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Enhancement of antidepressant-like activity by joint administration of imipramine and magnesium in the forced swim test: Behavioral and pharmacokinetic studies in mice

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Abstract

The effect of joint administration of imipramine (IMI) and magnesium (Mg) on antidepressant-like activity was studied in mice using forced swim test (FST). Mg doses ineffective per se (5 and 10 mg/kg) given jointly with IMI also at ineffective doses (10 and 15 mg/kg) resulted in a potent reduction in the immobility time. Since these combined treatments did not influence locomotor activity, the antidepressant-like activity was not due to non-specific behavioral activation. Moreover, we estimated the effect of joint administration of magnesium and IMI in FST on serum and brain magnesium, IMI and its active metabolite desipramine (DMI) concentrations in mice. Swim stress (mice subjected to FST) increased the magnesium concentration in serum and decreased it in the brain compared to naive animals. Moreover administration of IMI increased (normalized) magnesium brain concentration, without influence on the serum level. Joint administration of IMI and magnesium did not influence magnesium (compared with FST) or IMI and DMI (compared with IMI treatment alone) concentrations in both examined tissues.

The present data demonstrated an enhancement of the antidepressant-like effect by joint administration of IMI and magnesium in the FST, and further indicate the particular role of magnesium in the antidepressant action. Since there was no increase in IMI, DMI or magnesium concentration after joint administration of magnesium and IMI, the data suggest that pharmacodynamic rather than pharmacokinetic interaction between magnesium and IMI is accountable for behavioral effect in the FST.

Keywords: Magnesium; Imipramine; Forced swim test; Serum; Brain; Magnesium concentration; Imipramine concentration; Desipramine concentration; Mice

1. Introduction

Magnesium (Mg) is an important intracellular bioelement, which activates about 300 different enzymes (Ryan, 1991). It is responsible for metabolism of ATP providing energy for muscles, is essential for biosynthesis and maintenance of nucleic acids' structure, and is also necessary for protein synthesis (e.g., Grubbs and Maguire,

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1987; Vermon, 1998). Magnesium plays an important role in signal transmission in the central nervous system (CNS) where it acts as the NMDA receptor antagonist. Magnesium blocks the activation of NMDA receptor ion channel in a voltage-dependent manner (Burnashev et al., 1992; Mori et al., 1992; Sobolevskii and Khodorov, 2002). Disturbances of magnesium metabolism are now considered to contribute to many disorders. Symptoms and signs of hypomagnesaemia usually occur when serum total magnesium levels fall below 0.5 mmol/l (i.e., 1.215 mg/100 ml), and frequently ventricular arrhythmias torsades de pointes, convulsions, neuromuscular hyperexcitability, apathy, muscle cramp and increased stress susceptibility were observed (Fawcett et al., 1999; Iannello and Belfiore, 2001; Johnson, 2001; Morris, 1992; Saris et al., 2000). Moreover, deficiency of magnesium ions has been related to affective disorders (Hall and Joffe, 1973; Kirov et al., 1994; Linder et al., 1989; Pavlinac et al., 1979). An association between low serum magnesium levels and depressive symptoms was shown (Frazer et al., 1983; Hasey et al., 1993; Kamei et al., 1998; Widmer et al., 1992).

In animals magnesium deficiency leads to a reduction in offensive and to an increase in defensive behavior (Kantak, 1988). Moreover, magnesium administration reduces immobility time in the forced swim test (FST) in mice (Decollogne et al., 1997; Poleszak et al., 2004), which suggests potential antidepressant activity in humans.

In the present study we investigated the antidepressant-like effects of combined treatment of imipramine (IMI) and magnesium in the FST in mice and evaluated the potential pharmacokinetic interaction between these two agents by examining brain and serum concentrations of magnesium, IMI and its active metabolite desipramine.

2. Experimental procedures

2.1. Animals

All procedures were approved by the Ethical Committee of the Medical Academy, Lublin and Institute of Pharmacology Polish Academy of Sciences, Krakow. 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.

2.2. Drug administration

Magnesium salt [chloride (Fluka, Poznań, Poland), sulfate (Fluka), hydroaspartate (Farmapol, Poznań, Poland)] alone or in a combined treatment with imipramine, were administered intraperitoneally (i.p.) 0.5 h before the test. Imipramine (Polfa, Kraków, Poland) was administered 1 h before the test. Control animals received an i.p. injection of saline (vehicle). All vehicle and drug solutions were administered at a volume of 10 ml/kg.

2.3. Forced swim test

The studies were carried out on mice according to the method of Porsolt et al. (1977). 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.

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 light beams crossed by the mice was recorded as the locomotor activity.

2.5. Imipramine and desipramine determination

Serum and brain concentrations of imipramine and its metabolite desipramine were assayed by HPLC according to the method described by Szymura-Oleksiak et al. (2001) with a slight modification. After pretreatment and FST animals were sacrificed, brains removed and frozen on dry ice. Serum was isolated by centrifugation at 5000×*g* for 10 min at 4 °C, 1 h after collection and coagulation of trunk blood, then 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 bidistilled water) or 1 ml of brain homogenate containing both compounds were 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–hexan–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 a P100 isocratic pump, a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 50-μl sample loop, a 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 × 4.6 mm) with the same packing material. The mobile phase was 50 mM potassium dihydrogen phosphate, pH 4.5: acetonitrile

Table 1
The effects of joint administration of imipramine (IMI) and magnesium (Mg) on the total duration of immobility in the forced swim test in mice

Treatment	Dose (mg/kg)	Immobility time (s)
A. Vehicle+Vehicle	–	191.2±6.5
IMI+Vehicle	30	131.8±16.09**
IMI+Vehicle	15	156.5±17.89
B. Vehicle+Vehicle	–	169.0±4.8
IMI+Magnesium chloride	IMI 15+Mg 10	116.9±18.65**
IMI+Magnesium chloride	IMI 15+Mg 5	123.5±7.62**
IMI+Magnesium chloride	IMI 10+Mg 10	113.3±11.8**
IMI+Magnesium chloride	IMI 10+Mg 5	129.5±10.10*
IMI+Magnesium chloride	IMI 5+Mg 10	147.8±12.44
IMI+Magnesium chloride	IMI 5+Mg 5	183.5±6.7
C. Vehicle+Vehicle	–	190.7±3.36
IMI+Magnesium sulfate	IMI 15+Mg 10	127.7±14.91**
D. Vehicle+Vehicle	–	142.9±6.08
IMI+Magnesium hydroaspartate	IMI 15+Mg 10	99.13±6.03**

Magnesium (chloride, sulfate, hydroaspartate) and IMI were administered i.p. 0.5 h and 1 h, respectively, before the test. The values represent means±S.E.M. ($n=6-23$ mice per group). ANOVA: $F(2,25)=5.796$, $P=0.009$ (experiment A); $F(6,64)=8.15$, $P<0.0001$ (experiment B); t-test $T(14)=4.125$, $P<0.01$ (experiment C) and t-test: $T(18)=5.111$, $P<0.01$ (experiment D). *$P<0.05$, **$P<0.01$ versus the control vehicle-treated group.

(57:43 v/v) at a flow rate of 1.0 ml/min. Desipramine hydrochloride (DMI) were purchased from Sigma-Aldrich (St. Louis, MO, USA), mianserin hydrochloride (MS) was a gift from Organon (Oss, The Netherlands), imipramine hydrochloride (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.

2.6. Determination of serum magnesium concentration

Total magnesium concentration in blood serum and the whole brain was determined by xylidyl blue method (Hulanicki, 1993). After pretreatment and FST animals were sacrificed, brains removed and frozen on dry ice. Serum was isolated by centrifugation at 5000×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 (Heidolph DIAX 900 homogenizer, Austria) in four volumes of ice-cold 0.01 M Tris–HCl buffer, pH 7.4 at 26,000 rev/min for 3 min and centrifuged at 21,000×g for 30 min at 4 °C. Ten microliters 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 (Specord M40, Carl Zeiss Jena, Germany). The magnesium concentrations were calculated either as mg/100 ml (serum) or μg/g of fresh tissue (brain).

2.7. Statistics

Obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by Dunnett's or Student–Neuman–Keuls post hoc test. All results are presented as means±S.E.M. $P<0.05$ was considered as statistically significant.

3. Results

3.1. Behavioral studies

The effects of combined administration of IMI and magnesium (Mg) on total duration of immobility in mice are shown in Table 1. IMI administered alone at the dose of 30 mg/kg reduced the immobility time in mice but at the dose of 15 mg/kg, it had no significant effect in the FST. The following doses of IMI/Mg in mg/kg: 15/10, 15/5, 10/10, 10/5 induced statistically significant reduction of the immobility time in mice. The following combinations of IMI/Mg: 5/10 and 5/5 mg/kg were ineffective in this test.

The effects of magnesium and combined administration of IMI and Mg on spontaneous locomotor activity in mice are shown in Table 2. Combined administration of IMI/Mg: 15/5, 10/10, 10/5 or 15/10 mg/kg had no effect on locomotor activity in mice.

3.2. Biochemical studies

Effects of combined administration of magnesium and IMI on serum and brain magnesium concentrations in mice subjected to FST are shown in Table 3. Swim stress (mice subjected to FST) increased the magnesium concentration in serum and decreased it in brain compared to naïve animals. Moreover, administration of IMI increased (normalized) the concentration of magnesium in the brain without the

Table 2
The effects of magnesium (Mg) and its joint administration with imipramine (IMI) on spontaneous locomotor activity in mice

Treatment	Dose, mg/kg	Activity counts	
		5 min	10 min
Vehicle+Vehicle		82.9±4.8	126.0±9.9
IMI+Mg chloride	IMI 15+Mg 5	109.3±12.3	147.3±19.1
IMI+Mg chloride	IMI 10+Mg 10	65.6±9.8	94.5±12.2
IMI+Mg chloride	IMI 10+Mg 5	88.5±5.42	114.4±11.6
Vehicle+Vehicle		88.4±4.9	134.6±5.5
IMI+Mg hydroaspartate	IMI 15+Mg 10	91.9±5.6	118.2±10.1

Magnesium and IMI were administered i.p. 0.5 h and 1 h, respectively, before the test. The values represent means±S.E.M. ($n=8$ mice per group). ANOVA: $F(3,28)=4.331$, $P<0.01$; t-test: $T(14)=0.4704$ for 5 min counts and $F(3,28)=4.373$, $P<0.01$; t-test: $T(14)=1.426$ for 10 min counts.

Table 3
The effect of joint administration of magnesium (Mg) and imipramine (IMI) on serum and brain Mg concentrations in mice subjected to the forced swim test (FST)

Treatment	Mg concentration	
	Serum (mg/100 ml)	Brain (μg/g)
Naive	2.8±0.1	129.1±5.1
VEH+VEH+FST	3.2±0.2[a]	107.5±2.6[a]
VEH+Mg+FST	3.5±0.1	112.6±4.8
IMI+VEH+FST	3.5±0.2	125.8±3.7[b]
Mg+IMI+FST	3.7±0.2	110.6±6.0

Magnesium hydroaspartate (10 mgMg/kg) and IMI (15 mg/kg) were administered i.p. 0.5 h and 1 h, respectively, before the FST. Immediately after the FST animals were sacrificed, serum collected, brain removed and frozen on dry ice. The values represent means±S.E.M. (n=5–6 mice per group). ANOVA revealed $F(4,22)=7.367$, $P=0.0006$ for serum and $F(4,23)=4.402$, $P=0.0087$ for brain Mg concentrations. VEH—vehicle.
[a] $P<0.05$ vs. naive group.
[b] $P<0.05$ vs. VEH+FST group (Student–Newman–Keuls test).

influence on the serum level. Joint administration of IMI and magnesium did not influence magnesium (compared with FST) concentrations in both examined tissues.

Effects of combined administration of magnesium and IMI on serum and brain IMI and DMI concentrations in mice subjected to FST are shown in Table 4. Co-administration of IMI with magnesium did not influence IMI and DMI (compared with IMI treatment alone) concentrations in either brain or serum.

4. Discussion

Depressive disorders are nowadays one of the most disabling medical illnesses. Antidepressant therapy includes drugs with a diversity of pharmacological mechanisms. The non-selective inhibitors of biogenic amine reuptake have been the mainstays for the treatment of depression (Hollister and Csernansky, 1990). Currently, several new classes of antidepressants are available, which act as selective biogenic amine reuptake inhibitors (Stahl, 1997). Unfortunately, commonly used antidepressant therapy is effective in only 60–70% of patients and produces a variety of unwanted side effects (Hollister and Csernansky, 1990). Thus, the search for new more effective therapeutic strategies has been in progress. Behavioral and neurochemical data have related NMDA-mediated neurotransmission with depression (Nowak et al., 1993, 1995, 1998; Paul et al., 1994; Skolnick et al., 1996; Skolnick, 1999; Skolnick et al., 2001; Trullas and Skolnick, 1990). Functional antagonists of the NMDA receptor complex act as antidepressants in a variety of screen tests and animal models of depression (Maj et al., 1992a; Layer et al., 1995; Papp and Moryl, 1994, 1996; Skolnick et al., 1996; Trullas and Skolnick, 1990). Furthermore, combined administration of NMDA antagonists with antidepressants shows a synergistic action (Maj et al., 1992b). Thus, antidepressant-like properties of NMDA receptor ligands suggest the involvement of the glutamatergic system in the mechanism of the antidepressant action (Pilc et al., 2002).

Likewise organic antagonists of the NMDA receptor complex, inorganic magnesium, another inhibitor of NMDA receptor function (Novak et al., 1984) is also involved in the pathophysiology and treatment of depression. Data from both experimental and epidemiological studies suggest that disturbances in magnesium metabolism have been reported in affective disorders (Hashizume and Mori, 1990; Murck, 2002). Several clinical studies have shown a decrease in magnesium concentration in blood of depressed patients (Frizel et al., 1969; Rasmussen et al., 1989; Widmer et al., 1995; Zięba et al., 2000). In animals, magnesium deficiency leads to a reduction in offensive and an increase in defensive behavior (Kantak, 1988). However, it was also described that mice with low erythrocyte levels of this ion showed a more restless behavior and a more aggressive behavior under stressful conditions (Henrotte et al., 1997). Moreover, it was shown that magnesium exhibits an antidepressant-like effect in the forced swim test (FST) in mice (Decollogne et al., 1997; Poleszak et al., 2004). Zinc, another inhibitor of the NMDA receptors (Harrison and Gibbson, 1994; Prasad, 1993), is active in animal tests and models of depression and enhanced the antidepressant-like activity of antidepressants in FST (Kroczka et al., 2000, 2001; Nowak et al., 2003; Szewczyk et al., 2002). The antidepressant activity of zinc was observed also in the clinical studies (Nowak et al., 2003). A lower zinc serum concentration was demonstrated in depressed patients, which was normalized after successful antidepressant therapy (Maes et al., 1997; Nowak et al., 1993, 1999; Nowak and Szewczyk, 2002).

In the present study, we observed the enhancement of antidepressant-like activity by joint administration of IMI and magnesium in the FST. Magnesium, ineffective per se, given jointly with IMI at ineffective doses resulted in a potent reduction in the immobility time. Since, these joint magnesium and IMI treatments did not influence locomotor activity, the results indicate a specific enhancement of antidepressant-like activity by such combined treatment.

Table 4
The effect of joint administration of magnesium (Mg) and imipramine (IMI) on serum and brain IMI and desipramine (DMI) concentrations in mice subjected to forced swim test (FST)

	IMI+VEH+FST	IMI+Mg+FST
Serum (μg/ml)		
IMI	0.15±0.04	0.15±0.02
DMI	0.05±0.006	0.04±0.003
Brain (μg/g)		
IMI	3.35±1.17	3.82±0.77
DMI	0.29±0.04	0.29±0.03

Magnesium hydroaspartate (10 mgMg/kg) and IMI (15 mg/kg) were administered i.p. 0.5 h and 1 h, respectively, before the FST. Immediately after the FST animals were sacrificed, serum collected, brains removed and frozen on dry ice. The values represent means±S.E.M. (n=6 mice per group).

The enhancement of this effect following combined treatment with these agents may have either a pharmacodymanic or pharmacokinetic basis. To examine the pharmacokinetic interaction, in the present study we measured the brain and serum concentrations of magnesium, IMI and its metabolite DMI in mice treated with joint administration of magnesium and IMI in FST. It is generally known that the concentration of magnesium within the brain is a highly regulated process. Its concentration in the cerebrospinal fluid is higher than that in plasma, pointing to the existence of active transport systems between both compartments (Morris, 1992; Oppelt et al., 1963). While prolonged magnesium administration resulted in only small changes in the cerebrospinal fluid concentration of this bioelement, a chronic dietary magnesium deficit leads to the proportional changes in the cerebrospinal fluid and brain cellular magnesium (Kemeny et al., 1961; Schain, 1964).

In the present study we have demonstrated that mice subjected to FST exhibited higher magnesium concentration in serum, and lower in the brain compared to naive animals. Thus, it could be speculated that stress caused redistribution of magnesium from brain to peripheral tissue (serum). Interestingly, administration of IMI increased (normalized) concentration of this ion in the brain, without influence on the serum level. Joint administration of IMI and magnesium did not influence magnesium concentration (compared to FST). Similarly, this combined treatment did not affect IMI or DMI concentrations in both examined tissues (compared with IMI treatment alone). Therefore, probably pharmacodynamic interaction rather than kinetic changes are likely responsible for the behavioral effect of the combined magnesium and IMI treatment.

The present data demonstrated that joint administration of IMI and magnesium produced an enhancement of the antidepressant-like effect in the FST, and further indicated the particular role of magnesium in the antidepressant action. Since there was no increase in IMI, DMI or magnesium concentration after joint administration of magnesium and IMI, the data suggest that pharmacodynamic rather than pharmacokinetic interaction between magnesium and IMI is accountable for behavioral enhancement in the FST.

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