aleksandra Szopa[1], Sylwia Wośko[1], Jarosław Dudka[2], Andrzej Wróbel[3], Tomasz Oniszczuk[4], Piotr Wlaź[5]

[1]Department of Applied Pharmacy, Medical University of Lublin, Chodźki 1, PL 20-093 Lublin, Poland

[2]Medical Biology Unit, [3] Second Department of Gynecology, Medical University of Lublin, Jaczewskiego 8, PL 20-054 Lublin, Poland

[4] Department of Food Process Engineering, Lublin University of Life Sciences, Doświadczalna 44, PL 20-236 Lublin, Poland

[5] Department of Animal Physiology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Akademicka 19, PL 20-033 Lublin, Poland

Correspondence: Ewa Poleszak, e-mail: ewa.poleszak@umlub.pl

Abstract:
Background: The purpose of this study was to determine how the activation of different regulatory domains of the NMDA complex affects the antianxiety effect of antagonists acting at its distinct binding sites.
Methods: The anxiolytic-like activity was assessed by the elevated plus-maze test in mice.
Results: The anxiolytic activity of CGP 37849 (a competitive NMDA receptor antagonist) and L-701,324 (an antagonist at glycine site) was confirmed, but effects of both were significantly reduced by *N*-methyl-D-aspartic acid (NMDA) or by D-serine agonists at glutamate and glycine site of the NMDA receptor complex, respectively.
Conclusion: The obtained data suggest that stimulation of the glutamate or glycine recognition site of the NMDA receptor complex significantly decreases the antianxiety properties of antagonists of either site.

Key words:
NMDA receptor ligands, elevated plus-maze test, anxiety, mice

Abbreviations: AMPA – 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, CGP 37849 – DL-(E)-amino-4-methyl-5-phosphono-3-pentenoic acid, DS – D-serine, *icv* – intracerebroventricularly, *ip* – intraperitoneally, L-701,324 – 7-chloro-4-hydroxy-3-(3-phenoxy)phenylquinolin-2[1H]-one, NMDA – *N*-methyl-D-aspartate

Introduction

Results of a number of studies performed in recent years indicate that the glutamatergic neurotransmission *via* ionotropic receptors is involved in the patho-

physiology of anxiety, depression and fear conditioning [11, 13, 31, 40, 45]. Three types of ionotropic glutamate receptors are found in mammalian brain: *N*-methyl-D-aspartate (NMDA), 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate [4, 9]. Among them, NMDA receptor, a complex ion channel, is particularly known as the one which plays a key role in anxiety disorders [2, 12]. Several binding sites for structurally different ligands have been recognized within NMDA receptor, including a high affinity site for glutamate and NMDA, a glycine site physiologically activated by glycine or D-serine (DS), a polyamine site for spermine and spermidine [38, 43]. For activation of NMDA receptor, both glutamate and glycine sites should be occupied simultaneously, as well as depolarization of the membrane by AMPA receptors at the same synapse is required [14]. Under depolarizing conditions, the blockage of ion channels is relieved and the inflow of sodium and calcium ions through the NMDA receptor pore is permitted [15, 41].

The preclinical and clinical data provide strong evidences that diverse antagonists and partial agonists, acting at different sites of NMDA receptor complex exhibit anxiolytic-like activity. Quite often they are compared to benzodiazepines or barbiturates [12, 17, 36].

The aim of our study was to determine how the activation of different regulatory domains of the NMDA complex affects the antianxiety effect of a competitive glutamate site antagonist (CGP 37849) and a glycine site antagonist (L-701,324) in the elevated plus-maze test.

Materials and Methods

Animals

The experiments were conducted on naive, adult male albino Swiss mice weighing 25–30 g. The animals were housed in the environmentally controlled rooms with a 12 h light/dark cycle, in groups of 10 in standard cages. Throughout the study, the animals were given *ad libitum* access to water and food. The experiment began after at least 1-week acclimation period in the laboratory conditions. Each experimental group consisted of 9–12 animals. Each animal was tested only once. The experimental protocol was approved by the Local Ethics Committee at the Medical University of Lublin. All procedures involving animals and their care were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and Polish legislation acts concerning animal experimentation.

Drugs administration

Three separate experimental studies were conducted: (i) evaluation of the effects of joint administration of D-serine and CGP 37849 and joint administration of D-serine and L-701,324, (ii) evaluation of the effect of joint administration of CGP 37849 and NMDA and (iii) evaluation of the effect of joint administration of L-701,324 and NMDA. L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenylquinolin-2[1H]-one, 4 mg/kg, Sigma) was suspended in a 1% aqueous solution of Tween 80 (POCH), while NMDA (75 mg/kg, Sigma) and CGP 37849 (DL-(E)-amino-4-methyl-5-phosphono-3-pentenoic acid, 0.625 mg/kg, Abcam Biochemicals) were dissolved in physiological saline. The solutions were administered intraperitoneally (*ip*) 60 min before behavioral testing. DS (100 nmol/mouse, Sigma) was dissolved in saline and administered intracerebroventricularly (*icv*) 15 min before the test. The *icv* administration was performed according to a modified method described by Lipman and Spencer [20]. Briefly, a 10 µl type 701 glass Hamilton microsyringe with the 26 gauge needle shortened to a length of 7 mm was used. Rigid PVC tubing was put on the needle to limit its penetration to 3 mm. The injection site was approximately 2 mm posterior to and 1 mm lateral (left) of the bregma. The doses of drugs were selected on the basis of the results of previous experiments [29, 32]. All solutions were prepared immediately prior to the experiment. Animals from the control groups received an *ip* or *icv* injections of saline (vehicle). In order to avoid the risk of obtaining false results caused by an additional activation of glutamatergic system after *icv* administration, each animal in the experiments with DS was given an *icv* injection – either DS or vehicle, depending on the tested group. The volume of vehicle or drug solutions for *ip* administration was 10 ml/kg and for *icv* administration was 5 µl per mouse.

Procedure

The elevated plus maze test was conducted as described previously [22]. The elevated plus maze appa-

ratus in the shape of the horizontal cross was made of black polyvinyl chloride. It consisted of two open arms (30 × 5 cm) and two opposite arms (30 × 5 cm) enclosed by 15-cm high walls, which extended from a central platform (5 cm square). The apparatus was elevated 38 cm above floor level on a stable base. The mice were individually tested under the red light. The maze was carefully cleaned after each experiment. At the beginning of a test, a mouse was placed in the center of the apparatus facing one of the enclosed arms and allowed to freely explore the maze for 5 min. The number of open arm entries and the time spent in these arms were recorded. Arm entry was defined as placing all four paws within the boundaries of the arm. The following parameters being the standard anxiety indices were calculated as follows: the percentage of open arm time (the ratio of time spent in the open arms to total time spent in any arm × 100), the percentage of open arm entries (the ratio of entries into open arms to total entries × 100) [39]. As the mice are naturally afraid of open space, an increase in time spent in the open arms or a greater number of open arm entries were associated with an anxiolytic effect. The overall motor activity was measured by a number of closed arm entries. The total number of entries into either type of an arm was also recorded.

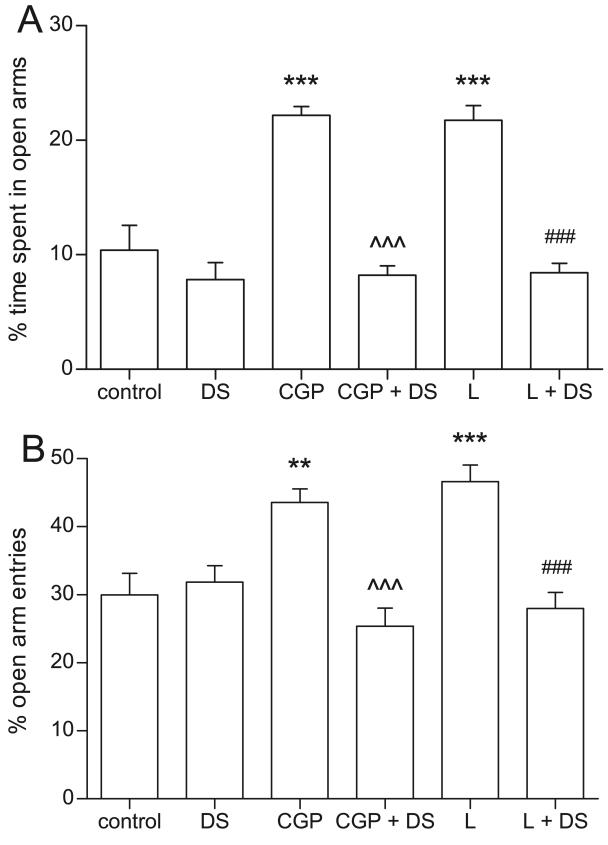

Fig. 1. Effect of joint administration of D-serine (DS) and CGP 37849 (CGP) and joint administration of D-serine (DS) and L-701,324 (L) in the elevated plus-maze procedure in mice [the percentage of the time spent in the open arms (**A**), and the percentage of the open arms entries (**B**)]. DS (100 nmol per mouse) was administered *icv* 15 min before the test and CGP 37849 (0.625 mg/kg) or L-701,324 (4 mg/kg) was administered *ip* 60 min before the test. Each animal in the experiments with DS was given an *icv* injection – either DS or vehicle, depending on the tested group. The values represent the mean + SEM (n = 9–10 mice per group). ** $p < 0.01$, *** $p < 0.001$ *vs.* control, ^^^ $p < 0.001$ *vs.* CGP 37849, ### $p < 0.001$ *vs.* L-701,324 (Student-Newman-Keuls *post-hoc* test)

Data analysis

As the main goal of our study was not to determine the overall factor p values and indicate the overall effect of DS or NMDA but to point out the significant differences between individual pairs of the tested groups, the statistical assessment was basically concentrating on the multiple comparisons. Therefore, a one-way analysis of variance (ANOVA) was used, followed by Student-Newman-Keuls *post-hoc* test. All results are presented as the means ± standard error of the mean (SEM). A $p < 0.05$ was considered statistically significant. All statistical calculations were performed with GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Effect of DS on the anxiolytic-like activity of CGP 37849 in the elevated plus-maze test

Data depicted in Figure 1 show that DS administered at a dose of 100 nmol/mouse did not significantly change either the percentage of time spent in open arms or the percentage of the open arm entries, whereas CGP 37849 given singly at a dose of 0.625 mg/kg significantly increased both the percentage of the time spent in the open arms and the percentage of the open arm entries in comparison with the vehicle-treated group. The anxiolytic effect of CGP 37849 was significantly decreased by DS when administered together, which was observed in the reduced number of the open arm entries and a shorter time spent in the open arms.

One-way ANOVA demonstrated statistically significant differences between tested groups in relation to the percentage of the time spent in open arms [ANOVA: F (3, 34) = 22.42, p < 0.0001] as well as in the relation to the percentage of the open arm entries [ANOVA: F (3, 36) = 8.901, p = 0.0002].

Effect of DS on the anxiolytic-like activity of L-701,324 in the elevated plus-maze test

The antianxiety effect produced by L-701,324 given alone was significantly reversed by co-administration of DS which was illustrated in Figure 1.

One-way ANOVA revealed a statistically significant differences between tested groups in relation to the percentage of the time spent in open arms [ANOVA: F (3, 33) = 16.97, p < 0.0001] as well as in the relation to the percentage of the open arm entries [ANOVA: F (3, 36) = 10.41, p < 0.0001].

Effect of NMDA on the anxiolytic-like activity of CGP 37849 in the elevated plus-maze test

Figure 2 illustrates that both the percentage of time spent in open arms and the percentage of entries into the open arms were altered by CGP 37849 administered *ip* at a dose of 0.625 mg/kg. The antianxiety effect induced by CGP 37849 was significantly reversed by NMDA given at a dose of 75 mg/kg.

One-way ANOVA pointed at a statistically significant differences between tested groups in relation to the percentage of the time spent in open arms [ANOVA: F (3, 36) = 46.07, p < 0.0001] as well as in the relation to the percentage of the open arm entries [ANOVA: F (3, 36) = 24.21, p < 0.0001].

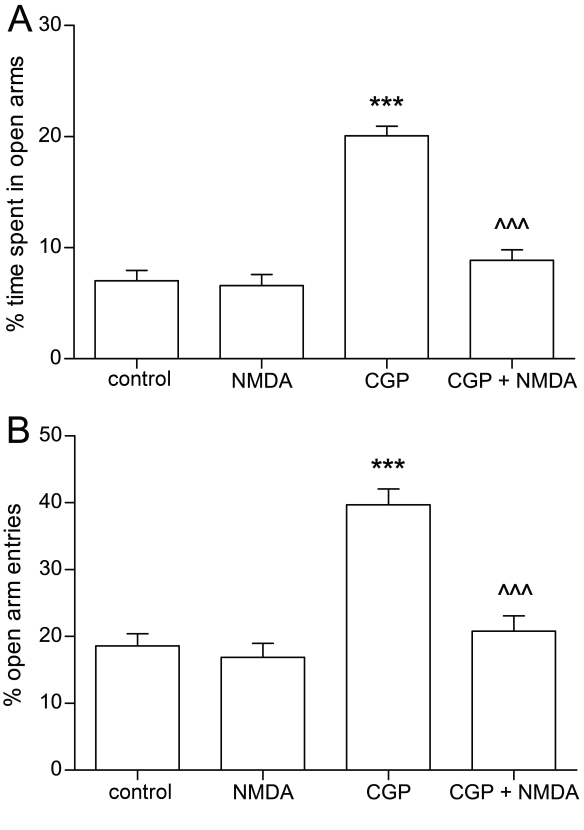

Fig. 2. Effect of joint administration of CGP 37849 (CGP) and NMDA in the elevated plus-maze procedure in mice [the percentage of the time spent in the open arms (**A**), and the percentage of the open arms entries (**B**)]. Both CGP 37849 (0.6 mg/kg) and NMDA (75 mg/kg) were administered *ip* 60 min before the test. The values represent the mean + SEM (n = 10 mice per group).*** p < 0.001 *vs.* control, ^^^ p < 0.001 *vs.* CGP 37849 (Student-Newman-Keuls *post-hoc* test)

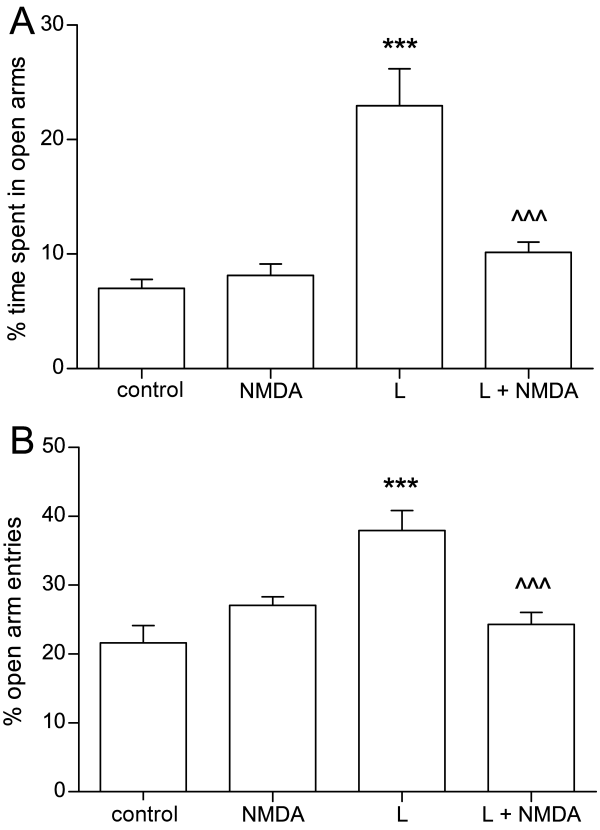

Fig. 3. Effect of joint administration of L-701,324 (L) and NMDA in the elevated plus-maze procedure in mice [the percentage of the time spent in the open arms (**A**), and the percentage of the open arms entries (**B**)]. Both L-701,324 (4 mg/kg) and NMDA (75 mg/kg) were administered *ip* 60 min before the test. The values represent the mean + SEM (n = 9–10 mice per group). *** p < 0.001 *vs.* control, ^^^ p < 0.001 *vs.* L-701,324 (Student-Newman-Keuls *post-hoc* test)

Effect of NMDA on the anxiolytic-like activity of L-701,324 in the elevated plus-maze test

L-701,324 exerted the anxiolytic-like effect when given alone at a dose of 4 mg/kg, which is presented in Figure 3. The joint administration of L-701,324 and NMDA resulted in diminished therapeutic effect of the first agent, measured by the percentage of the open arm entries and the total time spent in the open arms.

One-way ANOVA demonstrated statistically significant differences between tested groups in relation to the percentage of the time spent in open arms [ANOVA: $F(3, 34) = 17.95$, $p < 0.0001$] as well as in the relation to the percentage of the open arm entries [ANOVA: $F(3, 36) = 10.64$, $p < 0.0001$].

Effect of DS and NMDA ligands on the number of closed arm entries and the total number of arm entries

DS administered singly and in combination with the tested NMDA ligands did not alter the number of closed arm entries and the total number of arm entries as compared with the vehicle-treated group (Tab. 1).

Tab. 1. The number of the closed arm entries and the total arm entries for all experimental groups

Treatment (dose)	Number of closed arm entries	Number of total arm entries
A		
Control	8.30 ± 0.76	11.70 ± 0.99
DS (100 nmol/mouse)	7.70 ± 0.30	11.30 ± 0.49
CGP 37849 (0.625 mg/kg)	9.00 ± 0.77	15.80 ± 0.99 **
CGP 37849 + DS	9.40 ± 0.94	12.70 ± 0.91 #
	F (3, 36) = 1.048, p = 0.383	F (3, 36) = 5.494, p = 0.0033
	** $p < 0.01$ *vs.* control; # $p < 0.05$ *vs.* CGP 37849	
B		
Control	8.30 ± 0.76	11.70 ± 0.99
DS (100 nmol/mouse)	7.70 ± 0.30	11.30 ± 0.49
L-701,324 (4 mg/kg)	9.80 ± 0.99	17.50 ± 1.11 ***
L-701,324 + DS	8.00 ± 0.76	11.10 ± 1.03 ###
	F (3, 36) = 1.568, p = 0.2141	F (3, 36) = 10.80, $p < 0.0001$
	*** $p < 0.001$ *vs.* control; ### $p < 0.001$ *vs.* L-701,324	
C		
Control	10.20 ± 0.61	12.50 ± 0.78
NMDA (75 mg/kg)	9.80 ± 0.53	11.80 ± 0.57
CGP 37849 (0.625 mg/kg)	6.80 ± 0.61 *	11.10 ± 0.74
CGP 37849 + NMDA	15.20 ± 1.25 ***, ###, ^^^	19.00 ± 1.26 ***, ###, ^^^
	F (3, 36) = 18.61, $p < 0.0001$	F (3, 36) = 17.27, $p < 0.0001$
	* $p < 0.05$, *** $p < 0.001$ *vs.* control; ### $p < 0.001$ *vs.* CGP 37849; ^^^ $p < 0.001$ *vs.* NMDA	
D		
Control	10.80 ± 0.66	13.60 ± 0.56
NMDA (75 mg/kg)	10.40 ± 0.56	14.40 ± 0.77
L-701,324 (4 mg/kg)	11.20 ± 0.49	18.20 ± 0.71 ***
L-701,324 + NMDA	16.50 ± 0.64 ***, ###, ^^^	21.90 ± 0.72 ***, ###, ^^^
	F (3, 36) = 23.49, $p < 0.0001$	F (3, 36) = 29.96, $p < 0.0001$
	*** $p < 0.001$ *vs.* control; ### $p < 0.001$ *vs.* L-701,324; ^^^ $p < 0.001$ *vs.* NMDA	

Data represent the mean ± SEM, $n = 9–12$. CGP 37849, L-701,324 and *N*-methyl-D-aspartic acid (NMDA) were administered *ip* 60 min before the test. D-serine (DS) was administered *icv* 15 min before the test. The data were evaluated by the one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls *post-hoc* test

Both CGP 37849 and L-701,324 given alone did not influence the general activity described by the number of the closed arm entries.

Effect of NMDA and NMDA ligands on the number of closed arm entries and the total number of arm entries

Activity in the total arm entries and the closed arm entries were not different between mice receiving NMDA and the vehicle. Both CGP 37849 and L-701,324 given alone did not increase the general activity described by the number of closed arm entries. However, the joint administration of NMDA and the tested NMDA ligands had a significant effect on the number of closed arm entries and the total number of arm entries, as presented in Table 1.

Discussion

The elevated plus maze is one of the most popular unconditioned tests for examining anxiolytic and anxiogenic effects of different agents in rodent models [22, 27]. The brain structures – amygdala and hippocampus, are widely known to be implicated in regulation and control of anxious response [44]. Assessing the behavior of the genetically modified mice deprived of the NR1 or NR2B subunits of the NMDA receptor complex from hippocampal areas, Niewoehner et al. [25] and von Engelhardt et al. [42] proved the significant role of hippocampal NMDA receptors in anxiety. Nascimento Häckl and Carobrez [24] pointed the ventral (unlike dorsal) hippocampal NMDA receptors as the primarily implicated ones. The involvement of the amygdalar glutamatergic system in lasting increases in anxiety-like behavior of rats following the exposure to a stressor was revealed by Adamec et al. [1] – administration of a non-competitive antagonist of the NMDA receptor (MK-801) into the amygdala blocked the effects of predator stress. In addition, the anxiolytic-like effects of the NMDA receptor antagonists were also observed after microinjection into the dorsolateral periaqueductal gray [10, 23]. Encouraged by the above-mentioned experiments, we decided to evaluate the possible influence of two distinct NMDA receptor sites agonists on anxiolytic-like behavior in mice induced by ligands (antagonists) of the NMDA receptor complex.

An anxiolytic-like effect of CGP 37849, a conventional competitive antagonist of the glutamate site of NMDA receptor, was revealed in the present research. These findings are in agreement with results obtained by Przegaliński et al. [36] who performed their experiments in rat model. Several previous reports provide data concerning anxiety reducing properties of other competitive NMDA receptor antagonists [28, 36].

Our results confirmed the observations made in the course of the previous investigations [29, 31, 35], suggesting that the activity of NMDA receptor may be inhibited not only by antagonists of glutamate recognition site but also through blockage of the glycine modulatory site by its antagonists. L-701,324 as a high-affinity potent blocker of NMDA receptor induced an anxiety reducing response in laboratory animals after a single injection into the peritoneal cavity of 4 mg/kg. Moreover, similar effect after oral administration of L-701,324 has been reported [17]. According to the literature [32, 33], this agent produces an antidepressant effects, as well. It is encouraging, that administration of modulators of the glycine site is not associated with the inconvenient adverse effects typical for conventional antagonists of NMDA receptor [3, 6, 34].

Although NMDA excitatory amino acid agonist is known to produce anxiogenic-like effect [26, 29], it did not significantly influence the behavior of mice after acute 75 mg/kg dosing. However, this ineffective concentration significantly reduced the antianxiety properties of CGP 37849 and L-701,324 when co-administered. The similar results were achieved for co-treatment with DS, an agonist of the glycine binding site. Admittedly, the concentration of 10 mg/kg was too low to produce any significant effect when DS was given alone, but according to the literature [18], this substance is known to evoke a prominent anxiogenic-like effect in mice in multiple tests of anxiety. However, it cannot be excluded that the testing conditions applied in our study did not allow detecting the anxiogenesis induced by the low dose of DS. It is also possible that the so-called floor effect, defined as the lowest level of plus-maze performance that will not show any further decrease in the open arms exploration, was responsible for this lack of DS and NMDA effects. Thus, further investigations on this matter are needed. The outcomes of the present studies are in agreement with previous experiments which indicated that antidepressant-like activity of CGP 37849 and L-701,324 was significantly decreased by activation of glutamate or glycine biding site at NMDA receptor when measured in the forced

swim test in mice [30–32]. Furthermore, Poleszak et al. [31] noted that CGP 37849 and L-701,324 enhanced anxiety reducing effect of chlordiazepoxide in mice in the elevated plus-maze test.

It is well documented [21] that an increase of the time spent in the open arms or an augmented number of entries into the open arms suggest the anxiolytic-like effect of the drug only if there is no drug-related changes in the locomotor activity, which may confound the outcomes of the elevated plus-maze test. It may happen, that after administration of a substance producing a motor-stimulant effects, the tested animal would behave as if it was given an anxiolytic [7]. Under certain circumstances, the false negative results induced by the changes in the locomotor activity were noticed as well [8]. On the other hand, there are evidences that the total number of arm entries is a rather poor index of locomotor activity, charging simultaneously on both anxiety and locomotion factors [7, 37]. Some authors suggest using the number of closed arm entries – a relatively pure index of locomotor activity, as a better alternative [5, 19]. Therefore, the elevated number of the total arm entries observed for the animals treated with CGP 37849 or L-701,324 might have been the consequence of reflecting changes in anxiety. Particularly in view of the fact that the number of the closed arm entries was not increased for any group injected with CGP 37849 or L-701,324 alone. Observations made by other authors confirm that administration of low doses of CGP 37849 or L-701,324 does not affect the motor behavior of the animals [16, 17, 35]. Therefore, the anxiolytic effect observed for both tested compounds was not misinterpreted or induced by hyperlocomotion. When the anti-anxiety properties of CGP 37849 and L-701,324 were reversed by administration of NMDA, unexpectedly the locomotor activity of mice grew in a synergistic manner as compared to the single-treatment group. As it is not possible to explain this effect by analyzing only the outcomes of the behavioral tests, further pharmacokinetic investigations are planned. By contrast, concurrent therapy of DS and the tested NMDA ligands caused no alteration in the locomotor activity measured by the number of closed arm entries.

On the basis of the present results we can confirm the important role of NMDA/glutamate pathway in the anxiolytic effect of the antagonists of the NMDA receptor complex, since the stimulation of the glutamate or glycine recognition site of the NMDA receptor significantly decreases the antianxiety properties of their antagonists.

References:

1. Adamec RE, Burton P, Shallow T, Budgell J: Unilateral block of NMDA receptors in the amygdala prevents predator stress-induced lasting increases in anxiety-like behavior and unconditioned startle–effective hemisphere depends on the behavior. Physiol Behav, 1999, 65, 739–751.
2. Barkus C, McHugh SB, Sprengel R, Seeburg PH, Rawlins JN, Bannerman DM: Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion. Eur J Pharmacol, 2010, 626, 49–56.
3. Beardsley PM, Ratti E, Balster RL, Willetts J, Trist D: The selective glycine antagonist gavestinel lacks phencyclidine-like behavioral effects. Behav Pharmacol, 2002, 13, 583–592.
4. Cotman CW, Monaghan DT: Anatomical organization of excitatory amino acid receptors and their properties. Adv Exp Med Biol, 1986, 203, 237–252.
5. Cruz AP, Frei F, Graeff FG: Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav, 1994, 49, 171–176.
6. Danysz W, Parsons CG: Glycine and *N*-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol Rev, 1998, 50, 597–664.
7. Dawson GR, Crawford SP, Collinson N, Iversen SD, Tricklebank MD: Evidence that the anxiolytic-like effects of chlordiazepoxide on the elevated plus maze are confounded by increases in locomotor activity. Psychopharmacology (Berl), 1995, 118, 316–323.
8. Dawson GR, Crawford SP, Stanhope KJ, Iversen SD, Tricklebank MD: One-trial tolerance to the effects of chlordiazepoxide on the elevated plus maze may be due to locomotor habituation, not repeated drug exposure. Psychopharmacology (Berl), 1994, 113, 570–572.
9. Erreger K, Chen PE, Wyllie DJ, Traynelis SF: Glutamate receptor gating. Crit Rev Neurobiol, 2004, 16, 187–224.
10. Guimaraes FS, Carobrez AP, De Aguiar JC, Graeff FG: Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology (Berl), 1991, 103, 91–94.
11. Javitt DC: Glutamate as a therapeutic target in psychiatric disorders. Mol Psychiatry, 2004, 9, 984–979.
12. Jessa M, Nazar M, Bidziński A, Płaźnik A: The effects of repeated administration of diazepam, MK-801 and CGP 37849 on rat behavior in two models of anxiety. Eur Neuropsychopharmacol, 1996, 6, 55–61.
13. Kaster MP, Machado DG, Santos AR, Rodrigues AL: Involvement of NMDA receptors in the antidepressant-like action of adenosine. Pharmacol Rep, 2012, 64, 706–713.
14. Kemp JA, Leeson PD: The glycine site of the NMDA receptor – five years on. Trends Pharmacol Sci, 1993, 14, 20–25.
15. Kleckner NW, Dingledine R: Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. Science, 1988, 241, 835–837.
16. Kolasiewicz W, Maj J: Locomotor activity and motor disturbances induced by the competitive NMDA receptor antagonist CGP 37849. Pol J Pharmacol, 1996, 48, 467–479.

17. Kotlińska J, Liljequist S: A characterization of anxiolytic-like actions induced by the novel NMDA/glycine site antagonist, L-701,324. Psychopharmacology (Berl), 1998, 135, 175–181.
18. Labrie V, Clapcote SJ, Roder JC: Mutant mice with reduced NMDA-NR1 glycine affinity or lack of D-amino acid oxidase function exhibit altered anxiety-like behaviors. Pharmacol Biochem Behav, 2009, 91, 610–620.
19. Lin HQ, Burden PM, Christie MJ, Johnston GA: The anxiogenic-like and anxiolytic-like effects of MDMA on mice in the elevated plus-maze: a comparison with amphetamine. Pharmacol Biochem Behav, 1999, 62, 403–408.
20. Lipman JJ, Spencer PS: Rapid intracerebroventricular injection assisted by an automatic syringe. J Pharmacol Methods, 1980, 4, 327–333.
21. Lister RG: Ethologically based animal models of anxiety disorders. In: Psychopharmacology of anxiolytics and antidepressants. Ed. File SE, Pergamon Press, New York, 1991.
22. Lister RG: The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl), 1987, 92, 180–185.
23. Molchanov ML, Guimaraes FS: Anxiolytic-like effects of AP7 injected into the dorsolateral or ventrolateral columns of the periaqueductal gray of rats. Psychopharmacology (Berl), 2002, 160, 30–38.
24. Nascimento Hackl LP, Carobrez AP: Distinct ventral and dorsal hippocampus AP5 anxiolytic effects revealed in the elevated plus-maze task in rats. Neurobiol Learn Mem, 2007, 88, 177–185.
25. Niewoehner B, Single FN, Hvalby Ø, Jensen V, Meyer zum Alten Borgloh S, Seeburg PH, Rawlins JN et al.: Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. Eur J Neurosci, 2007, 25, 837–846.
26. Padovan CM, Del Bel EA, Guimaraes FS: Behavioral effects in the elevated plus maze of an NMDA antagonist injected into the dorsal hippocampus: influence of restraint stress. Pharmacol Biochem Behav, 2000, 67, 325–330.
27. Pellow S, Chopin P, File SE, Briley M: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods, 1985, 14, 149–167.
28. Płaźnik A, Palejko W, Nazar M, Jessa M: Effects of antagonists at the NMDA receptor complex in two models of anxiety. Eur Neuropsychopharmacol, 1994, 4, 503–512.
29. Poleszak E, Socała K, Szopa A, Wróbel A, Szewczyk B, Kasperek R, Blicharska E et al.: Involvement of NMDA receptor complex in the anxiolytic-like effects of chlordiazepoxide in mice. J Neural Transm, 2011, 118, 857–864.
30. Poleszak E, Szewczyk B, Wlaź A, Fidecka S, Wlaź P, Pilc A, Nowak G: D-serine, a selective glycine/*N*-methyl-D-aspartate receptor agonist, antagonizes the antidepressant-like effects of magnesium and zinc in mice. Pharmacol Rep, 2008, 60, 996–1000.
31. Poleszak E, Wlaź P, Szewczyk B, Wlaź A, Kasperek R, Wróbel A, Nowak G: A complex interaction between glycine/NMDA receptors and serotonergic/noradrenergic antidepressants in the forced swim test in mice. J Neural Transm, 2011, 118, 1535–1546.
32. Poleszak E, Wlaź P, Wróbel A, Dybała M, Sowa M, Fidecka S, Pilc A, Nowak G: Activation of the NMDA/glutamate receptor complex antagonizes the NMDA antagonist-induced antidepressant-like effects in the forced swim test. Pharmacol Rep, 2007, 59, 595–600.
33. Popik P, Wróbel M, Nowak G: Chronic treatment with antidepressants affects glycine/NMDA receptor function: behavioral evidence. Neuropharmacology, 2000, 39, 2278–2287.
34. Priestley T, Laughton P, Macaulay AJ, Hill RG, Kemp JA: Electrophysiological characterisation of the antagonist properties of two novel NMDA receptor glycine site antagonists, L-695,902 and L-701,324. Neuropharmacology, 1996, 35, 1573–1581.
35. Przegaliński E, Tatarczyńska E, Chojnacka-Wójcik E: Anxiolytic- and antidepressant-like effects of an antagonist at glycineB receptors. Pol J Pharmacol, 1998, 50, 349–354.
36. Przegaliński E, Tatarczyńska E, Chojnacka-Wójcik E: The influence of the benzodiazepine receptor antagonist flumazenil on the anxiolytic-like effects of CGP 37849 and ACPC in rats. Neuropharmacology, 2000, 39, 1858–1864.
37. Ramos A, Mormede P: Stress and emotionality: a multidimensional and genetic approach. Neurosci Biobehav Rev, 1998, 22, 33–57.
38. Reynolds IJ, Miller RJ: Multiple sites for the regulation of the *N*-methyl-D-aspartate receptor. Mol Pharmacol, 1988, 33, 581–584.
39. Rodgers RJ, Dalvi A: Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev, 1997, 21, 801–810.
40. Skolnick P, Popik P, Trullas R: Glutamate-based antidepressants: 20 years on. Trends Pharmacol Sci, 2009, 30, 563–569.
41. Szakacs R, Janka Z, Kalman J: The "blue" side of glutamatergic neurotransmission: NMDA receptor antagonists as possible novel therapeutics for major depression. Neuropsychopharmacol Hung, 2012, 14, 29–40.
42. von Engelhardt J, Doganci B, Jensen V, Hvalby Ø, Gongrich C, Taylor A, Barkus C et al.: Contribution of hippocampal and extra-hippocampal NR2B-containing NMDA receptors to performance on spatial learning tasks. Neuron, 2008, 60, 846–860.
43. Waxman EA, Lynch DR: *N*-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. Neuroscientist, 2005, 11, 37–49.
44. Yee BK, Zhu SW, Mohammed AH, Feldon J: Levels of neurotrophic factors in the hippocampus and amygdala correlate with anxiety- and fear-related behaviour in C57BL6 mice. J Neural Transm, 2007, 114, 431–444.
45. Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK: A randomized trial of an *N*-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry, 2006, 63, 856–864.

Received: December 21, 2012; **in the revised form:** March 26, 2013; **accepted:** April 11, 2013.