Pharmacological Reports
2008, 60, 865–871
ISSN 1734-1140

Antidepressant treatments-induced modifications of glutamatergic transmission in rat frontal cortex

Bartosz Bobula[1], Grzegorz Hess[1,2]

[1]Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

[2]Institute of Zoology, Jagiellonian University, Ingardena 6, PL 30-060 Kraków, Poland

Correspondence: Grzegorz Hess, e-mail: Hess@if-pan.krakow.pl

Abstract:
The effects of repeated administration of imipramine, citalopram, tianeptine and zinc hydroaspartate, lasting 7, 14 and 21 days, were studied *ex vivo* in rat frontal cortical slices prepared 48 h after the last dose of the drug. In a majority of cases the treatments resulted in a decrease in the amplitude of pharmacologically isolated N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4--isoxazolepropionic acid (AMPA)/kainate receptor-mediated components of the field potential. Zinc and tianeptine-induced effects were evident already after treatments lasting 7 days. Electroconvulsive shocks applied for 10 but not for 5 days reduced both the AMPA/kainate and the NMDA receptor-mediated components. The ratios of the amplitude of NMDA to AMPA/kainate component were altered to a different degree. These results indicate that repetitive treatment with antidepressants, zinc salt and electroconvulsive shocks results in an attenuation of glutamatergic synaptic transmission in the cerebral cortex, but the dynamics of the effects of these treatments vary.

Key words:
field potential, cortical slice, imipramine, citalopram, tianeptine, zinc, electroconvulsive shock

Introduction

A growing body of experimental data suggests that the pathophysiology of depression and the mechanisms of action of antidepressant drugs are related to alterations in glutamatergic transmission (reviewed in [25]). Clinical studies suggest a potential role for N-methyl-D-aspartate (NMDA) receptor antagonists in the treatment of depression (e.g., [6]). Repetitive administration of antidepressants, including imipramine and citalopram, results in a reduction in radioligand binding to rat cortical NMDA receptors [20, 23, 30] and in region-specific changes in the level of mRNAs encoding NMDA receptor subunits [10]. Moreover, NMDA receptor antagonists exhibit antidepressant-like actions in animal models and potentiate the effects of antidepressants [17, 35]. It has been hypothesized that the mechanism of antidepressant action involves dampening the function of NMDA receptors in the cerebral cortex [27–29]. More recent work has demonstrated that treatment with antidepressants results in an increased expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in rat hippocampus [18]. Since positive modulators of AMPA receptors exhibit antidepressant-like actions in animal models, these receptors have also been implicated in the mechanism of antidepressant action (reviewed in [1, 7]). It has been suggested that mice harboring a deletion of the AMPA re-

ceptor subunit GluR-A could represent a model of depression showing many behavioral and neurochemical features of the disease also postulated for humans [11].

We have previously demonstrated that treatments with a tricyclic drug, imipramine, or a selective serotonin reuptake inhibitor, citalopram, both lasting 14 days, decreased the amplitude of glutamatergic field potentials evoked in layer II/III by stimulation of underlying sites in layer V of *ex vivo* rat frontal cortical slices [8]. Effects of other classes of antidepressant treatments on glutamatergic transmission in rat frontal cortex have not yet been explored. One of those, the atypical antidepressant tianeptine, facilitates serotonin uptake, in contrast to the immediate effects of imipramine or citalopram [14]. Another effective means of treating depression is the therapy using the electroconvulsive shocks (ECS) [32]. Although the mechanism of action of ECS remains largely unknown, research in animal models points to ECS-induced modifications in G protein-mediated cyclic 3',5' adenosinomonophosphate (cAMP) signalling [24] and to increased expression of brain-derived neurotrophic factor (BDNF) [13]. Recent data indicate that administration of zinc salts results in antidepressant-like effects in rodent models of depression [16, 22, 31] most likely due to interactions of zinc with NMDA receptor (reviewed in [21]) and/or the involvement of zinc in the expression of BDNF [31].

In the present study, we aimed at finding the effects of repeated administration of tianeptine and zinc hydroaspartate on AMPA and NMDA receptor-mediated components of cortical field potentials. To assess the timecourse of the effects, *ex vivo* brain slices were investigated after 7, 14 and 21 days of treatment. To extend earlier data, effects of treatments with imipramine and citalopram of various duration were also investigated. Finally, we probed the influence of repeated ECS on AMPA and NMDA receptor-mediated components of field potentials.

Materials and Methods

Animals and treatment

Experiments were performed on male Wistar rats, weighing initially 80–90 g. Rats were housed under a 24 h light/darkness cycle (light on: 7:00–19:00) and had free access to standard food and tap water. Experiments were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences and were carried out in accordance with the European Community guidelines for the use of experimental animals and with the national law.

The following treatments were studied: imipramine (amount: 10 mg/kg); citalopram (amount: 10 mg/kg); tianeptine (amount: 10 mg/kg); and zinc hydroaspartate (amount: 65 mg/kg). Substances were dissolved in water and administered *per os* twice daily for 7, 14 and 21 days. Control animals received vehicle. In a separate group, ECS (150 mA, 0.3 s) was applied *via* earclip electrodes five times every second day or everyday for 10 days. Control animals did not receive ECS, but otherwise they were handled identically to ECS-treated rats.

Imipramine hydrochloride was obtained from Polfa, Poland, and zinc hydroaspartate was obtained from Farmapol, Poland. Citalopram hydrobromide and tianeptine were kindly provided by H. Lundbeck A/S and Servier Poland, respectively.

Slice preparation and recording

Slice preparation was conducted 48 h after last treatment session, as described in detail elsewhere [8, 36]. Briefly, rat brains after dissection were immersed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl (130), KCl (5), $CaCl_2$ (2.5), $MgSO_4$ (1.3), KH_2PO_4 (1.25), $NaHCO_3$ (26), and glucose (10), bubbled with carbogen (95% O_2, 5% CO_2) to pH 7.4. Frontal cortical slices (thickness: 450 μm) were cut in a coronal plane using a vibrating microtome and were stored submerged in ACSF at room temperature. Recordings were performed at 32 ± 0.5°C in a submersion-type chamber superfused with a slightly modified ACSF ([NaCl] increased to 132 mM and [KCl] decreased to 2 mM) at 2.5 ml/min.

A concentric bipolar Pt-Ir electrode (FHC, USA), was used to deliver constant voltage stimuli (0.1 Hz, 0.3 s, 4–15 V). The electrode was placed 2 mm lateral to the midline and 1.5 mm below the pial surface. Field potentials were recorded from overlying sites, 0.2 mm below cortical surface, using glass micropipettes filled with 2 M NaCl (2–5 MΩ). A slightly off-radial (approx. 50 μm) placement of the recording and

stimulating microelectrodes helped to diminish the contribution of antidromic activation to recorded responses. Recordings were amplified (Axoprobe, Axon Instruments, USA), bandpass filtered (1–500 Hz), acquired on a PC computer using the 1401 interface (CED, UK) and analyzed off-line using the Signal 2 software package (CED, UK).

For each slice, the recording was initially performed in standard ACSF followed by 25 min in ACSF containing 2 μM (±)-2-amino-4-methyl-5-phosphono-3--pentenoic acid (CGP-37849, Tocris, United Kingdom), to block NMDA receptors. Subsequently, to block AMPA/kainate receptors, the slice was perfused for 25 min with ACSF devoid of Mg^{2+} ions and containing 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]-quinoxaline-7-sulfonoamide (NBQX disodium salt, Tocris, United Kingdom).

The effects of the treatments on the magnitude of AMPA/kainate and NMDA receptor-mediated components of field potentials were measured using half-maximum responses, which were determined based on stimulus-response curves in each condition.

Statistical analysis was carried out using one-way ANOVA with *post-hoc* Tukey test.

Results

Incubation of rat frontal cortical slices, first in the ACSF containing CGP-37849 and then in the ACSF devoid of Mg^{2+} ions and containing NBQX, allowed for pharmacological isolation of AMPA/kainate and NMDA receptor-mediated components of the field potential, respectively (Fig. 1), in agreement with earlier results [8].

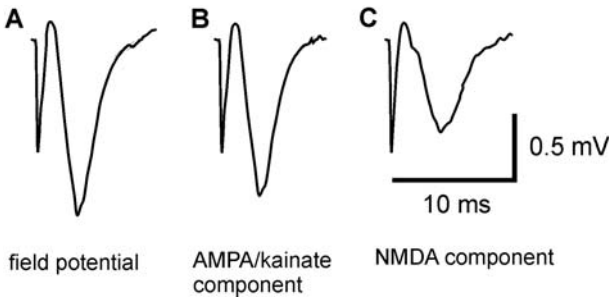

Fig. 1. Examples of recordings from a representative experiment. Shown are averages (n = 3) of half-maximum responses evoked in a single slice. **(A)** Field potential recorded in standard ACSF. **(B)** The response in ACSF containing CGP-37849 (labeled: AMPA/kainate component). **(C)** The response in ACSF containing NBQX and devoid of Mg^{2+} (labeled: NMDA component)

Treatment with imipramine (Fig. 2A) for 14 and 21 days induced a reduction of both the AMPA/kainate and the NMDA components. Changes observed after 21 days of imipramine administration were not more pronounced than those after 14 days. These changes resulted in a decrease of the NMDA to AMPA/kainate component ratio (Fig. 3A). Imipramine treatment lasting 7 days did not result in significant modifications of recorded responses.

Treatment with citalopram (Fig. 2B) resulted in a reduction of the AMPA/kainate component after 14 days and 21 days, but not after 7 days of drug administration; however, the change observed after 21 days of citalopram administration was not larger than the changes seen after just 14 days. Similarly, in the case of the NMDA component, a significant reduction was observed after citalopram treatment lasting 14 days and 21 days (Fig. 2B). These effects resulted in a decrease of the NMDA to AMPA/kainate component ratio after 14 days and 21 days of the treatment (Fig. 3B). Interestingly, 7 days of citalopram treatment induced an increase of the NMDA component (Fig. 2A), which resulted in an increase in the NMDA to AMPA/kainate component ratio at this timepoint (Fig. 3B).

A reduction of the AMPA/kainate component was evident already after 7 days of repetitive tianeptine treatment. This component remained low after treatments lasting 14 and 21 days (Fig. 2C). The effect observed after 14 days of tianeptine administration was larger than those after 7 and 21 days of treatment. Changes of the NMDA component were significant after 14 and 21 days of treatment (Fig. 2C). These effects resulted in an increase of the NMDA to AMPA/kainate component ratio after 7 days of tianeptine treatment and a reduction of this ratio after 21 days of the treatment (Fig. 3B). The NMDA to AMPA/kainate ratio was unchanged after tianeptine treatment lasting 14 days.

Zinc hydroaspartate administered for 7, 14 and 21 days induced a reduction of the AMPA/kainate component (Fig. 2D). A reduction of the NMDA component was also evident after treatments lasting 7 and 14, but not 21 days (Fig. 2D). There was no change in the NMDA to AMPA/kainate ratio after 7 and 14 days of zinc treatment, but after 21 days of treatment, this ratio was increased relative to control.

There was a slight, but insignificant decrease in the magnitude of both components after ECS was applied 5 times. In contrast, treatment with ECS times applied every day for 10 days significantly reduced both the AMPA/kainate and the NMDA components (Fig. 2E), resulting in a reduction of the NMDA to AMPA/kainate component ratio (Fig. 3E).

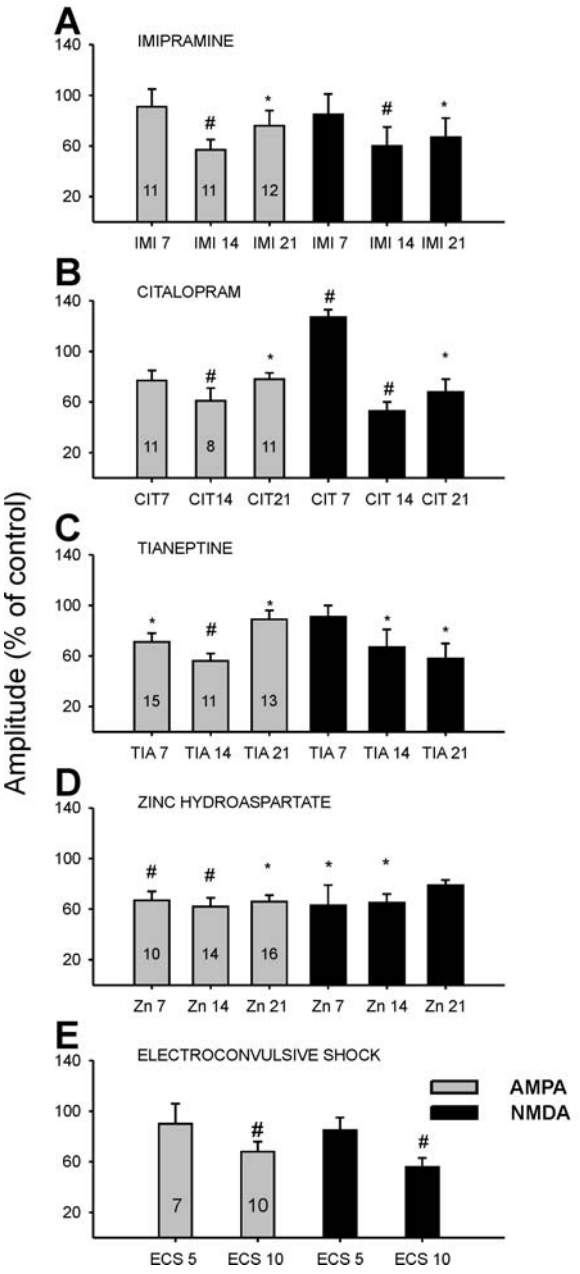

Fig. 2. Effects of treatment on the AMPA/kainate component (shaded bars) and the NMDA component (black bars) of responses recorded in *ex vivo* slices. Bars indicate a relative change (± SEM) in the amplitude of responses compared to control. **(A)** imipramine, **(B)** citalopram, **(C)** tianeptine, **(D)** zinc hydroaspartate, **(E)** electroconvulsive shock. Numbers on shaded bars indicate the number of slices in each group. The number of slices tested for both the AMPA/kainate and the NMDA components are the same. * $p < 0.05$, # $p < 0.01$, ANOVA

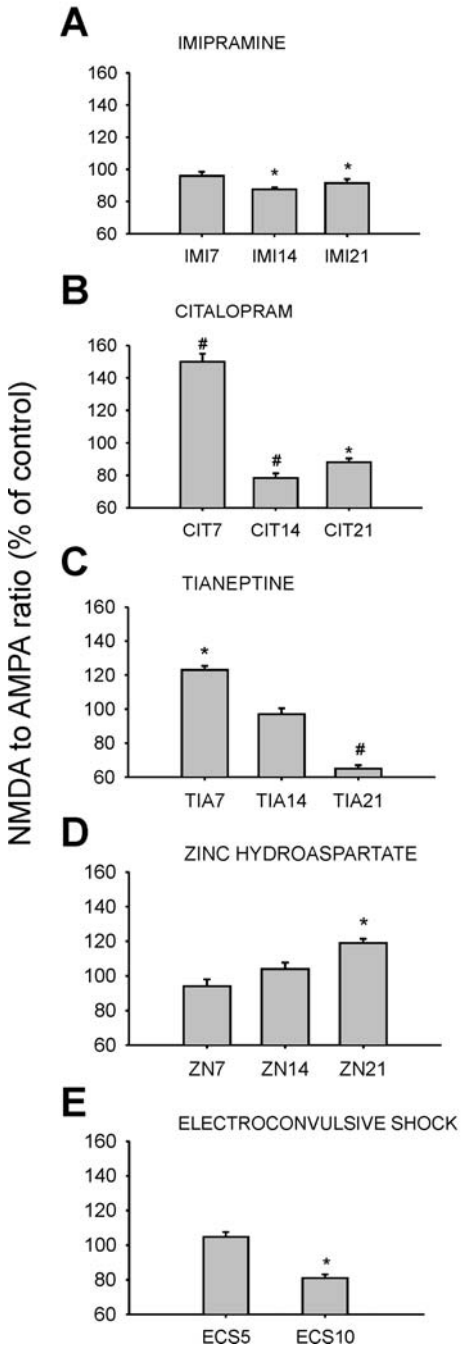

Fig. 3. Effects of treatment on the amplitude ratio of the NMDA to AMPA/kainate components compared to control. **(A)** imipramine, **(B)** citalopram, **(C)** tianeptine, **(D)** zinc hydroaspartate, **(E)** electroconvulsive shock. Number of slices tested – as in Figure 2. * $p < 0.05$, # $p < 0.01$, ANOVA

Discussion

These results extend our previous finding that repetitive treatments for 14 days with imipramine or citalopram result in a reduction of the magnitude of both AMPA/kainate and NMDA components of the field potential in rat frontal cortex, which persists for at least 2 days after the last administration of the drug [8]. The present data demonstrate a slow timecourse of the development of imipramine- and citalopram-induced adaptive changes, since the reduction of recorded responses was evident after 14 and 21 days, but not 7 days, of daily drug administration. A development of the effect, involving a decrease of both components of the field potential, was also evident in the case of ECS, where a decrease in the magnitude of responses occurred after ECS was repeated ten, but not five, times. The reductions of field potentials due to an atypical antidepressant, tianeptine, and a potentially antidepressive substance, zinc hydroaspartate, appeared faster and became evident after 7 days of treatment.

The underlying mechanisms may potentially involve both presynaptic modifications of glutamate release mechanisms and postsynaptic modifications of receptor properties. We have recently demonstrated that, in cortical slices obtained from rats treated with imipramine for 14 days, the mean frequency and the mean amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) in layer II/III pyramidal neurons were decreased. Imipramine treatment resulted in a prolongation of the rise and of the decay time constants of sEPSCs. Moreover, the treatment resulted in a reduction of the ratio of pharmacologically isolated NMDA to AMPA/kainate receptor-mediated stimulation-evoked excitatory postsynaptic currents. It has been concluded that imipramine induced mainly an attenuation of glutamate release from presynaptic terminals as well as an alteration of the reactivity of postsynaptic glutamate ionotropic receptors [34]. A decreased potassium- or veratridine-stimulated glutamate outflow in rat prefrontal cortex, consistent with a reduced release of synaptic glutamate, has also been reported to occur after repeated administration of imipramine [19] or citalopram [15].

The magnitude of adaptive changes seen in the present study differed between AMPA/kainate and NMDA components of the field potential, and thus these changes often resulted in modifications to the ratio of the components' amplitudes. Since a decreased glutamate release should affect both components to a similar degree, these effects are likely to result from adaptive processes modifying the functions of postsynaptic AMPA/kainate and NMDA receptors separately. Reductions in the NMDA to AMPA/kainate ratio were evident after 14 and 21 days of imipramine and citalopram administration and after tianeptine treatment lasting 21 days. On the other hand, 7 days of tianeptine and 21 days of zinc administration resulted in an increase in the NMDA to AMPA/kainate amplitude ratio. ECS repeated for 10, but not 5 days, also resulted in a decrease in the ratio of NMDA to AMPA/kainate components. Citalopram administration lasting 7 days resulted in a selective increase in the NMDA component, an effect that never occurred with other treatments and is hard to interpret. Collectively, these results suggest that the mechanisms underlying the effects of tested antidepressant treatments on the reactivity of postsynaptic ionotropic glutamate receptors are likely to be different.

Data regarding molecular mechanisms underlying the effect of antidepressants on glutamatergic transmission are scarce. In rat hippocampus, antidepressant drugs modify the interactions between syntaxin 1 and α-calcium/calmodulin-dependent protein kinase II as well as between syntaxin and Munc-18 [9]. Antidepressants also interfere with the function of presynaptic CaM kinase II [3]. These effects are likely to reduce the release of glutamate, which may account for a majority of the effects seen in the present study. A reduced release may also explain an apparent inconsistency of the present results, showing antidepressant treatment-induced decreases in the AMPA/kainate component of field potentials, with the reported antidepressant-induced increased expression of AMPA receptors [18] and antidepressant effects of ampakines, potentiators of AMPA receptor currents (reviewed in [1, 2]). The mechanism of action of antidepressant drugs may also involve altered phosphorylation of AMPA receptors [33]. Available data suggest different antidepressant-induced specific and regionally discrete changes in the expression of certain subunits of the AMPA receptor [4, 12, 18]. However, a selective decrease of the AMPA/kainate component of the field potential, seen in the present study in the case of tianeptine treatment for 7 days, indicates that in this particular case, while the release of glutamate remained unchanged, the number or functional properties of postsynaptic NMDA receptors underwent

downregulation. The effects of zinc hydroaspartate administration might be related to known interactions of Zn^{2+} with certain types of NMDA and AMPA receptors (reviewed in [26]). Interestingly, the mechanism of action of antidepressants on glutamate ionotropic receptors might involve activation of σ receptors (reviewed in [5]).

Thus, our results indicate that repetitive treatment with antidepressants, zinc salt and electroconvulsive shocks results in an attenuation of glutamatergic synaptic transmission in the cerebral cortex; however, the dynamics of the effects of these treatments vary.

Acknowledgments:
This work was supported by statutory funds from the Ministry of Science and Higher Education (MNiSW), Warszawa, Poland, awarded to the Institute of Pharmacology.

References:

1. Alt A, Witkin JM, Bleakman D: A role for AMPA receptors in mood disorders. Curr Pharm Des, 2005, 11, 1511–1527.
2. Arai AC, Kessler M: Pharmacology of ampakine modulators: from AMPA receptors to synapses and behavior. Curr Drug Targets, 2007, 8, 587–602.
3. Barbiero VS, Giambelli R, Musazzi L, Tiraboschi E, Tardito D, Perez J, Drago F et al.: Chronic antidepressants induce redistribution and differential activation of αCaM kinase II between presynaptic components. Neuropsychopharmacology, 2007, 32, 2511–2519.
4. Barbon A, Popoli M, La Via L, Moraschi S, Vallini I, Tardito D, Tiraboschi E et al.: Regulation of editing and expression of glutamate α-amino-propionic-acid (AMPA)/kainate receptors by antidepressant drugs. Biol Psychiatry, 2006, 59, 713–720.
5. Bermack JE, Debonnel G: The role of sigma receptors in depression. J Pharmacol Sci, 2005, 97, 317–336.
6. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH: Antidepressant effects of ketamine in depressed patients. Biol Psychiatry, 2000, 47, 351–354.
7. Bleakman D, Alt A, Witkin JM: AMPA receptors in the therapeutic management of depression. CNS Neurol Disord Drug Targets, 2007, 6, 117–126.
8. Bobula B, Tokarski K, Hess G: Repeated administration of antidepressants decreases field potentials in rat frontal cortex. Neuroscience, 2003, 120, 765–769.
9. Bonanno G, Giambelli R, Raiteri L, Tiraboschi E, Zappettini S, Musazzi L, Raiteri M et al.: Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. J Neurosci, 2005, 25, 3270–3279.
10. Boyer P-A, Skolnick P, Fossom LH: Chronic administration of imipramine and citalopram alters the expression of NMDA receptor subunit mRNAs in mouse brain. J Mol Neurosci, 1998, 10, 219–233.
11. Chourbaji S, Vogt MA, Fumagalli F, Sohr R, Frasca A, Brandwein C, Hortnagl H et al.: AMPA receptor subunit 1 (GluR-A) knockout mice model the glutamate hypothesis of depression. FASEB J, 2008, 22, 3129–3134.
12. Du J, Gray NA, Falke CA, Chen W, Yuan O, Szabo ST, Einat H, Manji HK: Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. J Neurosci, 2004, 24, 6578–6589.
13. Duman RS, Vaidya VA: Molecular and cellular actions of chronic electroconvulsive seizures. J ECT, 1998, 14, 181–193.
14. Fattaccini CM, Bolanos-Jimenez F, Gozlan H, Hamon M: Tianeptine stimulates uptake of 5-hydroxytryptamine in vivo in the rat brain. Neuropharmacology, 1990, 29, 1–8.
15. Gołembiowska K, Dziubina A: Effect of acute and chronic administration of citalopram on glutamate and aspartate release release in the rat prefrontal cortex. Pol J Pharmacol, 2000, 52, 441–448.
16. Kroczka B, Brański P, Pałucha A, Pilc A, Nowak G: Antidepressant-like properties of zinc in rodent forced swim test. Brain Res Bull, 2001, 55, 297–300.
17. Maj J, Rogóż Z, Skuza G, Sowińska H: Effects of MK-801 and antidepressant drugs in the forced swimming. Eur Neuropsychopharmacol, 1992, 2, 37–41.
18. Martinez-Turrillas, Frechilla D, Del Rio J: Chronic antidepressant treatment increases the membrane expression of AMPA receptors in rat hippocampus. Neuropharmacology, 2002, 43, 1230–1237.
19. Michael-Titus AT, Bains S, Jeetle J, Whelpton R: Imipramine and phenelzine decrease glutamate overflow in the prefrontal cortex – a possible mechanism of neuroprotection in major depression? Neuroscience, 2000, 100, 681–684.
20. Nowak G, Li Y, Paul IA: Adaptation of cortical but not hippocampal NMDA receptors after chronic citalopram treatment. Eur J Pharmacol, 1996, 295, 75–85.
21. Nowak G, Szewczyk B, Pilc A: Zinc and depression. An update. Pharmacol Rep, 2005, 57, 713–718.
22. Nowak G, Szewczyk B, Wierońska JM, Brański P, Pałucha A, Pilc A, Sadlik K, Piekoszewski W: Antidepressant-like effects of acute and chronic treatment with zinc in forced swim test and olfactory bulbectomy model in rats. Brain Res Bull, 2003, 61, 159–164.
23. Nowak G, Trullas R, Layer RT, Skolnick P, Paul IA: Adaptive changes in the N-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid. J Pharmacol Exp Ther, 1993, 265, 380–1386.
24. Ozawa H, Rasenick MM: Chronic electroconvulsive treatment augments coupling of the GTP-binding protein Gs to the catalytic moiety of adenylyl cyclase in a manner similar to that seen with chronic antidepressant drugs. J Neurochem, 1991, 56, 330–338.

25. Pałucha A, Pilc A, The involvement of glutamate in the pathophysiology of depression. Drug News Perspect, 2005, 18, 262–268.
26. Paoletti P, Vergnano AM, Barbour B, Casado M: Zinc at glutamatergic synapses. Neuroscience, 2008, in press. DOI: 10.1016/j.neuroscience.2008.01.061.
27. Petrie RXA, Reid IC, Stewart CA: The N-methyl-D-aspartate receptor, synaptic plasticity and depressive disorder. Pharmacol Ther, 2000, 87, 11–25.
28. Pittenger C, Sanacora G, Krystal JH: The NMDA receptor as a therapeutic target in major depressive disorder. CNS Neurol Disord Drug Targets, 2007, 6, 101–115.
29. Skolnick P: Antidepressants for the new millennium. Eur J Pharmacol, 1999, 375, 31–40.
30. Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R: Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. Pharmacopsychiatry, 1996, 29, 23–26.
31. Sowa-Kućma M, Legutko B, Szewczyk B, Novak K, Znojek P, Poleszak E, Papp M et al.: Antidepressant-like activity of zinc: further behavioral and molecular evidence. J Neural Transm, 2008, 115, 1621–1628.
32. Strober M, Rao U, DeAntonio M, Liston E, State M, Amaya-Jackson L, Latz S: Effects of electroconvulsive therapy in adolescents with severe endogenous depression resistant to pharmacotherapy. Biol Psychiatry, 1998, 43, 335–338.
33. Svenningsson P, Bateup H, Qi H, Takamiya K, Hugani RL, Spedding M, Roth BL et al.: Involvement of AMPA receptor phosphorylation in antidepressant actions with special reference to tianeptine. Eur J Neurosci, 2007, 26, 3509–3517.
34. Tokarski K, Bobula B, Wabno J, Hess G: Repeated administration of imipramine attenuates glutamatergic transmission in rat frontal cortex. Neuroscience, 2008, 153, 789–795.
35. Trullas R, Skolnick P: Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. Eur J Pharmacol, 1990, 185, 1–10.
36. Zahorodna A, Tokarski K, Hess G: Imipramine treatment ameliorates corticosterone-induced alterations in the effects of 5-HT_{1A} and 5-HT_4 receptor activation in the CA1 area of rat hippocampus. Eur Neuropsychopharmacol, 2006, 16, 383–390.

Received:
July 28, 2008; in revised form: December 1, 2008.