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Short communication

D-serine, a selective glycine/N-methyl-D-aspartate receptor agonist, antagonizes the antidepressant-like effects of magnesium and zinc in mice

Ewa Poleszak[1], Bernadeta Szewczyk[3], Aleksandra Wlaź[1], Sylwia Fidecka[1], Piotr Wlaź[2], Andrzej Pilc[4], Gabriel Nowak[3,5]

[1]Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Staszica 4, PL 20-081 Lublin, Poland

[2]Department of Animal Physiology, Institute of Biology, Maria Curie-Skłodowska University, Akademicka 19, PL 20-033 Lublin, Poland

[3]Laboratory of Neurobiology of Trace Elements, [4]Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland [5]Laboratory of Pharmacobiology, Departmentof Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University, Medyczna 9, PL 30-688 Kraków, Poland

Correspondence: Gabriel Nowak, e-mail: nowak@if-pan.krakow.pl

Abstract:
Zinc and magnesium are potent inhibitors of the N-methyl-D-aspartate (NMDA) receptor complex. Recent data demonstrate that both zinc and magnesium, like other NMDA receptor antagonists, exhibit antidepressant-like activity in rodent screening tests and depression models. In the present study, we investigated the effect of D-serine (agonist for the glycine$_B$ site of the NMDA receptor complex; 100 nmol/mouse, *icv*) on magnesium (30 mg/kg, *ip*)- and zinc (5 mg/kg, *ip*)-induced activity during a forced swim test (FST) in mice. The antidepressant-like effect observed during FST for both ions was abolished by D-serine co-treatment.
The present study indicates that the NMDA receptor complex, especially the glycine$_B$ site, plays a role in the antidepressant-like activity of magnesium and zinc in the FST in mice.

Key words:
NMDA receptor, glycine$_B$ site, magnesium, zinc, D-serine, forced swim test, mice

Introduction

The N-methyl-D-aspartate (NMDA) receptor is unique among ionotropic glutamate receptors in that it requires an obligate coagonist, glycine or D-serine to bind to a distinct site (the glycine$_B$ site) on the receptor. In the absence of glycine, the NMDA receptor channel opening is attenuated even in the presence of an adequate amount of glutamate (endogenous agonist) [4]. The other regulatory sites include sites within the ionophore where Mg^{2+} and phencyclidines (PCP, MK-801, ketamine) bind and produce a voltage-dependent open channel block. There is an additional site where Zn^{2+} acts to allosterically inhibit the agonist-induced response in a voltage-independent manner [4].

Several preclinical and human studies suggest that neurotransmission *via* the NMDA receptor is dysregulated in depression [21]. Moreover, compounds that target the NMDA receptor exhibit an antidepressant-like activity in animal tests and depression models [21]. Ketamine, one of the antagonists of the NMDA receptor, is also effective in human depression [1, 36]. In recent years, numerous experiments have also demonstrated the antidepressant-like effects of zinc and magnesium, inorganic inhibitors of the NMDA receptor, in several screening tests and models of depression [3, 5, 8, 9, 18, 24, 31] (for review [17, 33]). They not only exhibit antidepressant-like activity but also enhance the effect of classic antidepressants in these behavioral paradigms [3, 8, 23, 26, 31, 32].

Clinical data, although in limited number of reports, have also demonstrated antidepressant activity of these ions. Thus, magnesium was reported to be effective for unipolar and bipolar affective disorders [2, 6, 7]. Moreover our previous clinical study demonstrated the benefit of zinc supplementation in the treatment of unipolar depression [16].

Our previous study has shown that activation of the NMDA receptor complex *via* the glutamate (NMDA) or the glycine$_B$ (D-serine) sites abolish the antidepressant-like effect of CGP 37849 and L-701,324, antagonists of the NMDA receptor complex [27]. The involvement of the NMDA/glutamate pathway in the antidepressant-like activity of magnesium was also shown in forced swim test (FST) [25]. Thus, the antidepressant-like effect of magnesium was antagonized by NMDA co-treatment during this test [25]. So far, no data have demonstrated direct evidence for the role of NMDA receptor in the antidepressant-like activity of zinc.

In the present study, we examined the involvement of the glycine/NMDA site in the antidepressant-like activity of magnesium and zinc during FST. For this purpose, mice were co-treated with a selective glycine/NMDA receptor agonist D-serine [4, 35] and effective doses of magnesium or zinc and tested in that model.

Materials and Methods

Animals

The experiments were carried out on male Albino Swiss mice (25–30 g). The animals were kept on a natural day-night cycle with free access to food and water. Each experimental group consisted of 8–12 animals. The experimental protocol was approved by the Ethical Committee of the Medical University, Lublin, and all the procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drug administration

Magnesium hydroaspartate (Farmapol, Poznań, Poland) was administered intraperitoneally (*ip*) 30 min before the test, and zinc hydroaspartate (Farmapol, Poznań, Poland) was administered *ip* 1 h before the test. Dosages of magnesium and zinc refer to pure magnesium and zinc ions. D-serine (Sigma, USA) was administered intracerebroventricularly (*icv*) 15 min before the test. The *icv* administration was performed according to a modified method described by Lipman and Spencer [11]. Control animals received an *ip* and *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 (FST)

The study was carried out in mice according to the method described by Porsolt et al. [28]. Mice were dropped 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

Locomotor activity of mice was measured with photoresistor actometers (circular cages, diameter 25 cm, two light beams). The animals were placed individually in an actometer, and activity was measured during two consecutive 5-min intervals in order to characterize the dynamics of changes in locomotor activity. The number of times the mouse crossed the light beams was recorded as its locomotor activity.

Statistics

All results are presented as the means ± standard error of the mean (SEM). The obtained data were evaluated by the two-way analysis of variance (ANOVA) followed by the Bonferroni test, $p < 0.05$ was considered to be significant.

Results and Discussion

Several preclinical data indicated that functional NMDA receptor antagonists are effective in tests that predict antidepressant activity in humans and animal models of depression. The antagonists acting at glutamate receptor and different modulatory sites of the NMDA receptor complex are active in the FST and tail suspension test in rodents [10, 13–15, 29, 34]. Moreover, such activity was also demonstrated in animal models of chronic mild stress [20, 22], chronic unpredictable stress [19], and olfactory bulbectomy [30]).

Our previous study showed that NMDA receptor antagonists, which act at various modulatory sites on the NMDA receptor complex, enhanced the antidepressant-like action of magnesium in this test [25]. Moreover, the antidepressant-like activity of magnesium was antagonized by co-treatment with NMDA [25]. In turn, our present data demonstrate that glycine$_B$ co-transmitter site activation in the NMDA receptor complex by D-serine abolishes the antidepressant-like activity of magnesium. Magnesium at a dose of 30 mg/kg significantly reduced the immobility time in FST in mice (Fig. 1). D-serine, an agonist of the glycine$_B$ site, given alone at a dose of 100 nmol per mouse had no significant effect on the immobility time, while it did antagonize the effect of magnesium (Fig. 1). These data indicate that magnesium behaved as a typical NMDA antagonist in FST [27] and confirmed that the antidepressant-like activity of magnesium is correlated with the reduction of the activity of the NMDA receptor complex [25]. However, it should be mentioned that the serotonergic system is also involved in the antidepressant-like activity of magnesium during the FST [23].

In the present study, we also demonstrated that the activation of the glycine$_B$ co-transmitter site of the NMDA receptor complex by D-serine abolishes the antidepressant-like activity of zinc. Zinc at a dose of 5 mg/kg significantly reduced the immobility time mice in FST (Fig. 2). D-serine, an agonist of the glycine$_B$ site, given alone at a dose of 100 nmol/mouse had no significant effect on immobility time, while it antagonized the effect of zinc (Fig. 2). These data represent the first direct demonstration that the antidepressant-like activity of zinc is correlated with an alteration in the function of the NMDA receptor. Rosa et al. [31] suggested involvement of the NMDA/nitric oxide pathway in the antidepressant-like activity of zinc

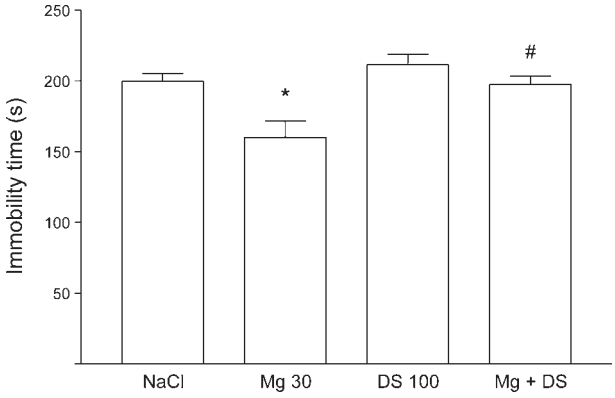

Fig. 1. Effect of joint administration of magnesium and D-serine on immobility time during the FST. The values represent the means ± SEM (n = 8–9 mice per group). Two-way ANOVA showed a significant effect of D-serine $F(1, 31) = 9.2$, $p = 0.0049$, significant effect of magnesium $F(1, 31) = 10.81$, $p = 0.0025$, and no interaction $F(1, 31) = 2.53$, $p = 0.1216$. * $p < 0.01$ *vs.* saline; # $p < 0.01$ *vs.* Mg group (Bonferroni test)

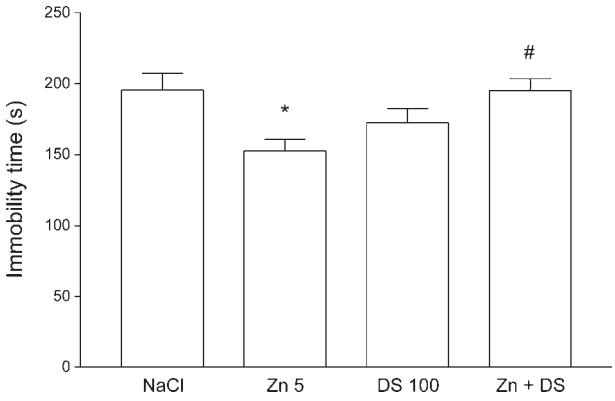

Fig. 2. Effect of joint administration of zinc and D-serine on immobility time during the FST. The values represent the means ± SEM (n = 9–10 mice per group). Two-way ANOVA showed an insignificant effect of D-serine $F(1, 35) = 1.01$, $p = 0.3214$, insignificant effect of zinc $F(1, 35) = 1.1$, $p = 0.3022$, and significant interaction $F(1, 35) = 11.6$, $p = 0.0017$. * $p < 0.01$ *vs.* saline; # $p < 0.01$ *vs.* Zn group (Bonferroni test)

Tab. 1. Effect of magnesium, zinc, and D-serine administration on spontaneous locomotor activity in mice

Treatment	Dose	Activity counts	
		5 min	10 min
Control	–	99 ± 9.2	145.2 ± 13
Magnesium	30 mg/kg	77 ± 9.9	116.4 ± 23.4
D-serine	100 nmol/mouse	55.7 ± 9.1**	82.1 ± 15.4*
Magnesium + D-serine	30 mg/kg + 100 nmol/mouse	72.7 ± 11	101 ± 14.5
Zinc	5 mg/kg	61.3 ± 9.6*	82.3 ± 18.1*
Zinc + D-serine	5 mg/kg + 100 nmol/mouse	35.3 ± 5.8***	47.4 ± 6***

Magnesium was administered *ip* 30 min, zinc *ip* 1 h, and D-serine *icv* 15 min before the test. Control animals received two injections (*ip* and *icv*) at respective times. The values represent the means ± SEM of 8–10 mice per group. Two-way ANOVA showed a significant effect of D-serine $F(1, 47) = 10.8$, $p = 0.0019$, significant effect of treatment $F(1, 47) = 6.26$, $p = 0.0039$, and no interaction $F(1, 47) = 2.26$, $p = 0.1152$, for 5 min; and significant effect of D-serine $F(1, 47) = 9.48$, $p = 0.0035$, significant effect of treatment, $F(1, 47) = 6.57$, $p = 0.0031$, and no interaction $F(1, 47) = 1.26$, $p = 0.2927$, for 10 min; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* control group (Bonferroni test)

during FST; however, their data were indirect. The mechanism of zinc, apart from affecting NMDA/glutamate, may also involve the adenosine system since adenosine receptor antagonists abolish and agonists enhance zinc antidepressant-like action during the FST [12].

D-serine and zinc (but not magnesium) administration reduces locomotor activity (Tab. 1). Combined administration of magnesium and D-serine did not alter such activity in comparison to the control or magnesium alone (Tab. 1). Combined administration of zinc and D-serine maintained a reduced locomotor activity in both treatments (Tab. 1). Thus, we can assume that D-serine selectively antagonizes the antidepressant-like activity of both magnesium and zinc, having no effect on the locomotor activity induced by these ions.

In conclusion, our study demonstrated that activation of the NMDA receptor complex (by D-serine) abolishes the antidepressant-like effects of magnesium and zinc during the FST test in mice. This indicates the major role of the NMDA/glutamate pathway (particularly $glycine_B$ sites) in the antidepressant activity of these two ions.

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