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Short communication

Immobility stress induces depression-like behavior in the forced swim test in mice: effect of magnesium and imipramine

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Abstract:
Previously, we demonstrated antidepressant-like effect of magnesium (Mg) in the forced swim test (FST). Moreover, the joint administration of Mg and imipramine (IMI) at ineffective doses *per se*, resulted in a potent reduction in the immobility time in this test. In the present study, we examined the effect of immobility stress (IS), and Mg and/or IMI administration on FST behavior. IS induced enhancement of immobility time, which was reversed by Mg or IMI at doses ineffective in non-stressed mice (10 mg/kg and 15 mg/kg, respectively). The joint administration of Mg and IMI was effective in both IS and non-stressed animals in FST. IS did not significantly alter locomotor activity, while IMI or Mg + IMI treatment in IS mice reduced this activity. We also measured serum and brain Mg, IMI and its metabolite desipramine (DMI) concentration in mice subjected to FST and injected with Mg + IMI, both restrained and non-restrained. In the present study we demonstrated a significant increase (by 68%) in the brain IMI and a slight, non-significant reduction in DMI concentration in IS + Mg + IMI + FST *vs*. Mg + IMI + FST groups, which might indicate the reduction in brain IMI metabolism. The IS-induced reduction in brain IMI metabolism did not participate in the activity in FST, since no differences in such activity were noticed between IS + Mg + IMI + FST and Mg + IMI + FST groups.
The present data suggest that IS-induced increase in immobility time in FST is more sensitive for detection antidepressant-like activity. However, further studies are needed to examine the effect of other antidepressants in such an experimental paradigm.

Key words:
immobility stress, forced swim test, magnesium, imipramine, serum, brain, magnesium concentration, imipramine concentration, desipramine concentration, mice

Introduction

Psychological stress is proposed to play an important role in the development of affective disorders in humans [49]. Stressful events play a prominent role in the provocation of depression [6]. Using stress to induce a feeling of loss of control might result in a behavioral state analogous to depression [60]. Restrain is one of the most frequently employed experimental animal models of depression which involves the application of uncontrollable stress, and is thought to induce a depressive behavioral state [1]. It was shown that the restrain is associated with increased extracellular glutamate concentrations in the brain [25] and glutamatergic NMDA receptors are involved in stress responses [36, 41]. In clinical studies, high glutamate levels in the central nervous system (CNS) of depressed patients were reported [2, 23, 28]. Moreover the NMDA receptor abnormalities were observed in human suicide victims [35] and major depressives [21]. On the other hand, an antagonist of the NMDA receptor complex, ketamine, is effective in human depression [3]. In animals, functional antagonists of the NMDA receptor complex act as antidepressants in a variety of screen tests and animal models of depression [22, 26, 27, 42, 53, 59]. In addition, zinc, being an inorganic inhibitor of the NMDA receptors [11, 50], was shown to be active in animal tests and models of depression and enhanced the antidepressant-like activity of antidepressants in the forced swim test (FST) [19, 20, 38, 39, 57]. Its antidepressant activity was documented in the clinical studies as well [37].

Magnesium (Mg) blocks the activation of NMDA receptor ion channel in a voltage-dependent manner [4, 32, 55]. Mg deficiency has been related to affective disorders [9, 17, 24, 33, 44] and a close relationship between low serum Mg levels and depressive symptoms was demonstrated [8, 10, 14, 51, 61, 62]. In animals, Mg deficiency leads to a reduction in offensive and to an increase in defensive behavior [15]. Moreover, Mg administration reduces immobility time in the (FST) in mice and rats [7, 46, 47] and enhances the antidepressant-like activity of imipramine (IMI) [47].

The aim of our present study was to examine the effect of immobility stress (IS), and Mg and/or IMI administration on FST behavior in mice.

Materials and Methods

Animals

All procedures were approved by the Ethical Committee of the Medical Academy, Lublin. The experiments were carried out on male Albino Swiss mice (25–30 g). The animals were kept on a natural day-night cycle with free access to food and water.

Experimental procedures

Drug administration

Magnesium hydroaspartate (Farmapol, Poznań, Poland) alone or combined with IMI, was administered intraperitoneally (*ip*) 0.5 h before the test. IMI (Polfa, Kraków, Poland) was administered 1h before the test. Control animals received *ip* injections of saline (vehicle) at respective pretreatment times. All vehicle and drug solutions were administered in a volume of 10 ml/kg.

Restraint apparatus and experimental procedure

The mice were immobilized by placing in a well-ventilated Plexiglas tube (10 cm long, 2.8 cm in diameter, 0.5 cm wall) for 2 h at a time (acute stress). The animal was not physically compressed and did not experience pain. Immediately after restraint, they were moved back to the home cages and subsequently tested. Non-stressed animals were placed separately in different cage for 2 h and then moved back to their home cages. The main test (forced swim test) was performed 1 h after immobilization.

Forced Swim Test

The studies were carried out on mice according to the slightly modified method of Porsolt and co-workers [48]. Mice were placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water, maintained at 23–25°C. The animals were left in the cylinder for 6 min. After the first 2 min the total duration of immobility was measured during a 4-min test. The mouse was judged to be immobile when it

remained floating passively, performing slow motion to keep head above the water.

Imipramine and desipramine determination

Serum and brain concentrations of IMI and its metabolite desipramine (DMI) were assayed by HPLC according to the method described by Szymura-Oleksiak and co-workers [58] with a slight modification. After pretreatment and FST, the animals were sacrificed, their brains were removed and frozen on dry ice. Serum was isolated by centrifugation at 5,000 g for 10 min at 4°C, 1 h after collection and coagulation of trunk blood, then it was frozen at –20°C. The brains were homogenized in 0.1 M phosphate buffered saline (PBS, 1:4 w/v) and 0.2 ml of serum (diluted 1:1 with redistilled water) or 1 ml of brain homogenate containing both compounds was mixed with mianserin as an internal standard (20 µl of 0.4 µg/ml or 2 µg/g in methanol for serum and brain, respectively). The samples were alkalized with 2 M sodium hydroxide and extracted with 5 ml of ethyl acetate-hexane-isoamyl alcohol (50:49:1 v/v). After centrifugation (30 min, 1800 × g), the organic layer was transferred to a new tube, then evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was dissolved in 100 µl of mobile phase, and 50 µl of this solution were injected into the HPLC system.

The HPLC system (Thermo Separation Products, San Jose, CA, USA) consisted of P100 isocratic pump, Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 50-µl sample loop, UV100 variable-wavelength UV/VIS detector, operating at 254 nm and a SP4400 (ChromJet) integrator. All analyses were performed at ambient temperature on a 250 mm × 4.6 mm Supelcosil LC PCN column (Supelco Inc., Bellefonte, PA, USA) with 5 µm particles, protected with a guard-column (20 mm x 4.6 mm) with the same packing material. The mobile phase was composed of 50 mM potassium dihydrogen phosphate, pH 4.5 : acetonitrile (57:43 v/v) at a flow rate of 1.0 ml/min. DMI was purchased from Sigma-Aldrich (St. Louis, MO, USA), mianserin hydrochloride (MS) was a gift from Organon (Oss, The Netherlands), IMI from Polfa (Poland). All HPLC solvents and reagents were obtained from Merck (Darmstadt, Germany).

Under these conditions, the approximate retention times (min) were: MS – 12.11, DMI – 13.26, and IMI – 15.06. The calibration curves were linear in the tested IMI and DMI concentration ranges, i.e. from 0.05 to 0.5 µg/ml for serum and from 0.1 to 6 µg/g for brain homogenate. The assay was reproducible with low intra- and inter-day variation (coefficient of variation less than 10%) and the recovery of both compounds ranged from 80 to 90% for serum and from 60 to 70% for brain homogenate.

Determination of magnesium concentration

Total Mg concentration in blood serum and the whole brain was determined by xylidyl blue method of Hulanicki [13]. After pretreatment and FST animals were sacrificed, their brains were removed and frozen on dry ice. Serum was isolated by centrifugation at 5,000 g for 10 min at 4°C, 1 h after collection and coagulation of trunk blood, then frozen at –20°C. A week later the brains were homogenized (PRO 200, PRO Scientific Inc., Connecticut, USA) in four volumes of ice-cold 0.01 M Tris-HCl buffer, pH 7.4 pH, at 26,000 rpm for 3 min and centrifuged at 21,000 × g for 30 min at 4°C. Ten µl of thawed serum or brain supernatant 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 (Hitachi U-3010, Tokyo, Japan). The Mg concentrations were calculated either as mg/100 ml (serum) or µg/g of fresh tissue (brain).

Statistics

The obtained data were evaluated by the Student's *t*-test or one-way analysis of variance (ANOVA), followed by Dunnett's or Student-Newman-Keuls *post-hoc* test. All results are presented as the means ± SEM. A $p < 0.05$ was considered as statistically significant.

Results and Discussion

Depression is a chronic mental disease affecting more than 10% of population [30]. Antidepressant therapy includes drugs with a diverse pharmacological mechanisms. The most currently used antidepressants affect either the uptake or metabolism of biogenic amines [5, 16]. The tricyclic antidepressants (e.g., IMI) non-selectively block the reuptake of biogenic amines [12], and a new class of antidepressants act as

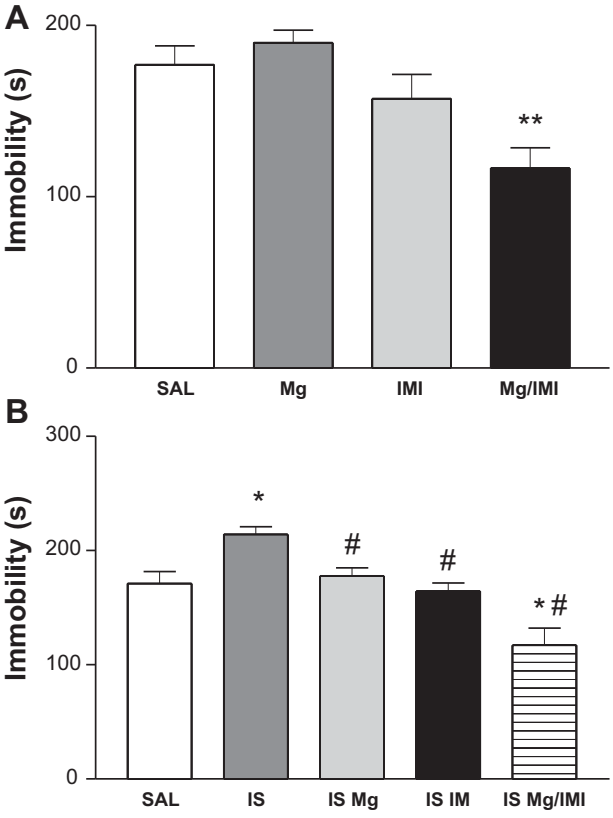

Fig. 1. The effect of immobility stress (IS), and administration of magnesium (Mg) and imipramine (IMI) on immobility time in the forced swim test (FST) in mice. IS was performed 1 h before FST. Magnesium hydroaspartate (10 mg Mg/kg) and IMI (15 mg/kg) were administered *ip* 0.5 h and 1 h, respectively, before the FST. The values represent the means ± SEM (n = 9–10 mice per group). ANOVA: $F(3,36) = 8.514$, $p = 0.0003$ (**A**) and $F(4,41) = 12.95$, $p < 0.0001$ (**B**) * $p < 0.05$, ** $p < 0.01$ *vs.* saline (SAL) group; # $p < 0.01$ *vs.* IS group (Newman-Keuls Multiple Comparison Test)

selective reuptake inhibitors [56]. The search for an effective and rapid antidepressant therapy is still not fully satisfactory. Commonly-used antidepressants have a delayed onset of action, produce a variety of unwanted side effects and numerous studies have indicated that approximately 30% of patients do not respond to these agents [12, 52].

It is known that stressful life events have been reported to facilitate the evolution of depressive illness [45]. In animals restraint stress is being used as a model of depression. It is believed to be the most severe type of stress in rodent models and has a comparative effect in humans, and this type of stress was used in the present study. Stress can influence the CNS functions by altering a number of neurotransmitter, endocrine and neuroendocrine systems, mainly monoamine systems, steroids and neurosteroids [18, 29]. A significant increase in glutamate levels in the different regions of the brain, e.g. significant increase in extracellular levels of glutamate following swimming in animals has been recently documented [31]. Thus the new perspectives for therapy of depression were the findings that NMDA receptors may be involved in the action of antidepressant drugs. Preclinical data have suggested that compounds which reduce transmission at NMDA receptors exhibit antidepressant-like action [34, 36, 40, 43, 52–54, 59].

The involvement of Mg in the antidepressant effects was demonstrated previously. In the FST, Mg salt reduced the immobility time in a way similar to IMI and thus resembled the antidepressant-like activity of MK-801 [7]. In our previous study, we confirmed the antidepressant action of Mg and demonstrated, that joint administration of IMI and Mg, produced an enhancement of the antidepressant-like effects in the FST, and further indicated the particular role of Mg in the antidepressant action [46, 47].

In the present study IS induced a significant (21%) enhancement in immobility time in FST (Fig. 1B). Mg and IMI administered at the doses of 10 and 15 mg/kg, respectively, which were ineffective in non-stressed mice (Fig. 1A), normalized the increased immobility time in IS mice (Fig. 1B). The joint administration of Mg and IMI significantly reduced immobility time in both non-stressed and stressed groups of animals (Fig. 1).

IS did not significantly alter locomotor activity, while IMI or Mg + IMI treatment in IS mice reduced this activity (Tab. 1). Since there is no alteration in

Tab. 1. The effect of immobility stress (IS), and administration of magnesium (Mg) and/or imipramine (IMI) on locomotor activity in mice

Treatment	Dose (mg/kg)	Activity counts	
		5 min	10 min
Saline	–	148.9 ± 11.4	237.0 ± 21.9
IS Saline	–	124. 9 ± 8.2	200.3 ± 14.6
IS Mg	10	118.0 ± 7.1	183.3 ± 9.8
IS IMI	15	104.3 ± 11.7*	155.3 ± 20.4*
IS IMI/Mg	15/10	89.10 ± 5.8*,#	141.6 ± 17.5*

IS was performed 1 h before FST. Magnesium hydroaspartate (10 mg Mg/kg) and IMI (15 mg/kg) were administered *ip* 0.5 h and 1 h, respectively, before the activity measurement. The values represent the means ± SEM (n = 8–10 mice per group). ANOVA: $F(4,43) = 6.163$, $p = 0.0006$ (5 min); $F(4,43) = 4.805$, $p = 0.003$ (10 min) $p < 0.001$ *vs.* Saline group; # $p < 0.01$ *vs.* IS Saline group (Dunnett's Multiple Comparison Test)

motor activity induced by IS, the increased immobility in the FST indicate depression-like behavior. On the other hand, the reduced locomotor activity induced by IMI or Mg + IMI treatment indicates that psychostimulant activity is not involved in the antidepressant effect in this test.

We also measured serum and brain concentrations of Mg, IMI and its active metabolite DMI in mice subjected to FST and administered with Mg + IMI with or without IS. In the present study, we demonstrated a significant increase (by 68%) in the brain (but not in serum) IMI and a slight and nonsignificant reduction in DMI concentrations in IS + Mg + IMI + FST *vs.* Mg + IMI + FST groups (Tab. 2). Mg concentration was unchanged (Tab. 2). This may indicate that IS induced reduction in brain IMI biotransformation in mice treated with Mg + IMI and subjected to FST. Previously, no differences in brain or serum Mg, IMI or DMI concentrations between IMI + FST and IMI + Mg + FST groups were demonstrated [47]. Thus, the previous and present data suggest, that IS is responsible for reduction in IMI metabolism, which needs further investigations.

Tab. 2. The effect of immobility stress (IS) and joint administration of magnesium (Mg) and imipramine (IMI) on serum and brain Mg, IMI and desipramine (DMI) concentrations in mice subjected to the forced swim test (FST)

group	Brain		
	Mg [μg/g]	IMI [μg/g]	DMI [μg/g]
Mg + IMI + FST	77.6 ± 2.7	2.48 ± 0.17	0.17 ± 0.02
IS + Mg + IMI + FST	78.4 ± 1.7	4.18 ± 0.39*	0.14 ± 0.03
		Serum	
group	Mg [mg/100ml]	IMI [ng/ml]	DMI [ng/ml]
Mg + IMI + FST	4.57 ± 0.16	231.7 ± 18.0	58.5 ± 7.9
IS + Mg + IMI + FST	5.34 ± 0.24	263.1 ± 30.8	62.0 ± 3.6

IS was performed 1 h before FST. Magnesium hydroaspartate (10 mg Mg/kg) and IMI (15 mg/kg) were administered *ip* 0.5 h and 1 h, respectively, before the FST. Immediately after the FST animals were sacrificed, serum was collected, brain was removed and frozen on dry ice. The values represent the means ± SEM (n = 9–10 mice per group). * $p < 0.05$ *vs.* Mg + IMI + FST group (Student's *t*-test)

The increase in brain concentration of IMI did not contribute to the changes in the activity in FST, since no differences in such activity were noticed between IS + Mg + IMI + FST and Mg + IMI + FST groups (Fig. 1; 117 ± 15.1 s and 116.8 ± 11.9 s, respectively). Similarly, lack of relationship between brain IMI/DMI concentration and activity in FST in mice was demonstrated previously [63]. Thus, also in IS-induced "sensitivity" to Mg or IMI activity in FST in mice, pharmacodynamic rather than pharmacokinetic mechanism is responsible for this effect.

Nevertheless, the present data suggest that the IS-induced increase in immobility time in FST is more sensitive for detection of antidepressant-like activity, however, further studies are needed to examine the effect of other antidepressants in such an experimental paradigm.

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