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ORIGINAL ARTICLE

The involvement of NMDA and AMPA receptors in the mechanism of antidepressant-like action of zinc in the forced swim test

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Abstract Antidepressant-like activity of zinc in the forced swim test (FST) was demonstrated previously. Enhancement of such activity by joint administration of zinc and antidepressants was also shown. However, mechanisms involved in this activity have not yet been established. The present study examined the involvement of the NMDA and AMPA receptors in zinc activity in the FST in mice and rats. Additionally, the influence of zinc on both glutamate and aspartate release in the rat brain was also determined. Zinc-induced antidepressant-like activity in the FST in both mice and rats was antagonized by *N*-methyl-D-aspartic acid (NMDA, 75 mg/kg, i.p.) administration. Moreover, low and ineffective doses of NMDA antagonists (CGP 37849, L-701,324, D-cycloserine, and MK-801) administered together with ineffective doses of zinc exhibit a significant reduction of immobility time in the FST. Additionally, we have demonstrated the reduction of immobility time by AMPA receptor potentiator, CX 614. The antidepressant-like activity of both CX 614 and zinc in the FST was abolished by NBQX (an antagonist of AMPA receptor, 10 mg/kg, i.p.), while the combined treatment of sub-effective doses of zinc and CX 614 significantly reduces the immobility time in the FST. The present study also demonstrated that zinc administration potentiated a veratridine-evoked glutamate and aspartate release in the rat's prefrontal cortex and hippocampus. The present study further suggests the antidepressant properties of zinc and indicates the involvement of the NMDA and AMPA glutamatergic receptors in this activity.

Keywords Forced swim test · Antidepressant · Zinc · NMDA · AMPA · Glutamate receptors

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Introduction

About 30–50% of patients with depression does not respond, or only partially respond, to their treatment with antidepressant drugs (Keitner et al. 2006; Rubio et al. 2004). Additionally, all clinically used antidepressant drugs exert multiple unwanted side effects (Hollister and

Csernansky 1990) and their mechanism of action is not fully understood (Vizi 2000; Hindmarch 2001; Nestler et al. 2002). Thus, the search for new antidepressant therapies and new antidepressant drugs is still in progress. The data gathered during recent years pointed to the involvement of glutamatergic transmission in the pathophysiology of depression (Hansen et al. 1983; Kugaya and Sanacora 2005; Petrie et al. 2000; Sanacora et al. 2003; Zarate et al. 2002; Hashimoto 2009; Machado-Vieira et al. 2009). Accumulated evidence demonstrated that *N*-methyl-D-aspartate (NMDA) receptor ligands (functional antagonists) interacting with different components of the NMDA receptor–ionophore complex produced antidepressant-like effects. The preclinical experiments showed that noncompetitive (MK-801) and competitive antagonists (CGP 37849), as well as antagonists (L-701,324) and partial agonists of the glycine/NMDA site (D-cycloserine) produced antidepressant-like effects in the forced swim test (FST) (Lopes et al. 1997; Panconi et al. 1993; Poleszak et al. 2007a; Przegalinski et al. 1998; Trullas and Skolnick 1990). Furthermore, ketamine (a noncompetitive antagonist of the NMDA receptor complex) and CP-101,606 (a selective antagonist of NR2B subunit of NMDA receptor) is effective in human depression (Berman et al. 2000; Zarate et al. 2006; Preskorn et al. 2008). In addition, inorganic inhibitors of the NMDA receptor function (magnesium and zinc) exhibit antidepressant-like effects in preclinical screening paradigms. Magnesium was active in the FST in mice (following both acute and chronic treatment) and in rats (acute treatment) (Decollogne et al. 1997; Poleszak et al. 2004, 2005a). In addition, the enhancement of its activity by joint administration of antidepressant drugs (Poleszak et al. 2005b; Poleszak 2007; Cardoso et al. 2009) and the NMDA receptor antagonist (Poleszak et al. 2007a) was demonstrated.

The role of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in depression and antidepressant treatment was also demonstrated. The antidepressant fluoxetine positively modulate AMPA receptors (Bleakman et al. 2007). Positive AMPA receptor modulators (potentiators) produced antidepressant-like effects in the FST and tail suspension test (TST) (Alt et al. 2005). Moreover, NBQX, an AMPA receptor antagonist, has been found to block the antidepressant-like effect of the NMDA receptor antagonists (Maeng et al. 2008, see Skolnick 2008), which suggests that this effect may be mediated by an increased AMPA-to-NMDA receptor throughput in neuronal circuits (Maeng et al. 2008).

Zinc produced antidepressant-like activity both in tests (the FST and TST) and some models (olfactory bulbectomy, chronic unpredictable stress and chronic mild stress) of depression (Cieslik et al. 2007; Franco et al. 2008; Kroczka et al. 2000, 2001; Nowak et al. 2003b; Rosa et al. 2003; Sowa-Kucma et al. 2008). Moreover, zinc, similar to magnesium, enhanced the activity of antidepressant drugs (Kroczka et al. 2001; Szewczyk et al. 2002, 2009; Cieslik et al. 2007; Cunha et al. 2008). On the other hand, zinc deficiency induced depressive-like behavior and neurochemical changes (Corniola et al. 2008; Tassabehji et al. 2008; Whittle et al. 2009).

In the clinical conditions, the low serum zinc levels in patients with depressive symptoms were identified (Hansen et al. 1983, 1994, 1997, 1999; Nowak and Schlegel-Zawadzka 1999) and successful antidepressant therapy may normalize the lowered serum zinc concentrations (Maes et al. 1994; McLoughlin and Hodge 1990; Nowak and Schlegel-Zawadzka 1999; Nowak et al. 2003a). It seems clear that reduced levels of serum zinc is linked to depression, and zinc administration may have antidepressant-like activity, but the mechanism of the action of zinc in the central nervous system is not fully elucidated. The recent hypothesis suggested that the antidepressant-like activity of zinc is connected with glutamatergic and serotonergic neurotransmission (Levenson 2006a, b, Szewczyk et al. 2008).

The aim of the present study is to examine the involvement of the glutamate system in the mechanism of the antidepressant action of zinc. Therefore, we investigated the influence of the NMDA agonist (*N*-methyl-D-aspartic acid) on zinc-induced activity in the FST and the effect of NMDA antagonists with sub-effective doses of zinc. In addition, we studied the influence of AMPA receptor antagonist and a positive AMPA receptor modulator on zinc activity in FST. Moreover, the influence of zinc on the glutamate and aspartate release induced by the depolarizing agent veratridine was also examined.

Experimental procedures

Animals

All procedures were approved by the Ethical Committee of the Medical University, Lublin and by the Ethics Committee of the Institute of Pharmacology, Krakow. The experiments were carried out on adult male Albino Swiss mice (25–30 g) or on adult male Wistar rats (300–350 g). The animals were housed under conditions of constant temperature (20–22°C), controlled 12:12 light–dark cycle and free access to food and water.

Drug administration

Zinc hydroaspartate (Farmapol, Poznań, Poland) was administered intraperitoneally (i.p.) and dosages were referred to pure zinc ions. 7-chloro-4-hydroxy-3-(3-phenoxy) phenylquinolin-2[1H]-one (L-701,324, Sigma) was

suspended in a 1% aqueous solution of tween 80 and administered i.p. 60 min before test. *N*-methyl-D-aspartic acid (NMDA, Sigma), DL-/E/-amino-4-methyl-5-phosphono-3-pentenoic acid; (CGP 37849, Ciba-Geigy, Basel), l5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801, Sigma), D-cycloserine (d-4-amino-3-isoxazolidone) (Sigma), NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2, 3-dione (Tocris House, Bristol, UK) and CX614 [2,3,6a,7,8,9-Hexahydro-11H-[1,4]dioxino[2,3-g]pyrrolo [2,1-b][1,3]benzoxazin-11-one] (Cortex Pharmaceuticals, USA) were dissolved in 0.9% saline and administered i.p. 1 h before the test. The doses of compounds were selected based on the results published previously (Dybala et al. 2008; Nowak et al. 2003b; Poleszak et al. 2007a; Szewczyk et al. 2009). Control animals received an i.p. injection of saline (vehicle). The volume of vehicles or drug solutions for i.p. administrations was 10 ml/kg in mice and 2 ml/kg in rats.

Forced swim test

The studies were carried out on mice according to the method of Porsolt et al. (1977) and on rats according to the method of Porsolt et al. (1978). Mice were propped individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water, maintained at 23–25°C. The rats were placed in glass cylinders (height 40 cm, diameter 18 m) containing 15 cm of water, maintained 23–25°C. Mice 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 total duration of immobility in rats was measured during a 5-min test, following 15 min of pre-test carried out 24 h earlier. The mouse or the rats was judged to be immobile when it remained floating passively in the water.

Locomotor activity

Locomotor activity of mice was measured with photoresistor actimeters (circular cages, diameter 25 cm, two light beams). The animals were placed individually in an actimeter for 6 min. The number of crossings the light beams by the mice was recorded as the locomotor activity.

Locomotor activity of rats was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, USA) linked on-line to a compatible IBM-PC. Each cage (43 × 44 × 25 cm) was surrounded with a 15 × 15 array of photocell beams located 3 cm from the floor surface as reported previously. Interruptions of these photobeams resulted in horizontal activity defined as distance traveled (in cm). Before locomotor activity was recorded, rats were injected with tested compounds in their home cages and at the appropriate time were transferred to the experimental cages. Locomotor activity was recorded for 5 min and analyzed using Auto-track software (Columbus Instruments, USA).

Statistics

The obtained data were evaluated by the Student *t* test, one-way or two-way analysis of variance (ANOVA) followed by Bonferroni test, where appropriate. All results are presented as mean ± SEM. $P < 0.05$ was considered as statistically significant.

Surgery, microdialysis, and analytical procedure

Male Wistar rats (250–280 g) were anesthetized 1 day before the experiment with ketamine (75 mg/kg i.m.) and xylazine (10 mg/kg i.m.) and were placed into stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was retracted and holes were drilled through the skull for insertion of vertical Microdialysis probe into prefrontal cortex (PFCx) (2.7 mm anterior from the bregma, 0.6 mm lateral from the sagittal suture and −5.5 mm ventral from the dural surface) or hippocampus −3.8 mm anterior from the bregma, 3.4 mm lateral from the sagittal suture and −4.0 mm ventral from the dural surface). Microdialysis probes were constructed by inserting two fused silica tubes [30 and 35 mm long, 150 μm outer diameter (o.d.); Polymicro Technologies Inc., Phoenix, AZ, USA] into a Microdialysis fiber (220 μm o.d., AN69, Hospal, Bologna, Italy). The tube assembly was placed in a stainless steel cannula (22 gauge, 10 mm) forming the shaft of the probe. Portions of the inlet and outlet tubes were individually placed inside polyethylene PE-10 tubing and were glued for protection. The free end of the dialysis fiber was sealed and 4 mm of the exposed length used for dialysis in the PFCx or 3 mm in the hippocampus.

One day after surgery and probe implantation, the inlet of the dialysis probes was connected to a syringe pump (BAS, IN, USA) which delivered an artificial cerebrospinal fluid (aCSF) composed of [in mM]: NaCl 145, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2; pH 7.4 at a flow 1.5 μl/min. After 3 h of washing period, when the extracellular level of glutamate and aspartate became stable, three baseline samples were collected every 30 min from freely moving animals. Next, zinc hydroaspartate (Farmapol, Poznan, Poland) at a dose 65 mg/kg (11.3 mg/kg of zinc) was administered intraperitoneally. Then, Veratridine (Sigma, Steinheim, Germany) at a concentration 250 μM was given for 30 min trough the dialyzing probe. At the end of the experiment, the rats were killed and their brains were histologically examined to validate the correct probe placement.

Dialysates (20 μl) were derivative with 4-dimethylaminobenzene-4′-sulfonyl chloride (DABS-Cl) at 70°C for

12 min, according to Knecht and Chang (1986). Dabsylated amino acids were separated on a Ultrasphere ODS (4.6 × 150 mm, 3 µm) (Supelco, Poznań, Poland) by gradient elution, with solvent A (10 mM citric acid, 4% dimethylformamide) and solvent B (acetonitrile) (Merck, Darmstadt, Germany). Dabsylated compounds were detected by absorbance at 436 nm on Amino Acid System Gold (Beckman, Fullerton, CA, USA).

The values, expressed as a percentage of the basal level, were mean ± SEM. A statistical evaluation was carried out by repeated measures ANOVA and Tukey's post hoc test. $P < 0.05$ was considered as statistically significant.

Results

Effect of joint administration of zinc and NMDA on the total duration of immobility in the FST in mice

The effects of combined administration of zinc and NMDA on the total duration of immobility in mice are shown in Fig. 1. Zinc (5 mg/kg) induced a significant reduction in the immobility time. NMDA (75 mg/kg) administered alone had no effect on the immobility time, while significantly antagonized the effect induced by zinc treatment (Fig. 1). Two-way ANOVA revealed significant effect of zinc $F(1,28) = 8.31$, $P = 0.0075$), significant effect of NMDA $F(1,28) = 19.45$, $P = 0.0001$ and no interaction $F(1,28) = 2.090$, $P = 0.1590$.

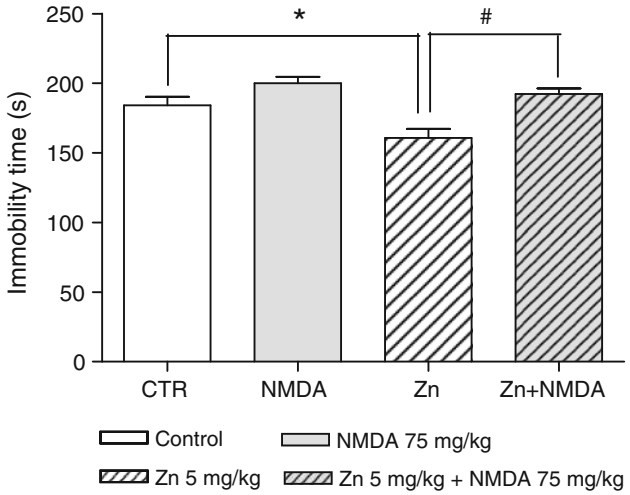

Fig. 1 Effect of joint administration of zinc and NMDA on the immobility time in the FST in mice. Zinc (5 mg/kg) and NMDA (75 mg/kg) were administered i.p. 45 and 60 min, respectively, before the test. The values represent mean ± SEM ($n = 7$–8 mice per group). $^{*}P < 0.05$ versus control group; $^{\#}P < 0.01$ versus Zn group

Effect of joint administration of zinc and L-701,324 on the total duration of immobility in the FST in mice

The effects of combined administration of zinc and L-701,324 (the glycine B receptor antagonist) on the total duration of immobility in mice are shown in Fig. 2a. Zinc at the dose of 2.5 mg/kg or L-701,324 at the dose of 1 mg/kg given alone was ineffective. The combined administration of zinc and L-701,324 significantly reduced the immobility time in FST (Fig. 2a). Two-way ANOVA revealed significant effect of zinc [$F(1,39) = 15.55$, $P = 0.0003$], significant effect of L-701,324 $F(1,39) = 8.06$, $P = 0.0072$ and significant interaction $F(1,39) = 4.43$, $P = 0.0491$.

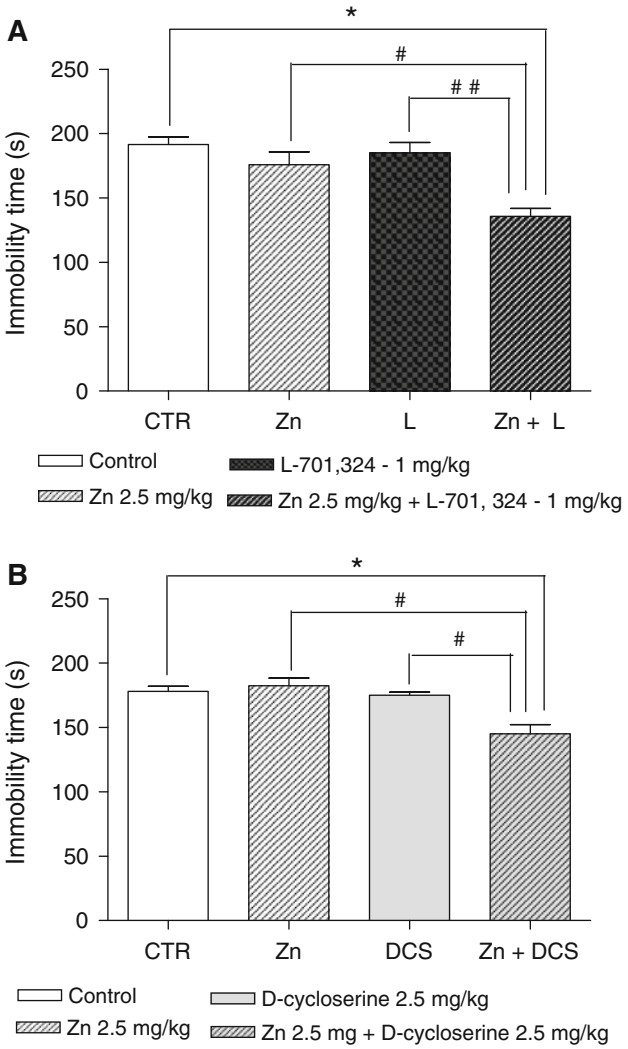

Fig. 2 Effect of joint administration of zinc and L-701,324 (1 mg/kg) (**a**) or D-cycloserine (2.5 mg/kg) (**b**) on the immobility time in the FST in mice. Zinc (2.5 mg/kg, i.p.) was administered 45 min and L-701,324 (i.p.) or D-cycloserine 60 min before the test. The values represent mean ± SEM ($n = 8$–12 mice per group) A: $^{*}P < 0.05$ versus control group; $^{\#}P < 0.01$; $^{\#\#}P < 0.001$ versus Zn + L-701,324 group. B: $^{*}P < 0.001$ versus control group; $^{\#}P < 0.01$; $^{\#\#}P < 0.001$ versus Zn + D-cycloserine group

Effect of joint administration of zinc and D-cycloserine on the total duration of immobility in the FST in mice

The effects of combined administration of zinc and D-cycloserine (a partial agonist at glycine B receptors) on the total duration of immobility in mice are shown in Fig. 2b. Both zinc and D-cycloserine given alone at the dose of 2.5 mg/kg had no effect on the immobility time. The combined administration of zinc with D-cycloserine significantly reduced the immobility time in FST (Fig. 2b). Two-way ANOVA revealed significant effect of zinc [$F(1,32) = 5.885$, $P = 0.0211$], significant effect of D-cycloserine $F(1,32) = 14.70$, $P = 0.0006$ and significant interaction $F(1,32) = 10.65$, $P = 0.0026$.

Effect of joint administration of zinc and CGP 37849 on the total duration of immobility in the FST in mice

The effects of combined administration of zinc and CGP 37849 (the competitive NMDA receptor antagonist) on the total duration of immobility in mice are shown in Fig. 3a. The combined administration of zinc (2.5 mg/kg) and CGP 37849 (0.3 mg/kg) significantly reduced the immobility time in FST [Student t test $t(20) = 5.250$, $P < 0.0001$]. Administration of CGP 37849 (0.3 mg/kg) alone did not alter the immobility time (previously published in Poleszak et al. 2007a, performed at the same experimental conditions).

Effect of joint administration of zinc and MK-801 on the total immobility duration in the FST in mice

The effects of combined administration of zinc and MK-801 (a noncompetitive NMDA receptor antagonist) on the total duration of immobility in mice are shown in Fig. 3b. Zinc (2.5 mg/kg) had no effect on the immobility time. The combined administration of zinc (2.5 mg/kg) with MK-801 (0.05 mg/kg) significantly reduced the immobility time in FST [ANOVA, $F(2,26) = 18.43$, $P < 0.001$, Fig. 4a]. Administration of MK-801 (0.05 mg/kg) alone did not alter the immobility time (previously published in Poleszak et al. 2007a, performed at the same experimental conditions).

Effect of CX 614 treatment on the total duration of immobility in the FST in mice

The effects of CX 614 (potentiator of AMPA receptor) treatment on the total duration of immobility in mice are shown in Fig. 4. ANOVA: $F(3,28) = 4.556$, $P = 0.0101$. CX 614 administered at a dose of 4 mg/kg induced statistically significant reduction in immobility time in FST in mice. The dose of 1 and 2 mg/kg had no effect on the immobility time in this test.

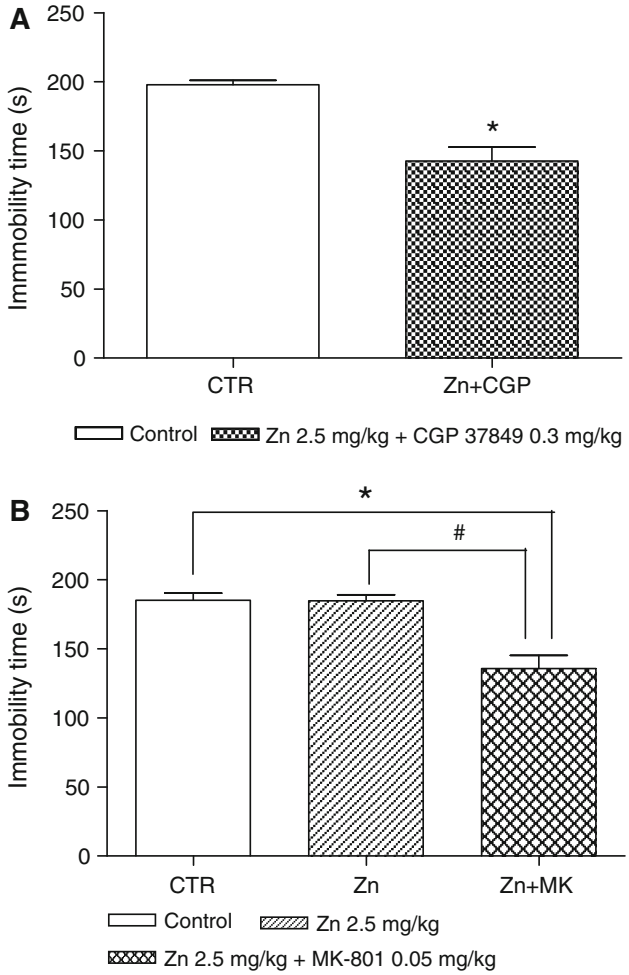

Fig. 3 Effect of joint administration of zinc and CGP 37849 (0.3 mg/kg) (**a**) or MK-801 (0.05 mg/kg) (**b**) on the immobility time in the FST in mice. Zinc (2.5 mg/kg, i.p.) was administered 45 min and CGP 37849 or MK-801 (i.p.) 60 min before the test. The values represent mean ± SEM ($n = 9$–11 mice per group). $^{*}P < 0.001$ versus control group, $^{\#}P < 0.001$ versus Zn + MK-801 group

Effect of joint administration of CX 614 and NBQX on the total immobility duration in the FST in mice

The effects of combined administration of CX 614 and NBQX (antagonist of the AMPA receptor) on the total duration of immobility in mice are shown in Fig. 5. CX 614 (4 mg/kg) induced a significant reduction in the immobility time (Fig. 6). NBQX (10 mg/kg) administered alone had no effect on the immobility time, while significantly antagonized the effect induced by zinc treatment (Fig. 5). Two-way ANOVA revealed significant effect of CX 614 $F(1,31) = 8.96$, $P = 0.0054$, no significant effect of NBQX $F(1,31) = 2.98$, $P = 0.0944$ and no interaction $F(1,31) = 2.86$, $P = 0.1008$.

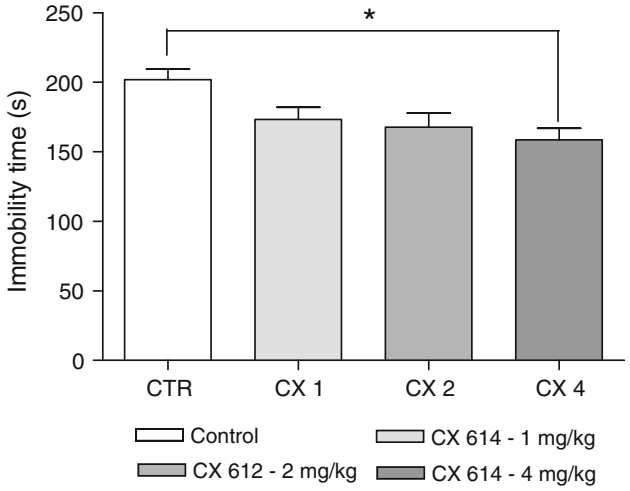

Fig. 4 Effect of CX 614 (1; 2; 4 mg/kg) administration on the immobility time in FST in mice. CX 614 was administered i.p. 45 min before the test. The values represent mean ± SEM ($n = 8$ mice per group). *$P < 0.05$ versus control group

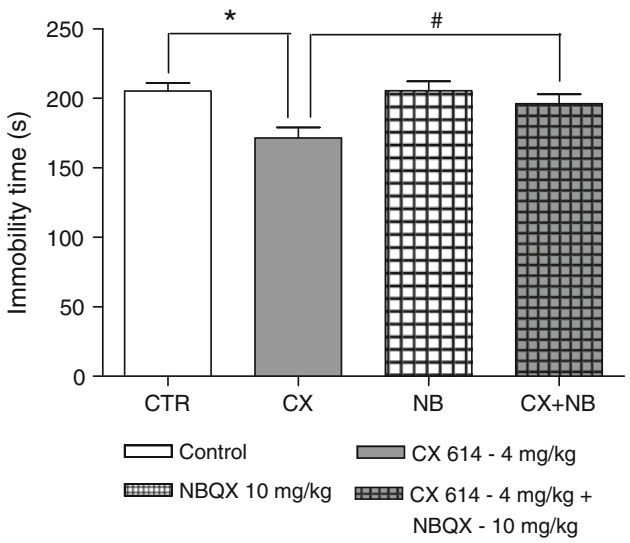

Fig. 5 Effect of joint administration of CX 614 and NBQX on the immobility time in the FST in mice. CX 614 (4 mg/kg) and NBQX (10 mg/kg) were administered (i.p.) 45 and 60 min, respectively, before the test. The values represent mean ± SEM ($n = 6$–12 mice per group). *$P < 0.001$ versus control group; #$P < 0.05$ versus CX 614 group

Effect of joint administration of zinc and NBQX on the total immobility duration in the FST in mice

The effects of combined administration of zinc and NBQX (antagonist of the AMPA receptor) on the total duration of immobility in mice are shown in Fig. 6. Zinc (5 mg/kg induced a significant reduction in the immobility time (Fig. 7). NBQX (10 mg/kg) administered alone had no effect on the immobility time, while significantly antagonized the effect induced by zinc treatment (Fig. 6). Two-way ANOVA

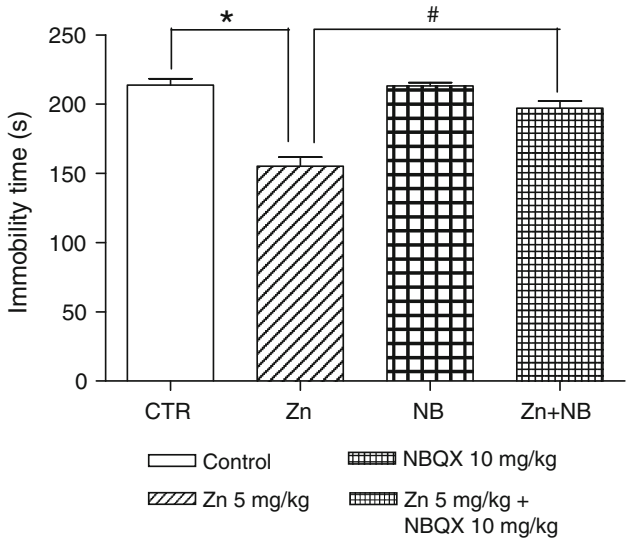

Fig. 6 Effect of joint administration of zinc and NBQX on the immobility time in the FST in mice. Zinc (5 mg/kg) and NBQX (10 mg/kg) were administered (i.p) 45 and 60 min, respectively, before the test. The values represent mean ± SEM ($n = 5$–8 mice per group). *$P < 0.001$ versus control group; #$P < 0.001$ versus Zn group

revealed significant effect of zinc $F(1,22) = 40$, $P = 0.001$, significant effect of NBQX $F(1,22) = 13.25$, $P = 0.0014$ and significant interaction $F(1,22) = 14.01$, $P = 0.0011$.

Effect of joint administration of zinc and CX614 on the total immobility duration in the FST in mice

The effects of combined administration of zinc and CX 614 (a positive AMPA receptor modulator) on the total duration of immobility in mice are shown in Fig. 7. Both zinc (2.5 mg/kg) and CX 614 (1 mg/kg) given alone had no effect on the immobility time. The combined administration of zinc with CX614 significantly reduced the immobility time in FST (Fig. 7). Two-way ANOVA revealed no significant effect of zinc $F(1,41) = 0.08$, $P = 0.7756$), significant effect of CX 614 $F(1,41) = 13.16$, $P = 0.0008$ and no significant interaction $F(1,41) = 1.97$, $P = 0.1681$.

Effect of joint administration of zinc and NMDA on the total duration of immobility in the FST in rats

The effects of combined administration of zinc and NMDA on the total duration of immobility in rats are shown in Fig. 8. Zinc (11.3 mg/kg) induced a significant reduction in the immobility time. NMDA (75 mg/kg) administered alone had no effect on the immobility time, while significantly antagonized the effect induced by zinc treatment (Fig. 8). Two-way ANOVA revealed significant effect of zinc $F(1,27) = 15.32$, $P = 0.0006$), no effect of NMDA $F(1,27) = 0.79$, $P = 0.3830$ and very significant interaction $F(1,27) = 13.50$, $P = 0.0010$.

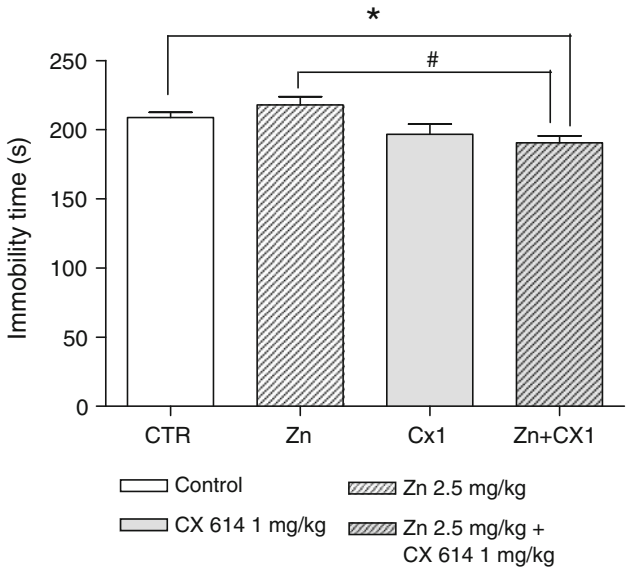

Fig. 7 Effect of joint administration of zinc and CX614 on the immobility time in the FST in mice. Zinc (2.5 mg/kg, i.p.) was administered 45 min and CX614 (1 mg/kg, i.p.) 60 min before the test. The values represent mean ± SEM ($n = 8$–18 mice per group). *$P < 0.05$ versus control group; #$P < 0.01$ versus Zn + CX614 group

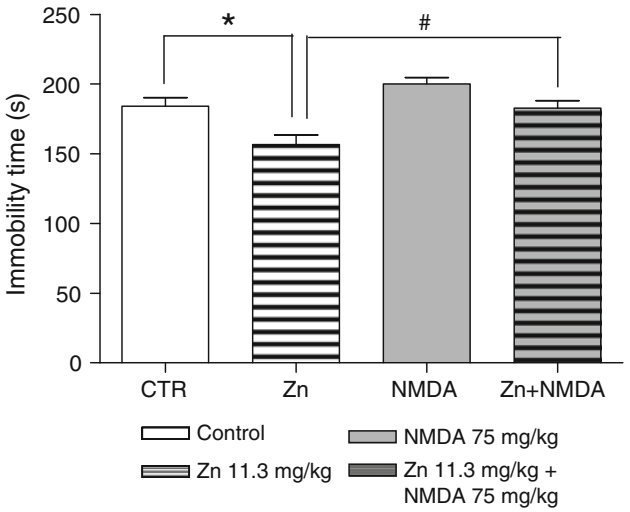

Fig. 8 Effect of joint administration of zinc and NMDA on the immobility time in the FST in rats. Zinc (11.3 mg/kg) and NMDA (75 mg/kg) were administered i.p. 40 and 60 min, respectively, before the test. The values represent mean ± SEM ($n = 8$–10 rats per group). *$P < 0.01$ versus control group; #$P < 0.05$ versus Zn group

Effect of treatment with zinc and glutamate receptor ligands on locomotor activity

The effect of the combined administration of Zn and NMDA or NBQX on spontaneous locomotor activity in mice is shown in Table 1. Zn treatment (5 mg/kg) reduced (by ~55%) the locomotor activity. Neither NMDA (A) nor NBQX (B) pre-treatment influenced Zn-induced locomotor alterations. Two-way ANOVA revealed no interaction [$F(1,20) = 0.53$, $P = 0.4740$], significant effect of Zn [$F(1,20) = 31.73$, $P = 0.0001$] and lack of NMDA effect [$F(1,20) = 0.01$, $P = 0.7425$ (A)], and no interaction [$F(1,20) = 0.09$, $P = 0.7728$], significant effect of Zn [$F(1,20) = 19.37$, $P = 0.0003$] and lack of NBQX effect [$F(1,20) = 0$, P = 0.9816 (B)]. L-701,324, D-cycloserine, CGP 37849, MK-801 or CX 614 administered alone or with zinc (2.5 mg/kg) did not alter the locomotor activity in mice (Poleszak et al. 2007a, data not shown). Zinc (11.3 mg/kg) and NMDA (75 mg/kg) alone or in combination of both agents did not affect locomotor activity in rats (Table 2). Two-way ANOVA revealed no effect of zinc $F(1,27) = 3.31$, $P = 0.0801$, no effect of NMDA $F(1,27) = 0.37$, $P = 0.5464$ and lack of interaction $F(1,27) = 0.41$, $P = 0.5278$.

Table 1 Effect of zinc and glutamate receptor ligands on locomotor activity in mice

Treatment	Dose (mg/kg)	Activity counts
A: control	–	147.0 ± 13.9
Zn	5	62.0 ± 13.7***
Zn + NMDA	5 + 75	76.2 ± 9.3**, #
NMDA	75	141.7 ± 15.7
B: control	–	128.4 ± 12.5
Zn	5	58.2 ± 21.0*
Zn + NBQX	5 + 10	52.8 ± 19.5*, #
NBQX	10	133.0 ± 13.8

Zinc (Zn) was administered 45 min and NMDA or NBQX 1 h before the test. The values represent mean ± SEM ($n = 6$ mice per group)
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control; # $P < 0.01$ versus NMDA or NBQX treated group

Effect of zinc on glutamate and aspartate release in rat prefrontal cortex

Veratridine, given via the microdialysis probe at a concentration of 250 μM for 30 min, increased the dialysate level of both glutamate and aspartate (Fig. 9a, b). Zinc significantly enhanced the veratridine-induced increase of both glutamate and aspartate level. The maximal effect was seen between 30 and 60 min of administration. Repeated measures ANOVA revealed $F(2,23) = 4.176$, $P = 0.0141$ for A and $F(2.23) = 9.088$, $P = 0004$ for B.

Effect of zinc on glutamate and aspartate release in rat hippocampus

Veratridine, given via the microdialysis probe at a concentration of 250 μM for 30 min, increased the dialysate level of both glutamate and aspartate (Fig. 10a, b). Zinc

Table 2 The effect of zinc and NMDA administration on locomotor activity in rats

Treatment	Dose (mg/kg)	Distance traveled (cm)
Control	–	408 ± 30
Zinc	11.3	331 ± 38
NMDA	75	369 ± 26
Zinc + NMDA	11.3 + 75	332 ± 28

Zinc (11.3 mg/kg) and NMDA (75 mg/kg) were administered i.p. 40 and 60 min, respectively, before the activity measurement. The values represent horizontal activity defined as distance traveled (cm) in the actometers within 5 min. Presented values are the mean ± SEM (n = 6–8 rats per group)

significantly enhanced the veratridine-induced increase of aspartate but not the glutamate level. The maximal effect was seen at 30 min of administration. Repeated measures ANOVA revealed $F(2,23) = 3.007$, $P = 0.0349$ for A and $F(2.23) = 9.937$, $P < 0.0001$ for B.

Discussion

Several lines of evidence in human and in animal studies indicate that glutamate homeostasis and glutamate neurotransmission have important function in physiology (Wu 2009) and are deregulated in depressive disorder (Hansen et al. 1983; Kugaya and Sanacora 2005; Petrie et al. 2000; Sanacora et al. 2003, 2008; Zarate et al. 2002). From several types of glutamate receptors, the contribution of the NMDA and AMPA receptors to depression and antidepressant treatment is especially demonstrated. Chronic antidepressant administration influences NMDA receptor function and receptor binding profiles as well as alterations in the mRNA expression that encodes NMDA receptor subunits (Nowak et al. 1995; Skolnick et al. 1996, 2009; Petrie et al. 2000). The NMDA competitive and noncompetitive antagonist, polyamine site antagonist and inorganic inhibitors of the NMDA receptor function demonstrated antidepressant-like effects in animal tests and models of depression, plus the combined administration of NMDA antagonists with antidepressants had a synergistic effect in these tests (Maj et al. 1992; Skolnick 1999; Skolnick et al. 1996, 2009; Poleszak et al. 2004; Poleszak et al. 2005; Szewczyk et al. 2008; Trullas and Skolnick 1990). Zinc, the same as magnesium, is an inorganic antagonist of the NMDA receptor and, similar to this ion, exhibits an antidepressant-like effect in the FST both in mice and rats (Kroczka et al. 2000, 2001; Nowak et al. 2003b; Rosa et al. 2003; Szewczyk et al. 2008).

Now, in order to investigate the NMDA pathway of the antidepressant-like action of zinc in the FST in mice we examined the influence of NMDA pre-treatment on the

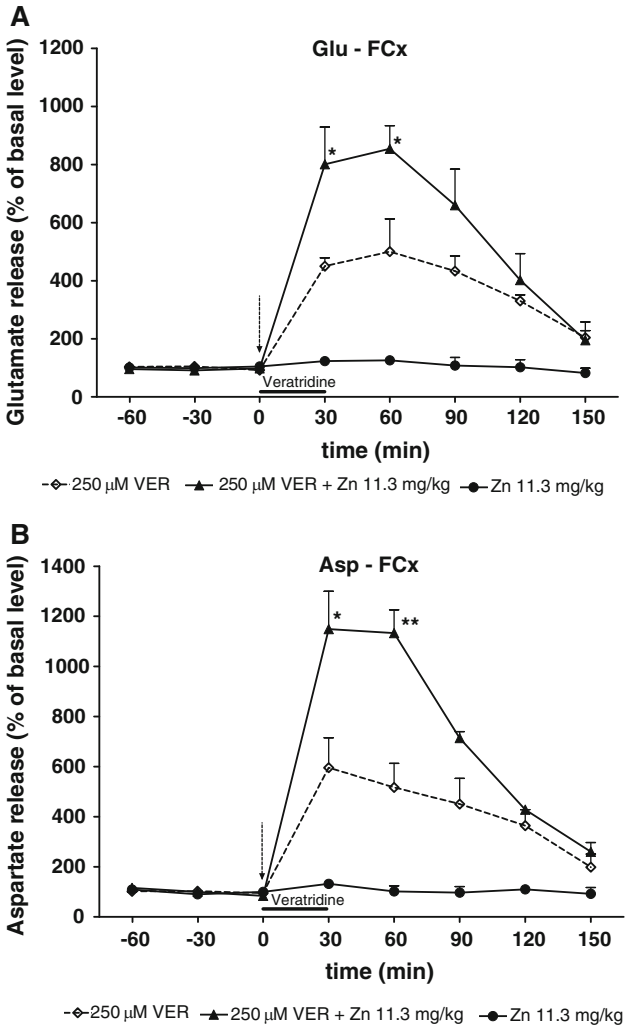

Fig. 9 Time-course of the effect of zinc on veratridine-evoked glutamate (**a**) and aspartate (**b**) release in rat prefrontal cortex. Veratridine (VER, 250 μM) was perfused via microdialysis probe for 30 min. Zinc hydroaspartate (Zn, 11.3 mg/kg) was given intraperitoneally at point "0" on x axis. Basal glutamate concentrations in dialysate were (in μM): 2.35 ± 0.03 (VER); 1.09 ± 0.09 (VER + Zn); 1.62 ± 0.36 (Zn). Basal aspartate concentrations in dialysate were (in nM): 0.63 ± 0.09 (VER); 0.29 ± 0.02 (VER + Zn); 0.44 ± 0.03 (Zn). Results are mean ± SEM (n = 5) expressed as a percentage of basal values. *$P < 0.05$, **$P < 0.01$ show significant difference between the veratridine-treated group (Tukey's post hoc test)

effect produced by zinc in this test and the effect of a combined administration of sub-effective doses of NMDA antagonists and zinc. Compounds used in our study act at various modulatory sites on the NMDA receptor complex. MK-801 blocks the neurophysiologic effects of the NMDA receptor complex by binding to a site in the ion channel of the receptor (Wong et al. 1986), CGP 37849 is the glutamate site antagonist (Fagg et al. 1990). Two other compounds L-701,324 and D-cycloserine function as an antagonist (first) and partial agonist (second) of glycine$_B$

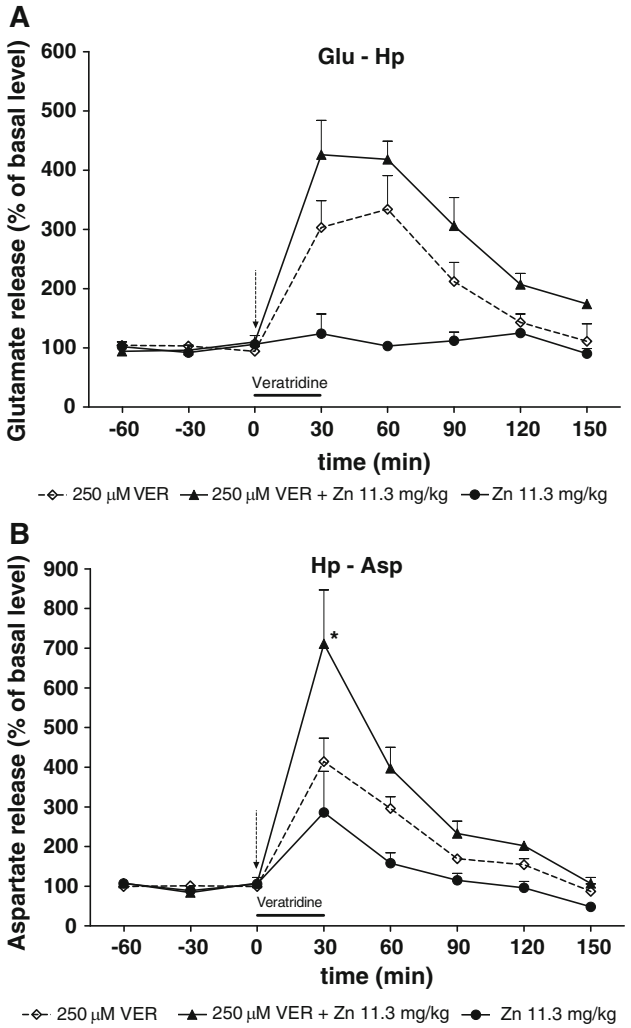

Fig. 10 Time-course of the effect of zinc on veratridine-evoked glutamate (**a**) and aspartate (**b**) release in the rat hippocampus. Veratridine (VER, 250 μM) was perfused via microdialysis probe for 30 min. Zinc hydroaspartate (Zn, 11.3 mg/kg) was given intraperitoneally at point "0" on *x* axis. Basal glutamate concentrations in dialysate were (in nM): 1.85 ± 0.1 (VER); 1.5 ± 0.14 (VER + Zn); 1.53 ± 0.15 (Zn). Basal aspartate concentrations in dialysate were (in nM): 0.48 ± 0.02 (VER); 0.58 ± 0.06 (VER + Zn); 0.52 ± 0.05 (Zn). Results are mean ± SEM ($n = 5$) expressed as a percentage of basal values. *$P < 0.05$ show significant difference between the veratridine-treated group (Tukey's post hoc test)

site on the NMDA receptor (Kulagowski et al. 1994; Kendig et al. 1956). Experiments described in this study showed that all used compounds significantly enhanced the antidepressant-like action of zinc in the FST in mice. Thus, the present data further demonstrated the importance of the NMDA receptor in the mechanism of antidepressant action produced by zinc in the FST in mice. Furthermore we reported that pre-treatment with *N*-methyl-D-aspartic acid (NMDA) also antagonized the zinc-induced antidepressant-like effect in the FST in mice, which supports the specificity of these results, while the data showing that NMDA blocks the antidepressant-like action of zinc in rats makes the data more universal. These results further confirm our hypothesis that the antidepressant-like effect of zinc is connected with a reduction of the NMDA receptor complex function. It should be mentioned that the NMDA-nitric oxide pathway might also be involved in the antidepressant activity of zinc. Zinc is an inhibitor of nitric oxide synthase (NOS) activity (Mittal et al. 1995) and Rosa et al. (2003) demonstrated the inhibition of antidepressant-like action of zinc in FST by L-arginine, which indicate the zinc-induced inhibition of NOS activity in this test. Thus, the antidepressant mechanism of zinc, like organic NOS inhibitors (e.g. Harkin et al. 1999; Füruzan et al. 2000), is related (at least in part) to NMDA-NO pathway.

The data are similar to the one described in our previous experiments (Poleszak et al. 2008) which demonstrated that co-treatment with D-serine (an agonist of the glycine$_B$ site of the NMDA receptor (Wolosker et al. 2008), antagonized zinc-induced antidepressant-like activity in the FST in mice. More importantly, the co-treatment of CGP 37849 or L-701,324 enhanced the antidepressant-like effects of magnesium, while the antidepressant-like effects induced by these compounds were also abolished by NMDA co-treatment (Poleszak et al. 2007b, 2008). Both this and the present data indicate that stimulation of the neurotransmitter glutamate site and glycine$_B$ co-transmitter site of the NMDA receptor complex abolishes the antidepressant-like activity of antagonists of either site.

The data by Poleszak et al. (2007a) and the current results indicate the pronounced similarities in the antidepressant-like action of two NMDA receptor antagonists, zinc and magnesium.

There is an increasing body of evidence implicated that another subtype of glutamate receptors—the AMPA receptor may also play an important role in depression and the action of antidepressant drugs. Although the data obtained from post-mortem studies are rather inconsistent in this field and show both an increase (Freed et al. 1993) and decrease (Meador-Woodruff et al. 2001) in the AMPA receptor related transduction pathway, much of the other data clearly demonstrated that compounds enhancing signaling through AMPA receptors (AMPA receptor potentiators) exhibit an antidepressant-like effect in animal tests and produced neuronal effects common for clinically effective antidepressants, such as neurotrophin induction and cell proliferation (see Alt et al. 2005, 2006; Bleakman et al. 2007; Skolnick 2008). The antidepressant fluoxetine was found to alter the AMPA receptor phosphorylation, which results in an increase in AMPA receptor signaling (Svenningsson et al. 2002). Additionally, the AMPA receptor antagonist NBQX was found to have blocked the antidepressant-like effect of the NMDA antagonists, such as ketamine, MK-801 and Ro-25,6981 (Meang et al. 2008),

although it did not do this with CGP 37849 (Dybala et al. 2008). Our present study demonstrated that another AMPA receptor potentiator, ampakine CX 614 (Arai et al. 2000; Jourdi et al. 2009), exhibits antidepressant-like activity in the FST. For the first time, we clearly demonstrated that such activity elicited by AMPA potentiator is antagonized by AMPA receptor antagonist, NBQX. Likewise, administration of NBQX antagonized the effect elicited by zinc in the FST in mice. Moreover, a low, ineffective dose of zinc administered together with a low and ineffective dose of CX614 (AMPA receptor potentiator) exhibits a significant reduction of immobility time in the FST. In our previous paper (Szewczyk et al. 2008), we speculated that zinc, as an enhancer of the AMPA receptors can activate these receptors directly and this may be one of the possible mechanisms involved in zinc-induced antidepressant activity. The results described above, displaying evidence of the particular role of the AMPA receptor in this activity, support our hypothesis that activation of the AMPA receptors may be a possible pathway for the antidepressant-like action of zinc.

The present study was also undertaken to see whether zinc can influence the release of glutamate and aspartate in the rat PFCx and hippocampus induced by the depolarizing agent veratridine using microdialysis in vivo. Veratridine causes a neurotransmitter release by the activation of voltage-dependent Na^+ channels and a rise in intracellular Ca^{2+} concentration, which leads to the Ca^{2+}-dependent vesicular release of neurotransmitters (Stier et al. 1996). The present study demonstrates that zinc significantly enhances the veratridine-evoked release of both glutamate and aspartate in the PFCx and a potentiated release of aspartate in the hippocampus. The effect of zinc on the veratridine-evoked release of glutamate in the hippocampus did not reach any significance; however, the trend to increase was also observed. Our data differ from that obtained for clinically effective antidepressant drugs, such as imipramine (IMI), desipramine (DMI), and citalopram (CIT). All these compounds inhibited the glutamate release in the PFCx, while the release of aspartate was affected only by DMI and CIT (Golembiowska and Zylewska 1999). On the other hand, we previously demonstrated that single i.p. zinc administration increases the extracellular (synaptic) zinc pool in the rat PFCx or hippocampus (Opoka et al. 2008; unpublished data). It was indicated that synaptic and extrasynaptic NMDA receptor localization may play a different role in neuronal plasticity and survival (Hardingham et al. 2002). Accordingly, Pittenger et al. (2007) suggested that NMDA antagonists may exhibit their antidepressant activity by antagonism of the extrasynaptic rather than synaptic NMDA receptors. In the case of zinc treatment when glutamate (and aspartate) and zinc is commonly increased in the synaptic regions, the following scenario can be proposed: all these agents compete to first reach the synaptic NMDA and AMPA receptors, stimulate the AMPA (glutamate and zinc) and exert differential effects on the NMDA receptors (glutamate stimulates, zinc inhibits), and that after diffusion toward the extrasynaptic NMDA receptors the inhibitory role of zinc is manifested, since the stimulatory glutamate is taken up by glial cells). However, such synaptic/extrasynaptic mechanisms need to be further examined in future studies.

Moreover, there are similarities between zinc and ketamine (NMDA antagonist) in affecting glutamate release. Ketamine increases extracellular glutamate level in the rat PFCx (Moghaddam et al. 1997), which was also conformed in human study (Rowland et al. 2005). In consequence, enhancement of glutamate release by ketamine may induce concomitant stimulation of non-NMDA glutamate receptors (AMPA) by glutamate and antagonism at NMDA receptor by ketamine. Such an effect may be also proposed for zinc antidepressant mechanism.

The experiments described in this paper further indicate the major role of the NMDA receptor in the mechanism of the antidepressant action of zinc and also indicates the participation of the AMPA receptor in this activity.

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