Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 78 (2004) 7–12
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Antidepressant- and anxiolytic-like activity of magnesium in mice

Ewa Poleszak[a], Bernadeta Szewczyk[b], Ewa Kędzierska[a], Piotr Wlaź[c], Andrzej Pilc[b,d], Gabriel Nowak[b,e,*]

[a]Department of Pharmacodynamics, Medical University School, Staszica 4, PL 20-081 Lublin, Poland
[b]Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, PL 31-343 Cracow, Poland
[c]Department of Animal Physiology, Institute of Biology, Maria Curie-Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[d]Institute of Public Health, Collegium Medicum, Jagiellonian University, Michalowskiego 12, PL 31-126 Cracow, Poland
[e]Department of Pharmacobiology, Collegium Medicum, Jagiellonian University, Medyczna 9, PL 30-688 Cracow, Poland

Received 7 October 2003; received in revised form 12 January 2004; accepted 14 January 2004

Available online 5 March 2004

Abstract

The antidepressant- and anxiolytic-like effects of magnesium, an *N*-methyl-D-aspartate (NMDA) glutamate receptor inhibitor, were studied in mice using the forced swim test and elevated plus-maze test, respectively. The doses of 20 and 30 mg Mg/kg, reduced immobility time in the forced swim test exerting antidepressant-like activity. In the elevated plus-maze test, magnesium at the same doses produced anxiolytic-like effect. The doses of magnesium active in both tests did not affect locomotor activity. To evaluate the tolerance to these effects, we also performed experiments on the following acute/chronic magnesium treatment schedule: chronic saline and saline challenge at 0.5 h before behavioral experiments or serum magnesium determination (S + S), chronic saline and magnesium challenge (S + Mg), chronic magnesium and saline challenge (Mg + S), chronic magnesium and magnesium challenge (Mg + Mg). The antidepressant- and anxiolytic-like effect of magnesium was demonstrated in groups treated acutely and chronically with magnesium (Mg + Mg), but not in the Mg + S group. Moreover, these effects seem to be connected with at least 58% increase in serum magnesium concentration.

The results indicate that magnesium induces the antidepressant- and anxiolytic-like effects without tolerance to these activities, which suggests a potential antidepressant and anxiolytic activity of magnesium in these disorders in humans.

Keywords: Magnesium; Forced swim test; Elevated plus-maze; Locomotor activity; Mice

1. Introduction

Magnesium (Mg) is an essential mineral that is needed for a broad variety of physiological functions. The symptoms of magnesium deficiency are variable and associated with nonspecific clinical signs. Very often, neuromuscular hyperexcitability, cardiac arrhythmias, increased muscle tension, muscle cramps, increased stress susceptibility, and headaches are observed (Iannello and Belfiore, 2001). Moreover, deficiency of Mg ions has been related to depressive disorders (Rasmussen et al., 1989; Hashizume and Mori, 1990). In animals, Mg reduces immobility in the forced swim test similarly to the conventional antidepressant, imipramine (Decollogne et al., 1997). Moreover, Mg depletion for 21 days in mice leads to an increase in anxiety- and depression-like behavior, manifested with a decreased struggling in the forced swim test and increased preference for the dark compartment in the light–dark test (Murck, 2002).

The main effect of magnesium is connected with its action on glutamate receptors. Magnesium is an inhibitor of the *N*-methyl-D-aspartate (NMDA) receptor (the ionotropic glutamate receptor). The activation of NMDA receptor ion channel is blocked by Mg^{2+} in a voltage-dependent manner (Mori et al., 1992). In vitro, this blockade operates at extracelullar Mg^{2+} concentrations of less than 1 mM, which are within the range of those found in the cerebrospinal fluid and plasma of humans and animals (Morris, 1992). It has been suggested that this

* Corresponding author. Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, PL 31-343 Cracow, Poland. Tel.: +48-12-6623215; fax: +48-12-6374500.
E-mail address: nowak@if-pan.krakow.pl (G. Nowak).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.01.006

mechanism is probably responsible for antidepressant-like effects in the forced swim test in mice (Decollogne et al., 1997).

Several clinical studies demonstrated low serum Mg level in depressed patients (e.g., Hashizume and Mori, 1990; Zięba et al., 2000); however, the data are inconsistent because no alterations or increases in blood Mg have been observed (Murck, 2002). A decrease in Mg concentration in the cerebrospinal fluid has been reported in subjects after suicidal attempts (Banki et al., 1985). Moreover, the mood-stabilizing properties of Mg have been demonstrated in case reports in patients with mania (Pavlinac et al., 1979).

Because depression and anxiety coexist in clinical practice (Zimmerman et al., 2000), the abovementioned data prompted us to more extensively examine antidepressant and anxiolytic activity of Mg in mice models, using the forced swim test and elevated plus-maze test, respectively.

2. Materials and methods

2.1. Animals

All procedures were approved by the Ethical Committee of the Medical University School, Lublin and the Institute of Pharmacology Polish Academy of Sciences, Cracow. The experiments were carried out on male Albino Swiss mice (25–30 g). The animals were kept under a normal day–night cycle with free access to food and water. Each experimental group consisted of 8–15 animals. Magnesium salts [chloride (Sigma), sulfate (Sigma), hydroaspartate (Farmapol)] were administered intraperitoneally 0.5 h before the test in acute experiments. Control animals received a vehicle. In chronic experiments, magnesium hydroaspartate was administered intraperitoneally for 14 days, once daily. Experiments were performed 24 h after the last magnesium or saline injections.

2.2. Forced swim test

The studies were carried out on mice according to the method of Porsolt et al. (1997). Mice were dropped individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water, maintained at 23–25 °C. Mice were left in the cylinder for 6 min. After the first 2 min, the total duration of immobility in rats was measured during a 4-min test. The mouse was judged to be immobile when it remained floating passively in the water.

2.3. Elevated plus-maze test

The studies were carried out on mice according to the method of Lister (1987). The plus-maze apparatus was made of Plexiglas and consisted of two open (30 × 5 cm) and two enclosed (30 × 5 × 15 cm) arms. The arms extended from a central platform of 5 × 5 cm. The apparatus was mounted on a Plexiglas base raising it 38.5 cm above the floor and illuminated by red light. The test consisted of placing a mouse in the center of the apparatus (facing a enclosed arm) and allowing it to freely explore. The number of entries into the open arms and the time spent in these arms were scored for a 5-min test period. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: the total number of arm entries; the percentage of arm entries into the open arms; the time spent in the open arms expressed as a percentage of the time spent in both the open and closed arms. Anxiolytic activity was indicated by increases in time spent in open arms or in number of open arm entries. Total number of entries into either type of arm was used as a measure of overall motor activity.

2.4. 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 for 10 min. Activity was measured at 5-min intervals to characterize dynamics of changes. The number of crossed light beams by the mice was recorded as the locomotor activity.

2.5. Determination of serum magnesium concentration

Total magnesium concentration in blood serum was determined by calmagite method, which is precise and has a high correlation coefficient with atomic absorption spectrometry (Ryan and Barbour, 1998). Serum was isolated by centrifugation 1 h after collection and coagulation of trunk blood. Ten microliters of serum was added to 1 ml of the commercially available reagent (Prohand, Lodz, Poland) and the absorbance of the solution was read at 520 nm in a spectrophotometer (Ultrospect 2000, Pharmacia Biotech).

2.6. Statistics

The obtained data were evaluated by the one-way or two-way analysis of variance (ANOVA), followed by Dunnett's multiple-comparisons test. All results are presented as means ± S.E.M. $P < .05$ was considered as statistically significant.

3. Results

3.1. Effects of magnesium in the forced swim test in mice

The effect of magnesium chloride, sulfate and hydroaspartate (at the doses 10, 20 and 30 mg Mg/kg) in the forced swimming test in mice is demonstrated in Table 1. The doses of 20 and 30 mg Mg/kg significantly reduced the immobility time, while the dose of 10 mg Mg/kg had no effect [ANOVA: $F(3,28) = 4.03$, $P < .01$; $F(5,36) = 12.55$, $P < .0001$; $F(3,28) = 20.29$, $P < .0001$ (for chloride, sulfate and hydroaspartate, respectively)].

Table 1
Effects of magnesium administration on the immobility time in the forced swimming test in mice

Compound	Dose (mg Mg/kg)	Immobility time (s)
Vehicle		188.3 ± 10.57
Magnesium chloride	10	172.0 ± 7.86
Magnesium chloride	20	148.4 ± 13.18*
Magnesium chloride	30	146.0 ± 7.48*
Vehicle		190.0 ± 3.36
Magnesium sulfate	10	175.0 ± 6.82
Magnesium sulfate	20	123.8 ± 10.92**
Magnesium sulfate	30	137.7 ± 9.70**
Vehicle		148.5 ± 4.52
Magnesium hydroaspartate	10	139.9 ± 7.61
Magnesium hydroaspartate	20	104.0 ± 8.18**
Magnesium hydroaspartate	30	88.88 ± 3.80**

Magnesium (chloride, sulfate and hydroaspartate) was administered 0.5 h before the test. The values represent means ± S.E.M. ($n = 10$ mice per group).
* $P < .05$ vs. the control vehicle-treated group (Dunnett's test).
** $P < .01$ vs. the control vehicle-treated group (Dunnett's test).

3.2. Effects of magnesium in the elevated plus-maze test

The effect of magnesium hydroaspartate (at the doses 10, 20 and 30 mg Mg/kg) in the elevated plus-maze test in mice

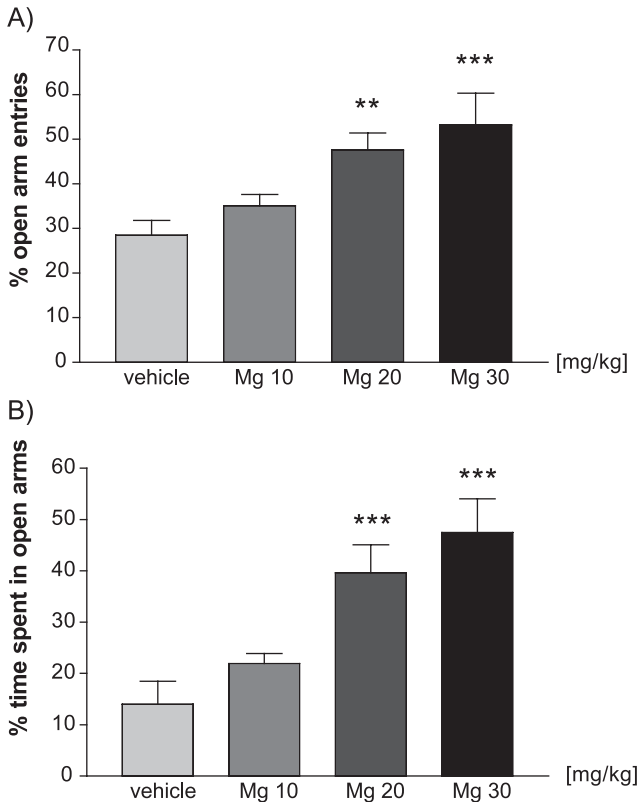

Fig. 1. Effects of magnesium administration on percentage of open arm entries (A) and percentage of time spent in open arms (B) in an elevated plus-maze procedure. Magnesium was administered 0.5 h before the test. Data represent means ± S.E.M. of 10 mice per group. ** $P < .01$, *** $P < .001$ vs. control vehicle-treated group (Dunnett's test).

Table 2
The effects of magnesium administration on spontaneous locomotor activity in mice

Compound	Dose mg Mg/kg	Activity counts	
		5 min	10 min
Vehicle		82.85 ± 4.76	126.0 ± 9.94
MgCl	20	78.25 ± 7.00	119.5 ± 3.40
MgCl	30	80.88 ± 7.41	135.6 ± 15.02

Magnesium (magnesium chloride) was administered 0.5 h before the test. The values represents means ± S.E.M. ($n = 10$ mice per group).

is demonstrated in Fig. 1. Magnesium dose-dependently increased the percentage of open-arm entries, and the results obtained with doses of 20 and 30 mg Mg/kg reached statistical significance [Fig. 1A; ANOVA: $F(3,26) = 5.83$, $P < .0035$]. There was also a dose-dependent increase in the percentage of time spent in the open arms and the effects of doses of 20 and 30 mg Mg/kg were statistically significant [Fig. 1B; ANOVA: $F(3,22) = 10.84$, $P < .0001$].

3.3. Effect of magnesium on spontaneous locomotor activity in mice

The effects of magnesium on spontaneous locomotor activity in mice are shown in Table 2. Magnesium administered at the doses of 20 and 30 mg Mg/kg did not influence the locomotor activity in mice.

3.4. Effect of acute and chronic magnesium administration on the immobility time in the forced swim test in mice

The effects of acute and chronic magnesium treatment on the immobility time in the forced swim test are presented in

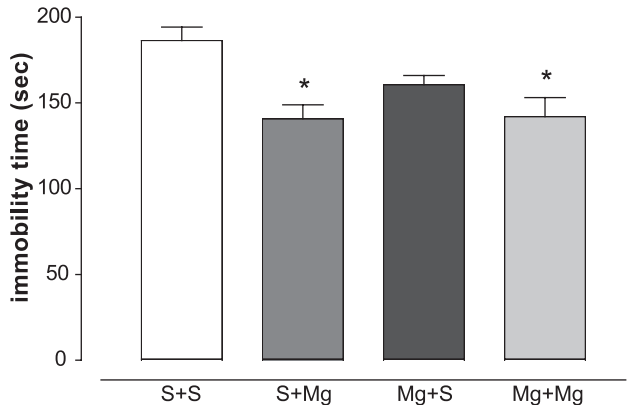

Fig. 2. Effect of acute and chronic treatment with magnesium on the immobility time in the forced swimming test in mice. The following treatment schedules were applied: chronic saline and saline challenge at 0.5 h before behavioral experiments (S + S), chronic saline and magnesium challenge (S + Mg), chronic magnesium and saline challenge (Mg + S), chronic magnesium and magnesium challenge (Mg + Mg). Magnesium (magnesium hydroaspartate) was administered at the dose of 30 mg Mg/kg. Data represent means ± S.E.M. ($n = 8–12$). * $P < .05$ vs. control (S + S) group (Dunnett's test).

Fig. 2. Two-way ANOVA revealed no chronic treatment effect [$F(1,38)=2.21$, $P=.1455$] and significant magnesium challenge effect [$F(1,38)=15.44$, $P=.0003$]. Moreover, there is no interaction between groups [$F(1,38)=2.73$, $P=.1070$]. Magnesium administered acutely (S + Mg) or chronically with magnesium challenge (Mg + Mg), but not with last Mg injection 24 h before saline challenge (Mg + S), significantly reduced the immobility time (Fig. 2).

3.5. Effect of acute and chronic magnesium administration on the time spent in the open arms of the elevated plus-maze in mice

The effects of acute and chronic magnesium treatment on percentage time spent in the open arms of the elevated plus-maze are presented in Fig. 3. Two-way ANOVA revealed no chronic treatment effect [$F(1,39)=0.0284$, $P=.8670$] and significant magnesium challenge effect [$F(1,39)=13.85$, $P=.0006$]. Moreover, there is no interaction between groups [$F(1,39)=0.2116$, $P=.6481$]. Magnesium administered acutely (S + Mg) or chronically with magnesium challenge (Mg + Mg) significantly reduced the time spent in the open arms. However, magnesium administered chronically with saline challenge (Mg + S) did not affect the time spent in the open arms (Fig. 3).

3.6. Effect of acute and chronic magnesium administration on serum magnesium level in mice

The effects of acute and chronic magnesium treatment on serum magnesium level are presented in Table 3 [ANOVA: $F(3,56)=34.351$, $P<.0001$]. Different magnesium treatment schedules, acute or chronic, significantly (by 39–74%) increased serum magnesium level.

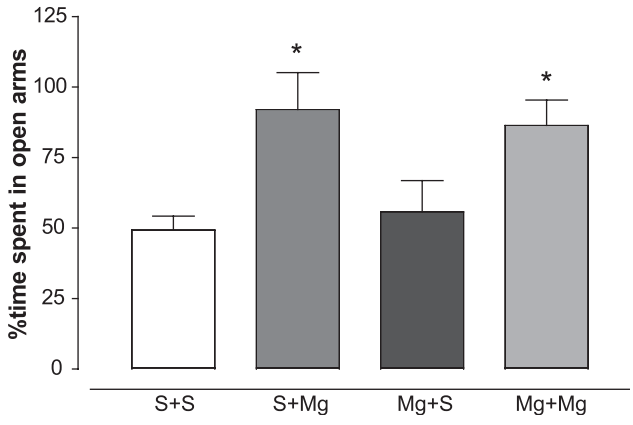

Fig. 3. Effect of acute and chronic treatment with magnesium on the time spent in open arms in the elevated plus-maze in mice. The following treatment schedules were applied: chronic saline and saline challenge at 0.5 h before behavioral experiments (S + S), chronic saline and magnesium challenge (S + Mg), chronic magnesium and saline challenge (Mg + S), chronic magnesium and magnesium challenge (Mg + Mg). Magnesium (magnesium hydroaspartate) was administered at the dose of 30 mg Mg/kg. Data represent means ± S.E.M. ($n=10-12$). *$P<.05$ vs. control (S + S) group (Dunnett's test).

Table 3
The effects of magnesium administration on serum magnesium level in mice

Treatment	Magnesium level (mg Mg/100 ml)	Percentage of control
S + S	2.11 ± 0.06	100
S + Mg	3.67 ± 0.14*	174
Mg + S	2.94 ± 0.10*	139
Mg + Mg	3.33 ± 0.14*	158

Magnesium (magnesium hydroaspartate) at the dose of 30 mg Mg/kg was administered according to the following schedule: chronic saline and saline challenge at 0.5 h before experiments (S + S), chronic saline and magnesium challenge (S + Mg), chronic magnesium and saline challenge (Mg + S), chronic magnesium and magnesium challenge (Mg + Mg). The values represents means ± SEM ($n=15$ mice per group).

*$P<.05$ vs. control (S + S) group (Dunnett's test).

4. Discussion

Antidepressant therapy includes drugs mostly affecting monoamine reuptake or metabolism, as well as nonpharmacologic management, such as electroconvulsive shock (Hollister and Csernansky, 1990). However, the mechanism of antidepressant therapy is still the matter of dispute (Vetulani and Nalepa, 2000). Recent data have suggested that glutamate NMDA receptor may be involved in the mechanism of action of antidepressant treatments (Skolnick et al., 1996, 2001; Skolnick, 1999). Several studies have demonstrated that the NMDA receptor antagonists exhibit antidepressant-like activity in tests/models, such as forced swim test, chronic unpredictable stress, chronic mild stress and olfactory bulbectomy (Layer et al., 1995; Maj et al., 1992a,b, 1994; Moryl et al., 1993; Ossowska et al., 1997; Papp and Moryl, 1994, 1996; Przegaliński et al., 1997; Redmond et al., 1997; Trullas and Skolnick, 1990). Moreover, zinc ion, an antagonist of NMDA receptor, is active in rodent forced swim test and olfactory bulbectomy model (Kroczka et al., 2000, 2001; Nowak et al., 2003). Besides antidepressant-like effects, the NMDA receptor antagonists exhibit anxiolytic-like activity, examined in the elevated plus-maze test (Dunn et al., 1989; Płaźnik et al., 1994; Wiley et al., 1995; Przegaliński et al., 1996; Pilc et al., 2002). Thus, antidepressant- and anxiolytic-like properties of NMDA receptor ligands suggest the involvement of glutamatergic system in the mechanism of antidepressant and anxiolytic action (Pilc et al., 2002).

Involvement of magnesium in the antidepressant effects was demonstrated previously (Decollogne et al., 1997). In the forced swim test, magnesium salts reduced the immobility time and this effect was comparable with that observed with imipramine or MK-801 (Decollogne et al., 1997). The effect of magnesium in the forced swim test was observed at its doses between 30 and 100 mg/kg

(Decollogne et al., 1997). Thus, the present results confirmed antidepressant-like activity of magnesium in the forced swim test also at the lower dose than previously demonstrated. The doses active in this test did not influence locomotor activity of animals. Hence, simple increase in motor activity does not participate in the antidepressant-like action.

The present study is the first demonstration of an anxiolytic-like activity of magnesium. The results showed that magnesium significantly increased the time spent in the open arms and the number of open arm entries without affecting motor activity.

Treatment of depression in humans requires chronic (weeks) administration of antidepressants for manifestation of the therapeutic effect (Oswald et al., 1972), thus, the neuronal adaptive alterations seem to participate in mechanism(s) of action of antidepressants. For this reason, it is important to examine if tolerance to the acute antidepressant-like effect develops after prolonged treatment with an antidepressant agent. Development of tolerance is also an important factor in anxiolytic activity (Pilc et al., 2002).

Our data demonstrate that chronic treatment with magnesium does not alter both measured behavioral responses to magnesium challenge 24-h after the last dose. Moreover, challenge with saline in chronically magnesium treated group does not exhibit behavioral effect. At that time, a 39% increase in serum magnesium level is present. Thus, the antidepressant- and anxiolytic-like activity of magnesium seems to be connected with a rise (by at least 58%) in serum magnesium concentration.

The results indicate that magnesium induces the antidepressant- and anxiolytic-like effects in animals without development of tolerance to these actions, which strongly suggests a potential antidepressant and anxiolytic activity of magnesium in these disorders in humans.

Our data further support the notion that inhibition of the NMDA receptor activity is involved in the mechanisms of both antidepressant and anxiolytic activity.

Acknowledgements

The authors thank "Farmapol" Sp. z o.o., Poznan, Poland for generous gift of magnesium hydroaspartate. B. Szewczyk is a grantee of the Foundation for Polish Science.

References

Banki CM, Vojnik M, Papp Z, Balla KZ, Arato M. Cerebrospinal fluid magnesium and calcium related to amine metabolites, diagnosis, and suicide attempts. Biol Psychiatry 1985;20:163–71.

Decollogne S, Tomas A, Lecerf C, Adamowicz E, Seman M. NMDA receptor complex blockade by oral administration of magnesium: comparison with MK-801. Pharmacol Biochem Behav 1997;58:261–8.

Dunn RW, Corbett R, Fielding S. Effects of 5-HT_{1A} receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur J Pharmacol 1989;169:1–10.

Hashizume N, Mori M. An analysis of hypermagnesemia and hypomagnesemia. Jpn J Med 1990;29:368–72.

Hollister LE, Csernansky JG. Clinical pharmacology of psychotherapeutic drugs. 3rd ed. New York: Churchill Livingstone; 1990.

Iannello S, Belfiore F. Hypomagnesemia. A review of pathophysiological, clinical and therapeutical aspects. Panminerva Med 2001;43:177–209.

Kroczka B, Zięba A, Dudek D, Pilc A, Nowak G. Zinc exhibits an antidepressant-like effects in the forced swimming test in mice. Pol J Pharmacol 2000;52:403–6.

Kroczka B, Branski P, Palucha A, Pilc A, Nowak G. Antidepressant-like properties of zinc in rodent forced swim test. Brain Res Bull 2001;55: 297–300.

Layer RT, Popik P, Olds T, Skolnick P. Antidepressant-like actions of the polyamide site NMDA antagonist, eliprodil (SL-82.0715). Pharmacol Biochem Behav 1995;52:621–7.

Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.

Maj J, Rogóż Z, Skuza G, Sowińska H. Effects of MK-801 and antidepressant drugs in the forced swimming testing rats. Eur Neuropsychpharmacol 1992a;2:37a–41a.

Maj J, Rogóż Z, Skuza G, Sowińska H. The effect of CGP37849 and CGP 39551, competitive NMDA receptor, antagonists in the forced swimming test. Pol J Pharmacol 1992b;44:337–46.

Maj J, Rogóż Z, Skuza G, Kołodziejczyk K. Some central effects of kynurenic acid, 7-chlorokynurenic acid and 5,7-dichloro-kynurenic acid, glycine side antagonists. Pol J Pharmacol 1994;46:115–24.

Mori H, Masaki H, Yamakura T, Mishina M. Identification by mutagenesis of a Mg^{2+}-block site of the NMDA receptor channel. Nature 1992; 358:673–5.

Morris ME. Brain and CSF magnesium concentrations during magnesium deficit in animals and humans: neurological symptoms. Magnes Res 1992;5:303–13.

Moryl E, Danysz W, Quack G. Potential antidepressive properties of amantadine, memantine and bifemelane. Pharmacol Toxicol 1993;72:394–7.

Murck H. Magnesium and affective disorders. Nutr Neurosci 2002;5: 375–89.

Nowak G, Szewczyk B, Wieronska JM, Branski P, Palucha A, Pilc A, et al. Antidepressant-like effects of acute and chronic treatment with zinc in forced swim test and olfactory bulbectomy model in rats. Brain Res Bull 2003;61:159–64.

Ossowska G, Klenk-Majewska B, Szymczyk G. The effects of NMDA antagonists on footshock-induced fighting behavior in chronically stressed rats. J Physiol Pharmacol 1997;48:127–35.

Oswald I, Brezinova V, Dunleavy DLF. On the slowness of action of tricyclic antidepressant drugs. Br J Psychiatry 1972;120:673–7.

Papp M, Moryl E. Antidepressant activity of non-competitive and competitive NMDA receptor antagonist in e chronic mild stress model of depression. Eur J Pharmacol 1994;263:1–7.

Papp M, Moryl E. Antidepressant-like effects of I-aminocyclopropanecarboxylic acid and D-cycloserine in an animal model of depression. Eur J Pharmacol 1996;316:145–51.

Pavlinac D, Langer R, Lenhard L, Deftos L. Magnesium in affective disorders. Biol Psychiatry 1979;14:657–61.

Pilc A, Klodzinska A, Nowak G. A role of glutamate in the treatment of anxiety and depression: focus on group I metabotropic glutamate (mGlu) receptors. Drugs Future 2002;27:753–63.

Płaźnik A, Pałejko W, Nazar M, Jessa M. Effects of antagonists at the NMDA receptor complex in two models of anxiety. Eur Neuropsychopharmacol 1994;4:503–12.

Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1997; 229:327–36.

Przegaliński E, Tatarczyńska E, Dere-Wesołek A, Chojnacka-Wójcik E. Anticonflict effects of a competitive NMDA receptor antagonist and a partial agonist at strychnine-insensitive glycine receptors. Pharmacol Biochem Behav 1996;54:73–7.

Przegaliński E, Tatarczyńska E, Deren-Wesołek A, Chojnacka-Wójcik E.

Antidepressant-like effects of a partial agonist at strychnine-insensitive glycine receptors and a competitive NMDA receptor antagonist. Neuropharmacology 1997;36:31–7.

Rasmussen HH, Mortensen PB, Jensen IW. Depression and magnesium deficiency. Int J Psychiatr Med 1989;19:57–63.

Redmond AM, Kelly JP, Leonard BE. Behavioural and neurochemical effects of dizocilpine in the olfactory bulbectomized rat model of depression. Pharmacol Biochem Behav 1997;58:355–9.

Ryan MF, Barbour H. Magnesium measurement in routine clinical practice. Ann Clin Biochem 1998;35:449–59.

Skolnick P. Antidepressants for the new millennium. Eur J Pharmacol 1999;375:31–40.

Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R. Adaptation of *N*-methyl-D-aspartate receptors following antidepressant treatment: implications for the pharmacotherapy of depression. Pharmacopsychiatry 1996;29:23–6.

Skolnick P, Legutko B, Li X, Bymaster FP. Current perspectives on the development of non-biogenic amine-based antidepressants. Pharmacol Res 2001;43:411–22.

Trullas R, Skolnick P. Functional antagonists at the NMDA receptor complex exhibit antidepressant action. Eur J Pharmacol 1990;185:1–10.

Vetulani J, Nalepa I. Antidepressants: past, present and future. Eur J Pharmacol 2000;405:351–63.

Wiley JL, Cristello AF, Balster RL. Effects of site-selective NMDA receptor antagonists in an elevated plus-maze model of anxiety in mice. Eur J Pharmacol 1995;294:101–7.

Zięba A, Kata R, Dudek D, Schlegel-Zawadzka M, Nowak G. Serum trace elements in animal models and human depression: III. Magnesium. Relationship with copper. Hum Psychopharmacol Clin Exp 2000;15:631–5.

Zimmerman M, Mcdermut W, Mattia JI. Frequency of anxiety disorders in psychiatric outpatients with major depressive disorder. Am J Psychiatry 2000;157:1337–40.