Antidepressant-like activity of magnesium in the chronic mild stress model in rats: alterations in the NMDA receptor subunits

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Abstract

Recent data suggests that the glutamatergic system is involved in the pathophysiology and treatment of major depressive disorder (MDD) and that the N-methyl-D-aspartate (NMDA) receptor is a potential target for antidepressant drugs. The magnesium ion blocks the ion channel of the NMDA receptor and prevents its excessive activation. Some preclinical and clinical evidence suggests also that magnesium may be useful in the treatment of depression. The present study investigated the effect of magnesium treatment (10, 15 and 20 mg/kg, given as magnesium hydroaspartate) in the chronic mild stress (CMS) model of depression in rats. Moreover, the effect of CMS and magnesium (with an effective dose) on the level of the proteins related to the glutamatergic system (GluN1, GluN2A, GluN2B and PSD-95) in the hippocampus, prefrontal cortex (PFC) and amygdala were examined. A significant reduction in the sucrose intake induced by CMS was increased by magnesium treatment at a dose of 15 mg/kg, beginning from the third week of administration. Magnesium did not affect this behavioural parameter in the control animals. CMS significantly increased the level of the GluN1 subunit in the amygdala (by 174%) and GluN2A in the hippocampus (by 191%), both of which were significantly attenuated by magnesium treatment. Moreover, magnesium treatment in CMS animals increased the level of GluN2B (by 116%) and PSD-95 (by 150%) in the PFC. The present results for the first time demonstrate the antidepressant-like activity of magnesium in the animal model of anhedonia (CMS), thus indicating the possible involvement of the NMDA/glutamatergic receptors in this activity.

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Introduction

Magnesium plays a fundamental role in the regulation of various processes that are necessary for organisms to function well. It is essential for the activity of about 300 enzymes (Ryan, 1991). An appropriate concentration of magnesium is required for muscles to work properly, acid–base balance maintenance, biosynthesis of proteins and the upkeep of the structure of nucleic acids (Grubbs and Maguire, 1987). Magnesium ions also have a key role in the modulation of neurotransmission in the central nervous system (e.g. the blocking of the ion channel pore of the NMDA receptor is the main site of action of magnesium ions (Szewczyk et al., 2012)).

As an important microelement, magnesium modulates many aspects of behaviour. Several clinical studies have indicated the role of magnesium in the pathophysiology and treatment of depression. Most depressed patients in these studies had abnormal serum/plasma magnesium concentrations. However, no simple correlation between depressive symptoms and magnesium concentration was found. A low blood magnesium level occurred mainly in patients with long-lasting and unipolar depression (Kirov et al., 1994); it was not observed in patients with acute depression (Linder et al., 1989; Hashizume and Mori, 1990; Hasey et al., 1993; Eby and Eby, 2006). In other studies, some fluctuations in the plasma concentration of magnesium were noticed, closely related to
the phase of the disease. Patients with depression had a reduced content of magnesium in their plasma, and their recovery correlated with normalization of the serum magnesium levels (Frizel et al., 1969; Hashizume and Mori, 1990). Also, in cases of rapid cycling bipolar disorder, treatment with magnesium correlated with an improved mood (Pavlinic et al., 1979). On the other hand, hypomagnesemia was found to induce depression- and anxiety-like behaviour (Singewald et al., 2004; Sartori et al., 2012). It has been reported that a decreased level of magnesium is associated with a reduction in offensive, and an increase in defensive, behaviour in animals (Kantak, 1988). It is important to note that both depression and anxiety-related behaviour were reversed by antidepressant and anxiolytic drugs, respectively (Singewald et al., 2004). Studies by Sartori et al. (2012) have revealed that magnesium deficiency induced an increase in the transcription of the corticotropin releasing hormone (CRH) in the paraventricular hypothalamic nucleus (PVN), and elevated adrenocorticotropic hormone (ACTH) plasma levels, indicating an enhanced set-point of the hypothalamic–pituitary–adrenal (HPA) axis. Additionally, magnesium deficient mice were more sensitive to anxiety-provoking situations. Dysregulation of the HPA axis evoked by magnesium deficiency was normalized by chronic desipramine or diazepam treatment (Sartori et al., 2012). This data indicated that dysregulation in the HPA axis may contribute to the hyper-emotionality in response to dietary induced hypomagnesaemia. It is also possible that it plays an important role in the induction of depressive-like symptoms observed in hypomagnesaemia.

Magnesium was found to exert antidepressant-like activity in the rodent screening tests. Administration of magnesium effectively reduced the immobility time in rodents in the forced swim test (FST) (Decollogne et al., 1997; Poleszak et al., 2004). Moreover, magnesium enhanced the antidepressant-like effect of antidepressants in the FST (Poleszak et al., 2005b, 2007). The best established mechanism involved in the antidepressant-like activity of magnesium seems to be the inhibitory modulation of glutamate signaling through the inhibition of N-methyl-d-aspartate (NMDA) receptors. It was found that the antidepressant-like activity evoked by magnesium in the FST was antagonized by administration of NMDA receptor agonists (NMDA or D-serine), while ineffective doses of magnesium, administered concomitantly with ineffective doses of NMDA receptor antagonist (CGP 37849, D-cycloserine, L-701,324, MK-801), show a significant reduction in the immobility time in the FST (Poleszak et al., 2007, 2008).

Since, so far, the antidepressant potential of magnesium has only been examined in the screening test (the FST), we decided to evaluate the antidepressant efficacy of magnesium in a more complex procedure like the chronic mild stress (CMS) model.

As noted above, a blockade of the ion channel of the NMDA receptor is the most well-known and established way in which magnesium affects the functioning of the central nervous system (CNS). This effect prevents hyperactivity of the NMDA receptor and protects nerve cells from an excessive influx of calcium into the interior (Szewczyk et al., 2012). In physiological conditions, magnesium ions can be removed from the pore of the channel in two distinct ways. The first possibility is the removal of magnesium by stimulation of the (2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid) (AMPA) receptors. The second manner is linked to the activity of protein kinase C (PKC). This way is independent of the cell membrane potential (Pittaluga et al., 2000). It has been reported that depletion of magnesium in spinal cord neurons caused an increase in the PKC-dependent activity of NMDA receptors (Begon et al., 2001). In addition Li-Smerin et al. (2001) discovered that an intracellular magnesium-dependent blockade of NMDA receptors increases with membrane depolarization of the cells (Li-Smerin et al., 2001).

Administration of magnesium evokes some effects that are similar to those caused by ketamine (a non-competitive NMDA receptor antagonist), whose antidepressant properties are well known. For example, magnesium and ketamine lead to an increase in cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) expression in PFC (Abumaria et al., 2011; Murck, 2013). These changes may be very important for achieving the antidepressant effects of both magnesium and ketamine (Abumaria et al., 2011).

The study by Kotermanski and Johnson (2009) indicates that the physiological concentration of magnesium is sufficient to block the entire pool of NMDA receptors, and that external supplementation in this case can be ineffective. However, this study suggests that sensitivity of the memantine (an NMDA-type open-channel blocker) to the heteromeric GluN1/2A and GluN1/2B NMDA receptors is inversely proportional to magnesium concentration and, in turn, that the NMDA receptors composed of GluN1/2C and GluN1/2D are more insensitive to magnesium blockade. As such, the therapeutic effects of noncompetitive NMDA antagonists may be due to the blockade of the above receptors (Kotermanski and Johnson, 2009). It was also found that the potency of ketamine to attenuate depressive symptoms is more pronounced in patients with a lower concentration of magnesium (Murck, 2013). As mentioned above, interactions between the concentration of magnesium and the affinity of ketamine or memantine to NMDA receptors are linked to extracellular concentrations of magnesium. It seems that such an approach ignores the very important issues concerning the intracellular role of magnesium. Numerous studies have confirmed that the function of the native NMDA receptor is the result of equilibrium between extracellular and intracellular concentration of magnesium.

As reported in recent post-mortem studies, changes in the amounts and the composition of the NMDA receptors...
are correlated with depressive disorders (human data) (Feyissa et al., 2009; Karolewicz et al., 2009) or depression-like behaviour (animal tests) (Tokita et al., 2012). These changes especially relate to GluN2A and GluN2B, as well as post-synaptic density protein-95 (PSD-95), which plays a key role in mediating trafficking, clustering, and downstream signaling events, following NMDA receptor activation (Paoletti et al., 2013). Some data indicate the essential role of PSD-95 and glutamate receptors both in the development of, and recovery from, depression. The excessive stimulation of extra-synaptic di-heteromeric GluN2B NMDA receptors conjugated with PSD-95 may activate neuronal nitric oxide synthase, an important factor leading to neuronal death (Fan et al., 2010; Paoletti et al., 2013). Conversely, activation of tri-heteromeric receptors GluN1/2A/2B in the synaptic layer leads to a higher level of PSD-95 and long term potentiation (Paoletti et al., 2013). Based on these findings, and the fact that magnesium may induce antidepressant actions via the NMDA receptor complex, the second aim of this study was to examine the effect of the CMS procedure and magnesium treatment on the expression of NMDA receptor subunits GluN1, GluN2A and GluN2B, as well as PSD-95 in the PFC, hippocampus and amygdala of rats. Additionally, serum magnesium concentration in rats subjected to the CMS procedure and magnesium treatment was examined.

Methods

Animals and housing

Male Wistar rats (Charles River, Germany) were brought into the laboratory one month before the start of the experiment. Apart from those described below, the animals were singly housed with food and water freely available, and were maintained on a 12 h light/dark cycle with constant temperature (22±2 °C) and humidity (50±5%) conditions. All procedures were undertaken in accordance with the guidelines of the National Institutes of Health Animal Care and Use Committee and were approved by the Ethic Committee of the Institute of Pharmacology Polish Academy of Science in Krakow. All the procedures were performed with a minimization of animal suffering and as few animals used as possible.

Stress procedure (Fig. 1)

After a period of 2 wk of adaptation to a laboratory and the housing conditions, the animals were first trained to consume a 1% sucrose solution; the training consisted of nine 1 h baseline tests in which sucrose was presented, in the home cage, following 14 h of food and water deprivation; and the sucrose intake was measured by weighing bottles containing the sucrose solution, at the end of the test. Subsequently, sucrose consumption was monitored under similar conditions, at weekly intervals throughout the whole experiment.

On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group of animals was subjected to the chronic mild stress procedure for a period of seven consecutive weeks. Each week of the stress regime consisted of: two periods of food or water deprivation, two periods of 45° cage tilt, two periods of intermittent illumination (lights on and of every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low intensity stroboscopic illumination (150 flashes/min) and three periods without stress. All of the stressors were of 10–14 h duration and were applied individually and continuously, day and night. The control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for the 14 h preceding each sucrose test, but otherwise food and water were freely available in the home cage.

Drug administration

On the basis of their sucrose intakes following an initial 2 wk of stress, both the stressed and the control groups were each divided further into matched subgroups (n=8), and during the subsequent 5 wk they received once daily i.p. injections of the vehicle (sterile saline, 1 ml/kg), magnesium (given as magnesium hydro-aspartate 10, 15 and 20 mg/kg). The drugs were administered at approximately 10.00 hours, and the weekly sucrose tests were carried out 24 h following the last drug injection. After 5 wk and 24 h following the last treatment, all of the animals were terminated and their brain and blood samples were collected for further
analysis. Stress was continued throughout the entire period of treatment.

**Immunobloting of NMDA receptor subunits and PSD-95**

24 h after the last dosage of magnesium, the animals were killed and their brains were collected. The hippocampus, amygdala and the prefrontal cortex were rapidly dissected. The tissues were frozen in dry ice and stored at −80 °C. In the next step, they were homogenized in ice in 2% solution of sodium dodecyl sulfate (SDS).

After that, the homogenates were denatured at 95 °C for 10 min and finally centrifuged for 5 min at 10000 r/min at 4 °C. After centrifugation, the supernatant was collected and the protein content was determined. For this assay, bicinchonic acid was used (Pierce). Next, the samples were fractionated by 10.0% SDS-polyacrylamide gel electrophoresis. In a further step, proteins were transferred to the nitrocellulose membrane (Invitrogen, UK). To block non-specific binding, 1% blocking solution was used (BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit), Roche, Switzerland). After blocking, the membranes were incubated overnight at 4 °C with the respective antibodies. The following antibodies were used: polyclonal anti-NMDAR1 antibody (Abcam), diluted 1:500; polyclonal anti-NMDAR2A antibody (Abcam), diluted 1:1000; polyclonal anti-NMDAR2B antibody (Abcam), diluted 1:1000; polyclonal anti-PSD-95 antibody (Abcam), diluted 1:2000. All antibodies were dissolved in the 0.5% blocking reagent (Roche). The next day, the membranes were washed three times for 10 min in Tris-buffered saline with Tween (TBS-T) and incubated for 30 min with an anti-mouse IgG-peroxidase conjugated/anti-rabbit IgG-peroxidase conjugated antibodies (diluted 1:7000). This set of secondary antibodies was also a component of BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit). After incubation, the membranes were washed three times for 10 min with TBS-T. In the last step, the blots were incubated with detection reagent (Roche). The signal from the tested proteins was visualized and measured using a Fuji-Las 1000 system and Fuji Image Gauge v.4.0 software. To check the transfer and loading, β-actin was indicated on each blot. For this, a primary monoclonal antibody (Millipore; 1:8000) was used. Further procedures were the same as for the other proteins. The final result is given as the ratio of the optical density of particular proteins to an optical density of β-actin.

**Serum magnesium determinations**

After animal termination, the serum was isolated by centrifugation at 1800 r/min for 30 min at 4 °C, 1 h after collection and coagulation of trunk blood, then frozen at −80 °C. The magnesium levels were determined by a colorimetric assay with chlorophosphonazo-III, using the Cobas c 501 analyser (Roche Diagnostics). The magnesium concentrations were presented as mg/l.

**Statistical analysis**

All results from the CMS obtained in this study were analysed by multiple analyses of the variance with three between-subject factors (stress/control, drug treatments and successive sucrose tests). Fisher’s LSD test was used for the post-hoc comparisons of the means. All immunoblotting (Western blot) results and serum magnesium concentrations were analysed by two-way ANOVA followed by the Bonferroni Multiple Comparison test. p<0.05 was considered as statistically significant.

**Results**

**Behavioural studies**

Chronic mild stress caused a gradual decrease in the consumption of 1% sucrose solution. In the final baseline test (Fig. 1), all animals drank approximately 13 g of sucrose solution (data not shown). Following an initial two weeks of stress, the intakes remained at a similar level in the controls but fell to approximately 8 g in the stressed animals (F(1,125)=96.515; p<0.001), and this difference between the control and the stressed animals administered the vehicle was maintained at a similar level for the remainder of the experiment (Fig. 2). When compared to the vehicle administration, magnesium had no significant effect on the sucrose intake in the control animals (treatment effect: F(3,618)=0.709; NS) and gradually increased the sucrose consumption in the stressed animals, resulting in a significant treatment effect (F(3,618)=24.461; p<0.001) and treatment×weeks interaction (F(3,618)=1.631; p=0.070). When compared to the Week 0 scores, increases in sucrose intake in the stressed animals administered the most active dose of 15 mg/kg of magnesium reached statistical significance after 3 wk of administration (p<0.05), and this effect was enhanced thereafter for four weeks (p<0.01) (Fig. 2). Under the same CMS experimental conditions, imipramine treatment (10 mg/kg) induced a significant increase in sucrose intake after four and five weeks of treatment (this data will be published separately). In summary, magnesium showed a potent activity in the CMS model of depression; and the compound gradually enhanced the sucrose intakes in the stressed animals and did not affect the behaviour of the control animals.

**Effect of CMS and administration of magnesium on the GluN1 subunit level (Fig. 3)**

CMS induces a non-significant increase of GluN1 subunit in the PFC (Fig. 3(a)) and administration of magnesium reduced the level of this subunit in stressed rats (p<0.05 vs. stress). Two-way ANOVA demonstrated no significant effect of stress (F(1,21)=0.09, p=0.7623), no significant effect
of magnesium ($F_{1.21}=3.35, p=0.0813$) and a borderline interaction ($F_{1.21}=4.26, p=0.0515$). As shown in Fig. 3(b), CMS or magnesium treatment do not affect the level of the GluN1 subunit in the hippocampus. Two-way ANOVA demonstrated no significant effect of stress ($F_{1.12}=0.92, p=0.3572$), no significant effect of magnesium ($F_{1.12}=2.89, p=0.1150$) and no significant interaction ($F_{1.12}=1.67, p=0.2205$). CMS significantly increases the level of the GluN1 subunit in the amygdala (Fig 3(c)) ($p<0.01$ vs. saline). Administration of magnesium reversed this effect ($p<0.05$ vs. stress). Two-way ANOVA demonstrated a significant effect of stress ($F_{1.19}=6.67, p=0.0183$), no significant effect of magnesium ($F_{1.19}=1.02, p=0.3241$) and significant interaction ($F_{1.19}=6.80, p=0.0173$).

**Effect of CMS and administration of magnesium on the GluN2A subunit level (Fig. 4)**

As shown in Fig. 4(a), CMS or magnesium treatment do not affect the level of the GluN2A subunit in the PFC. Two-way ANOVA demonstrated no significant effect of stress ($F_{1.28}=0.16, p=0.9325$), no significant effect of magnesium ($F_{1.28}=0.14, p=0.7129$) and significant interaction ($F_{1.28}=4.40, p=0.0450$). As shown in Fig. 4(b), CMS induces the significant elevation of the GluN2A subunit in the hippocampus ($p<0.05$ vs. saline) but magnesium treatment reversed this effect ($p<0.05$ vs. stress). Two-way ANOVA demonstrated a very significant effect of stress ($F_{1.30}=8.42, p=0.0069$), a significant effect of magnesium ($F_{1.30}=4.38, p=0.0449$) and significant interaction ($F_{1.30}=4.64, p=0.0394$). As shown in Fig. 4(c), CMS does not affect the level of the GluN2A subunit in stressed rats in amygdala. Two-way ANOVA demonstrated no significant effect of stress ($F_{1.18}=1, p=0.3314$), no significant effect of magnesium ($F_{1.18}=0.78, p=0.3895$) and no significant interaction ($F_{1.18}=0.71, p=0.4098$).

**Effect of CMS and administration of magnesium on the GluN2B subunit level (Fig. 5)**

As shown in Fig. 5(a), CMS procedure or magnesium alone do not affect the level of the GluN2B subunit in the PFC, but administration of magnesium increases GluN2B level in stressed rats ($p<0.05$ vs. stress). Two-way ANOVA demonstrated no significant effect of stress ($F_{1.33}=1.54, p=0.2233$), a significant effect of magnesium ($F_{1.33}=1.54, p=0.0353$) and no significant interaction ($F_{1.33}=3.60, p=0.0667$). As shown in Fig. 5(b), CMS or magnesium treatment do not affect the level of the GluN2B subunit in the hippocampus. Two-way ANOVA demonstrated no significant effect of stress ($F_{1.20}=0.46, p=0.5056$), no significant effect of magnesium ($F_{1.20}=0.52, p=0.4782$) and no significant interaction ($F_{1.20}=0.22, p=0.6419$). As shown in Fig. 5(c), CMS or magnesium treatment do not affect the level of the GluN2B subunit in the amygdala. Two-way ANOVA demonstrated no significant effect of stress ($F_{1.14}=0.93, p=0.3511$), no significant effect...
of magnesium ($F_{1,14}=0.11, p=0.7483$) and no significant interaction ($F_{1,14}=0.92, p=0.3535$).

**Effect of CMS and administration of magnesium on the PSD-95 level (Fig. 6)**

As shown in Fig. 6(a), CMS or magnesium alone do not affect the level of PSD-95 protein in the PFC, but administration of magnesium significantly increases PSD-95 in the stressed animals ($p<0.05$ vs. stress). Two-way ANOVA demonstrated no effect of stress ($F_{1,17}=1.70, p=0.2103$), a very significant effect of magnesium ($F_{1,17}=8.88, p=0.0084$) and no significant interaction ($F_{1,17}=1.41, p=0.2522$). As shown in Fig. 6(b), CMS or magnesium treatment do not affect the level of PSD-95 in the hippocampus. Two-way ANOVA demonstrated no significant effect of stress ($F_{1,24}=0.05, p=0.8327$), no significant effect of magnesium ($F_{1,24}=0.15, p=0.7011$) and no significant interaction ($F_{1,24}=0.07, p=0.7915$). As shown in Fig. 6(c), CMS or magnesium treatment does not affect the level of PSD-95 in the amygdala. Two-way ANOVA demonstrated no significant effect of stress ($F_{1,15}=0.03, p=0.8704$) and no significant interaction ($F_{1,15}=1.42, p=0.2522$).

**Representative Western blots in the rat brain**

Figure 7 demonstrates representative Western blotting of GluN1, GluN2A, GluN2B subunits and the PSD-95 protein.

**Effect of CMS and administration of magnesium on the magnesium serum level**

As shown in Table 1, CMS or magnesium treatment does not affect the serum magnesium concentration.
A decreased (by 7%) serum magnesium level was found in the stressed animals receiving magnesium treatment ($p<0.05$ vs. stress). Two-way ANOVA demonstrated a significant effect of stress ($F_{1,25}=5.35$, $p=0.0293$), a significant effect of magnesium ($F_{1,25}=6.0$, $p=0.0217$) and no significant interaction ($F_{1,25}=2.76$, $p=0.1091$).

**Discussion**

The mechanism of current antidepressant drugs is based on the interaction with the monoaminergic systems. Unfortunately, the present treatment strategies are inadequate for several reasons. The first problem associated with such therapy is a delayed onset of action. For this reason, monoaminergic drugs have limited applicability in severe cases of depression, such as patients with suicidal tendencies, when a rapid therapeutic effect is needed (Mathews et al., 2012). The next complications are linked to the many side effects caused by conventional drugs. The third main cause of the unsatisfactory antidepressant therapies currently available is the lack of efficacy in a large percentage of patients (Trivedi et al., 2006; Connolly and Thase, 2011; Mathews et al., 2012). These circumstances are forcing researchers to look for new solutions concerning new therapeutic possibilities, besides a better understanding of the etiology of depression. As shown earlier, magnesium has an antidepressant-like activity in the FST (Poleszak et al., 2005a,b). In the present study, we demonstrated the antidepressant-like activity of magnesium in the CMS model of depression in rats. It is surprising that the dose of 15 mg/kg of magnesium is more active than a higher dose (20 mg/kg), although an analogous situation is observed after ketamine administration. Antidepressant-like effects for ketamine were observed only at a low dose like 10 mg/kg, but not at 80 mg/kg.

**Fig. 4.** Changes in the protein levels of the GluN2A subunit of the NMDA receptor induced by CMS and magnesium treatment. The levels of the GluN2A subunits were measured in the prefrontal cortex, the hippocampus and amygdala of non-stressed and chronically stressed rats, treated for 5 wk with a vehicle or magnesium. The data expressed the mean of s.e.m. $^*p<0.05$ vs. saline, $^#p<0.05$ vs. stress (Bonferroni test).
It is worth emphasizing that magnesium showed antidepressant-like activity beginning at 3 wk after the commencement of treatment. In this model, tricyclic antidepressants (e.g. imipramine) have a similar onset of activity (Kubera et al., 1996; Sowa-Kucma et al., 2008), while others drugs (especially from the new generation) display a shorter onset time. For instance, escitalopram demonstrates antidepressant activity following 1 wk of treatment, citalopram 2 wk and fluoxetine 3 wk (Sanchez et al., 2003). Moreover, zinc, another inorganic NMDA receptor modulator, also exhibit efficacy in CMS 1 wk after the start of treatment (Sowa-Kucma et al., 2008). While referring to the results of the antidepressant activity of magnesium observed in CMS we examined the impact of an active dose of magnesium on the glutamatergic system (the NMDA receptor), both in the control and stressed animals. NMDA receptors have a tetrameric structure. Functional NMDA receptors are composed of four subunits in which two obligatory GluN1 subunits and two GluN2A, B, C, D or GluN3 proteins can be distinguished. There are many possible combinations of GluN2 subunits in the NMDA receptor complex and both homomeric and heteromeric complexes have been observed (Traynelis et al., 2010).

The present study showed that CMS induced a significant increase in the level of the GluN1 subunit in the amygdala, and slightly (a tendency without statistical significance) in the PFC, yet not in the hippocampus. As recent studies show, CMS increases the mRNA levels of GluN1 in the PFC and ventral hippocampus. It has also been shown that there are no changes in the GluN1 protein level in the synaptosomes of the ventral hippocampus (Calabrese et al., 2012). Some human data

![Figure 5](https://example.com/figure5.png)

**Fig. 5.** Changes in the protein levels of the GluN2B subunit of the NMDA receptor induced by CMS and magnesium treatment. The levels of the GluN2B subunit were measured in the prefrontal cortex, the hippocampus and amygdala of non-stressed and chronically stressed rats, treated for 5 wk with a vehicle or magnesium. The data expressed the mean of s.e.m. #p<0.05 vs. stress (Bonferroni test).
showed no alterations in the level of this subunit in depression (Feyissa et al., 2009; Karolewicz et al., 2009). The increased hippocampal level of the GluN2A subunit in our stress model (CMS for 7 wk) is in accordance with the findings of Calabrese et al. (2012), following similar stress (CMS for 6 wk) in the ventral hippocampus. On the other hand, another experiment using CMS did not demonstrate alterations in the hippocampal GluN2A, although the duration of the stress period was shorter (4 wk) (Yilmaz et al., 2011). The data concerning the influence of CMS on the GluN2B subunit are equivocal; an increase, a reduction and, as demonstrated in the present study, no change have all been observed. However, these studies differ according to the type and duration of stress, animal species and detection methods. Sterlemann et al. (2010) for example showed that 7 wk of a social stress paradigm induces an increase in hippocampal GluN2B mRNA and protein levels in stressed mice (Sterlemann et al., 2010). Alternatively, in the study by Quan et al. (2011) 21 d of chronic unpredictable stress (CUS) induce a reduction in the expression of GluN2B receptor in PFC of stressed rats (Quan et al., 2011). Furthermore human post-mortem data indicate that the level of both subunits (GluN2A, GluN2B) is reduced in the prefrontal cortex and that GluN2A is elevated in amygdala in depressed patients (Feyissa et al., 2009; Karolewicz et al., 2009). Thus, generally, the level of glutamate NMDA receptor subunits in the brains of animals under stressful conditions is different than that found in depressed humans, which may indicate different mechanisms involved in the response to different stress procedures and the pathophysiology of depression. These different constellations of changes of NMDA receptor subunits in different brain regions induced by stress events or depression may lead to a common pathological feature – the enhancement

Fig. 6. Changes in the level of PSD-95 induced by CMS and magnesium treatment. The levels of PSD-95 measured in the prefrontal cortex, the hippocampus and amygdala of non-stressed and chronically stressed rats, treated for 5 wk with a vehicle or magnesium. The data expressed the mean of s.e.m. #p<0.05 vs. stress (Bonferroni test).
of brain glutamate transmission (Feyissa et al., 2009; Karolewicz et al., 2009; Tokita et al., 2012).

In our study magnesium treatment normalized CMS-induced GluN1 and GluN2A alterations. Conventional antidepressants like venlafaxine, duloxetine or imipramine (although not escitalopram) also normalized stress-induced alterations in the NMDA subunits (Yilmaz et al., 2011; Calabrese et al., 2012) and our unpublished data). We have also noticed during the present study that magnesium increases GluN2B and PSD-95 (the protein responsible for anchoring and scaffolding the NMDA receptors to postsynaptic density) level in the prefrontal cortex in CMS (but not control) treated animals. Thus, a combination of stress and magnesium is able to enhance the expression of these two proteins, despite the fact that stress alone did not produce any alterations. Involvement of NMDA receptors containing GluN2B subunits in the pathophysiology of depression is rather complex. On the one hand triheterotrimERIC NMDA receptors GluN1/2A/2B are probably necessary for the mobilization of synaptic protein (for example PSD-95, GKAP) during synaptic pruning but, on the other hand extrasynaptic di-heteromeric GluN2B NMDA receptors conjugated with PSD-95 have a crucial role in the process of neuronal death (Paoletti et al., 2013). In view of these data, an increased level of PSD-95 and GluN2B in stress animals after magnesium treatment could be interpreted at least in two ways. First it could be a sign of elevation of extrasynaptic di-heteromeric GluN1/GluN2B NMDA receptors or, second, can be a sign of increased neuroplasticity. The first hypothesis seems to be unlikely, however due to the fact that chronic administration of magnesium (15 mg/kg) reversed changes invoked by stress. Moreover, we have witnessed no changes in the GluN2A subunit and a decrease of GluN1 receptors in the PFC. These arguments seem to indicate that the second hypothesis concerning changes in the composition of synaptic NMDA receptors and its responsibility for neuroplasticity is more reliable. Furthermore, the blockade of NMDA receptors has well-documented effects of intracellular signal pathways. Both ketamine and the GluN2B specific antagonist, Ro-6981, rapidly (1 h after administration) activated the mammalian target of the rapamycin (m-TOR) pathway. These events are associated with the translation in numerous proteins (PSD-95, a GluA1 subunit of AMPA receptors, Synapsin I, ARC) in the rat PFC (Li et al., 2010). Additionally, a NMDA/ketamine-dependent blockade led to deactivation of the eukaryotic elongation factor 2 (eEF2) in the hippocampus, reduced phosphorylation of eEF2 and disinhibition of translation of BDNF (Autry et al., 2011).

The functional differentiation of NMDA receptors because of the composition of the subunits is the most important problem concerning the relationship between NMDA antagonists and its antidepressant like-activity. The main source of information about pharmacological properties of NMDA antagonists comes from studies with ketamine and MK-801. These, non-specific NMDA receptor antagonists, were preferably tested for antidepressant activity. Unfortunately, however due to their potential to evoke side effects clinical application is limited (Paoletti et al., 2013). As has been reported in many studies a numerous side effects are the consequence of non-selective NMDA antagonism of these compounds (Quan et al., 2011; Lima-Ojeda et al., 2013). For instance, the psychotic effects of ketamine probably result from the blockade of extracortical di-heteromeric GluN2C/D NMDA receptors. Studies of rodents indicate on hyperactivity caused by MK-801 in the open field test (Lima-Ojeda et al., 2013). The upshot of this is that it is associated with positive symptoms in schizophrenia. In contrast, a Ro-6981 GluN2B-specific antagonist significantly reduced immobility time in the FST, and no hyperlocomotion in the open field test was shown (Lima-Ojeda et al., 2013). This excludes the situation that antidepressant-like activity in the FST is evoked by overall hyperactivity. Magnesium at a dose of 15 mg/kg also did not show hyperlocomotion in the open field test (our unpublished data). Additionally, in rodents, ‘global’ NMDA antagonists induce cortical neurotoxicity.
similar neuronal failures are absent after treatment with a GluN2B-specific antagonist (Lima-Ojeda et al., 2013). Despite the undoubted role of the blockade of the NMDA receptor in ketamine’s antidepressant-like activity, concomitant AMPA receptor activation seems to be necessary to achieve this effect. Pre-treatment with 2,3-dihydro-6-nitro-7-sulfoamylbenzo(f)-quinozaline (NBQX) AMPA receptor antagonists significantly diminished antidepressant-like behaviour for Ro-6981, ketamine and MK-801 in the FST (Maeng et al., 2008; Autry et al., 2011). In line with these results, GluA1 knock-out mice showed depressive-like behaviour in the learned helplessness paradigm (increased). These behavioural abnormalities correlate with a significant higher level of glutamate and an increased level of the GluN1 subunit in the hippocampus (Chourbaji et al., 2008).

Some preclinical studies have shown that GluN2A-knockout mice exhibit anti-depressant-like behaviour in the FST and tail suspension test (Boyce-Rustay and Holmes, 2006). The interpretation of these results may not be clear because other studies have shown hyperactivity in the open field test in GluN2A-knockout mice (Miyamoto et al., 2001). Inta et al. (2013) discovered that animals with GluN2A receptors without a C-terminal exhibit anxious-like behaviour but not depressive-like behaviour (Inta et al., 2013). Additionally, animals with C-terminal truncated GluN2A subunits did not show hyperactivity in the FST (Inta et al., 2013). But undoubtedly, analysis of our research may suggest a certain functional relationship between expression of the GluN2A subunit in stress animals and an elevated level of PSD-95 and GluN2B subunits after magnesium treatment and abnormalities of GluN1 in the amygdala. It is well established that pathways between the hippocampus and prefrontal cortex play a crucial role in memory, learning and emotions (Vertes, 2006). These structures are anatomically and functionally linked with the nucleus accumbens, the ventral tegmental area and the amygdala. Deregulation of these circuits, may impair the expression of motivating and rewarding behaviours and lead to anhedonia (Gorwood, 2008). Therefore we have noticed the elevation level of the GluN1 subunit and no changes in GluN2A and GluN2B in the amygdala after CMS. This markedly higher level of the GluN1 subunit may indicate intensified glutamate transmission and stimulation of neurons in the amygdala. This in turn may cause impairment of hippocampal CA1 LTP the crucial region involved in memory learning and emotion connecting with the medial prefrontal cortex. Moreover the amygdala electrolytic lesion (Kim et al., 2001) and microinfusion of the muscimol GABA agonist into the amygdala, blocks stress-induced impairments in the hippocampal LTP and spatial memory (Kim et al., 2005). The lesion of the basolateral amygdala is important for the fluoxetine-induced proliferation in hippocampal dentate gyrus cells. Only in lesion conditions was the fluoxetine proliferation effect achieved.

Furthermore, lesion of the basolateral amygdala leads to antidepressant-like activity in the FST (Castro et al., 2010). These effects display the potential involvement of the amygdala in the expression of depression-like behaviour and suggest that inhibition of amygdala neurons may have antidepressant effects (Kim et al., 2005).

A serum magnesium study showed no effect of the CMS procedure on the serum magnesium concentration in rats (both in the present data and (Zieba et al., 2000)), while the acute stress (FST) induced an increase in mouse serum (Poleszak et al., 2005b). In the present study, we demonstrated that chronic magnesium treatment in animals subjected to CMS reduced serum magnesium concentration when compared to a CMS stressed group. This may suggest a redistribution of magnesium pools (blood, different tissue or organs) induced by such chronic procedures, although this hypothesis needs further investigation.

The present study, for the first time, demonstrates the antidepressant-like activity of magnesium in the CMS animal model of anhedonia. It also indicates the possible involvement of NMDA/glutamatergic receptors (different alterations of the subunits in different brain regions) in this activity.

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Statement of Interest

None.

References


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