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Short communication

Effects of acute and chronic treatment with magnesium in the forced swim test in rats

Ewa Poleszak[1], Piotr Wlaź[2], Ewa Kędzierska[1], Maria Radziwon-Zaleska[6], Andrzej Pilc[3,4], Sylwia Fidecka[1], Gabriel Nowak[3,5]

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

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

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

[4]Institute of Public Health, Collegium Medicum, Jagiellonian University, Michałowskiego 12, PL 31-126 Kraków, Poland

[5]Department of Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University, Medyczna 9, PL 30-688 Kraków, Poland

[6]Department of Psychiatry, Medical University of Warsaw, Nowowiejska 27, PL 00-665, Warszawa, Poland

Correspondence: Ewa Poleszak, e-mail: ewa.poleszak@am.lublin.pl

Abstract:
The antidepressant-like activity of magnesium, the non-specific N-methyl-D-aspartate glutamate receptor antagonist, in the mice forced swim test was demonstrated previously. In the present study, the effects of this biometal were studied in the rat forced swim test. Magnesium ($MgCl_2$) at doses ranging from 15 to 50 mg Mg/kg reduced the immobility time in the forced swim test, thus exerting antidepressant-like activity. To evaluate tolerance to this effect, we also performed experiments with the following acute/chronic magnesium treatment schedule: 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). The antidepressant-like effect of magnesium was demonstrated in the group treated acutely with magnesium (S + Mg) but not in the chronically treated group (Mg + S) and (Mg + Mg). It is interesting to note that in Mg + Mg group serum concentration of magnesium was quite similar to the S + Mg group (6.44 *vs.* 6.08 mg/100 ml, respectively), which displayed antidepressant-like effect.
The results confirmed that magnesium administered acutely induced the antidepressant-like effects also in rats. However, contrary to mice, chronic treatment with magnesium induced tolerance to this effect in rats.

Key words:
Magnesium, depression, NMDA receptor, forced swim test, rats

Introduction

Magnesium (Mg) is the most abundant biometal in the organism that occurs physiologically in intracellular and extracellular fluids. It is a co-factor in hundreds of enzymatic reactions [24]. Magnesium deficiency is now considered to contribute to many diseases and the role of magnesium as a therapeutic agent is being tested in numerous clinical trials. Data from experi-

mental and epidemiological studies suggest that disturbances in magnesium metabolism occur in affective disorders [4, 14]. Several clinical studies reported decreases in magnesium concentration in the blood of depressed patients [3, 23, 29, 30]. In animals Mg deficiency leads to reduction in offensive and an increase in defensive behavior [9]. Mice with low erythrocyte Mg levels showed a more restless behavior and more aggressive behavior under stressful conditions [6]. In the forced swim test, Mg reduces immobility similarly to imipramine (IMI) or MK-801 [2, 21]. The mechanism of pharmacological action of Mg is probably connected with the non-competitive blockade of *N*-methyl-D-aspartate (NMDA) receptors [13, 15]. This mechanism is probably responsible for antidepressant-like effect in the forced swim test in mice [2].

Our previous results demonstrated the antidepressant-like activity of Mg in mice forced swim test without tolerance during chronic treatment and supported the notion that inhibition of the NMDA receptor activity is involved in the mechanisms of antidepressant activity. In the present study, we have investigated the effects of acute and chronic treatment of Mg in the forced swim test in rats.

Materials and Methods

Animals

All procedures were approved by the Ethical Committee of the Medical University School, Lublin. The experiments were carried out on male Wistar rats (180–220 g). The animals were kept on a natural day-night cycle with free access to food and water. Each experimental group consisted of 6–8 animals. Magnesium [as chloride (Fluka)] was administered intraperitoneally (*ip*). The doses refer to the elemental Mg and the volume of the injected solutions was 5 ml/kg. The following schedules of treatment were used: sub-chronic – three times 24, 5 and 1 h before the test; control animals received *ip* injections of saline (vehicle), and chronic – 14 times at 24-h intervals; saline and saline challenge took place 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).

Forced Swim Test

The studies were carried out on rats according to the method of Porsolt et al. [22]. The rats were placed individually in glass cylinders (40 cm high, 18 cm in diameter) containing 15 cm of water, maintained at 23–25°C. The rats were removed and returned to their home cages after 15 min in water. They were again placed in the cylinder 24 h later. The total duration of immobility in rats was measured during a 5-min test. The rats were judged to be immobile when they remained floating passively in the water.

Determination of magnesium concentration in serum

Total Mg concentration in serum was determined by xylidyl blue method [8]. Serum was separated by centrifugation at 5,000 × g for 10 min at 4°C, 1 h after collection and coagulation of trunk blood, and stored at –20°C until analysis. 10 µl of thawed serum was added to 1 ml of the commercially available reagent (Liquick Cor-Mg 30, Cormay, Lublin, Poland) and the absorbance of the solution was read at 520 nm in a spectrophotometer (Specord M40, Carl Zeiss, Jena, Germany). Mg concentrations were calculated as mg/100 ml.

Statistical analysis

The obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by post hoc Dunnett's (forced swim test) or Tukey-Kramer's (serum magnesium levels) multiple comparisons tests. All results are presented as the means ± SEM. A difference was considered significant when $p < 0.05$.

Results

Effects of magnesium in the forced swim test in rats

The effects of sub-chronic treatment with magnesium ($MgCl_2$) on the total duration of immobility time in rats are shown in Figure 1. Magnesium given three times at a dose of 7.5 mg/kg had no effect on the immobility time in rats. Magnesium at doses 15, 30 and 50 mg/kg significantly shortened the immobility time

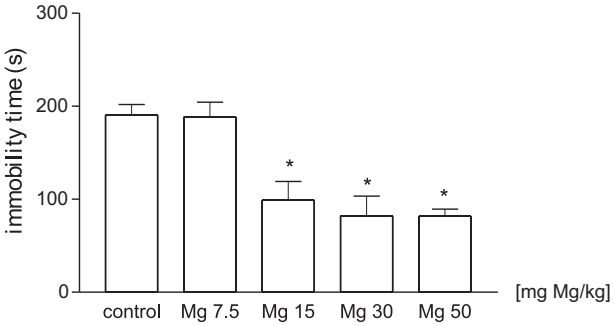

Fig. 1. The effects of sub-chronic treatment with magnesium ($MgCl_2$) on the total duration of immobility time in rats. Magnesium was given three times: 24 h, 5 h, and 1 h before the test. The immobility time was measured during a 5 min experimental session. Each column represents the mean value (± SEM) from 6–8 rats per group. ANOVA followed by Dunnett's test, * $p < 0.001$

by approximately 50%. Raw data were as follows: control: 109 s/5 min; magnesium 7.5 mg/kg, 188 s/5 min; magnesium 15 mg/kg, 99 s/5 min; magnesium 30 mg/kg, 82 s/5 min; magnesium 50 mg/kg, 81s/5 min. [ANOVA, $F(4, 39) = 13.74$; $p < 0.0001$].

Effects of acute and chronic magnesium administration on the immobility time in the forced swim test in rats

The effects of chronic treatment with magnesium ($MgCl_2$) on the total duration of immobility time in rats are shown in Figure 2. Magnesium administered

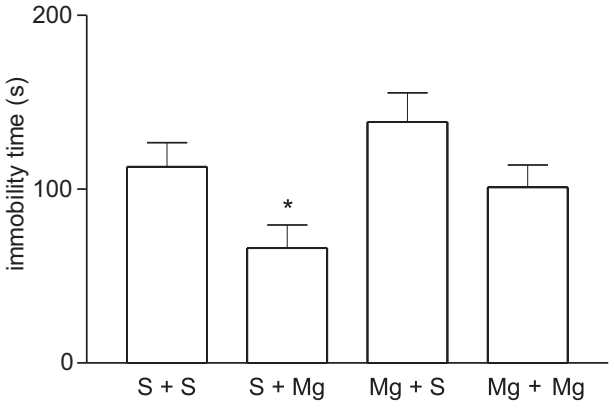

Fig. 2. The effects of acute and chronic treatment with magnesium ($MgCl_2$) on the total duration of immobility time in rats. The following treatment schedules were applied: chronic saline and saline challenge at 0.5 h before behavioral experiments (S + S), chronic saline and magnesium challenge (S + Mg), chronic magnesium and saline challenge (Mg + S), chronic magnesium and magnesium challenge (Mg + Mg). Magnesium was administered at the dose of 30 mg/kg. Each column represents the mean value (± SEM) from 6–8 rats per group. ANOVA followed by Dunnett's test, * $p < 0.01$

acutely (S + Mg) produced antidepressant-like effect. Administration of magnesium with magnesium challenge (Mg + Mg) and with last magnesium injection 24 h before saline challenge (Mg + S) did not influence the immobility time in rats. [ANOVA, $F(3, 18) = 5.36$; $p < 0.008$].

Effect of chronic magnesium administration on serum magnesium level in rats

The effects of acute and chronic magnesium treatment on serum magnesium concentration in rats subjected to FST are presented in Table 1 (ANOVA: $F(4, 22) = 126.09$, $p < 0.0001$). Swim stress (rats subjected to FST) increased (by 30%) the Mg concentration in serum compared to naive animals. Magnesium treatment schedules, acute or chronic, significantly (over 100%) increased serum magnesium level compared with control group (S + S). Chronic magnesium and saline challenge (Mg + S) did not change magnesium level compared with control group (S + S).

Tab. 1. The effects of magnesium administration on serum magnesium level in rats subjected to forced swim test (FST)

Treatment	Mg concentration	
	Serum (mg/100 ml)	Percentage of control
Naive (–FST)	2.67 ± 0.09	100
S + S (+ FST)	3.49 ± 0.22#	130
S + Mg (+ FST)	6.44 ± 0.08*	241
Mg + S (+ FST)	2.91 ± 0.12	109
Mg + Mg (+ FST)	6.08 ± 0.23 *	228

Magnesium (magnesium chloride) 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 represent the means ± SEM (n = 6–8 rats per group). ANOVA revealed $F(4, 22) = 126.09$, $p < 0.0001$ for serum concentrations. # $p < 0.05$ *vs.* naive group, * $p < 0.001$ *vs.* control (S + S) group (Tukey-Kramer test)

Discussion

Antidepressant therapy is remarkably diverse, encompassing both drugs and non-pharmacological interventions such as electroconvulsive shock (ESC) [7]. Since clinical efficacy of currently available antidepressants is not satisfactory, search for better drugs is

still in progress. From the beginning of the 1990s the glutamate receptors emerge as target for development of new antidepressants. Particularly, studies demonstrated that functional antagonists of NMDA receptor complex exhibit an antidepressant-like effects in an animal tests and models of depression [25–27]. Recent clinical study demonstrated that ketamine, a potent NMDA antagonist, exhibited rapid antidepressant effects in the depressed patients [1]. Also, zinc, the NMDA receptor antagonist, is active in animal tests and models of depression [10, 11, 16, 28]. A reduced zinc serum concentration was demonstrated in depressed patients, which was normalized only after successful antidepressant therapy [12, 18, 19]. Moreover, our preliminary clinical study indicated that zinc supplementation of antidepressant therapy significantly enhanced efficacy of this treatment in patients with unipolar depression [17].

Likewise, an involvement of magnesium in the pathophysiology and treatment of depression has been previously suggested. A lower magnesium plasma concentration was demonstrated in depressed patients, and the increased magnesium level was shown during recovery [3, 4]. Furthermore, magnesium used as supplementary therapy to lithium, benzodiazepines and neuroleptics in mania, significantly reduced the use of these drugs [5]. It was demonstrated previously that magnesium possesses the antidepressant-like effects. Magnesium salts reduced the immobility time in the forced swim test similarly to imipramine and MK-801 in mice [2]. Our previous study also demonstrated that magnesium had an antidepressant-like effect in the forced swim test in mice, when given at the dose 20–30 mg Mg/kg [21].

Our present study indicates that magnesium administered acutely or sub-chronically reduces the immobility time of rats in the forced swim test. In the treatment of clinical depression, chronic administration of antidepressants is necessary for the therapeutic effect to appear, as demonstrated by e.g. Oswald et al. [20]. For this reason, we examined whether tolerance to magnesium antidepressant-like effect develops after prolonged treatment. Chronic treatment with magnesium (magnesium challenge 24 h after the last magnesium dose, or saline challenge 24 h following the last magnesium injection) did not produce behavioral response. Antidepressant-like activity of magnesium was accompanied with a 111% rise in serum magnesium concentration in rats. However, similar increase by 98% in serum magnesium concentration was not reflected by the antidepressant effect. Thus, tolerance developed to the antidepressant action of magnesium, without significant alterations in magnesium serum concentration. Therefore, a pharmacokinetic component seems not to be responsible for the observed effect in rats. In comparison, in mice not subjected to FST, the magnesium antidepressant effects (acute and chronic) were observed when serum magnesium concentration rose by about 50% over the control [21]. The lack of effect after chronic magnesium treatment may be caused by a pharmacodynamic factor or by alterations in brain magnesium concentrations, which is not related to serum magnesium homeostasis.

The present results confirm that magnesium exhibits its antidepressant-like activity also in rats. However, contrary to mice, chronic treatment with magnesium induced tolerance to this effect in rats.

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