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NMDA Receptor Complex Blockade by Oral Administration of Magnesium: Comparison with MK-801

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DECOLLOGNE, S., A. TOMAS, C. LECERF, E. ADAMOWICZ AND M. SEMAN. *NMDA receptor complex blockade by oral administration of magnesium: Comparison with MK-801.* PHARMACOL BIOCHEM BEHAV **58**(1) 261–268, 1997.—The ion channel of the *N*-methyl-D-aspartate (NMDA) receptor complex is subject to a voltage-dependent regulation by Mg^{2+} cations. Under physiological conditions, this channel is supposed to be blocked by a high concentration of magnesium in extracellular fluids. A single dose of magnesium organic salts (i.e., aspartate, pyroglutamate, and lactate) given orally to normal mice rapidly increases the plasma Mg^{2+} level and reveals a significant dose-dependent antagonist effect of magnesium on the latency of NMDA-induced convulsions; this effect is similar to that seen after administration of the dizocilpine (MK-801) channel blocker. An anticonvulsant effect of Mg^{2+} treatment is also observed with strychnine-induced convulsions but not with bicuculline-, picrotoxin-, or pentylenetetrazol-induced convulsions. In the forced swimming test, Mg^{2+} salts reduce the immobility time in a way similar to imipramine and thus resemble the antidepressant-like activity of MK-801. This activity is masked at high doses of magnesium by a myorelaxant effect that is comparable to MK-801-induced ataxia. Potentiation of yohimbine fatal toxicity is another test commonly used to evaluate putative antidepressant drugs. Administration of Mg^{2+} salts, like administration of imipramine, strongly potentiates yohimbine lethality, in contrast to MK-801, which is only poorly active in this test. Neither Mg^{2+} nor MK-801 treatment can prevent reserpine-induced hypothermia. These data demonstrate that oral administration of magnesium to normal animals can antagonize NMDA-mediated responses and lead to antidepressant-like effects that are comparable to those of MK-801. This important regulatory role of Mg^{2+} in the central nervous system needs further investigation to evaluate the potential therapeutic advantages of magnesium supplementation in psychiatric disorders. © 1997 Elsevier Science Inc.

Magnesium organic salts MK-801 Mice NM DA antagonists Forced swimming test Anticonvulsant Absorption

RECEPTORS for *N*-methyl-D-aspartate (NMDA) are part of a group of receptors that mediate excitatory amino acid (EAA) synaptic transmission in the vertebrate central nervous system (6). They are involved in the induction of long-term potentiation of synaptic efficacy and are thus thought to play a key role in plasticity during development and learning (5). Selective antagonists have shown that the NMDA receptor complex is not simply a ligand-gated ion channel but is subject to additional regulatory factors (48). Both D-AP5 (D-2-amino-5-phosphono-pentanoic acid) and CPP (3-3-carboxypiperazine-4-yl-propyl-1-phosphonic acid) are illustrations of a class of drugs that act competitively at the agonist recognition site. HA-966 and 7-chlorokynurenic acid exemplify another class of inhibitors with a competitive action at a glycine co-agonist rec-

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ognition site. Phencyclidine (PCP) and dizocilpine (MK-801) are noncompetitive blockers that are active at the channel site.

Uniquely, the activated NMDA receptor ion channel, which is highly permeable to Ca^{2+}, is blocked by Mg^{2+} in a voltage-dependent manner (2,22,31). In vitro, this blockade operates at extracellular Mg^{2+} concentrations of less than 1 mM, which are within the range of those found in the cerebrospinal fluid (CSF) and plasma of humans and animals (32). It has thus been assumed that at membrane potentials close to resting, NMDA receptors are totally inhibited by Mg^{2+} present in extracellular fluids and that agonists induce little current flow. However, experimental data indicate that raising the concentration of Mg^{2+} above physiological levels causes further antagonism of response to NMDA (8,43). Moreover, NMDA antagonists display a variety of pharmacological and behavioral effects, including anticonvulsant (4,49) and antidepressant-like activities (25) in animals with normal magnesium levels. Conversely, magnesium deficiency is associated with behavioral alterations in patients (11), as in experimental animal models (1).

These observations prompted us to evaluate the effect of oral administration of magnesium organic salts, which are commonly used in clinics, on convulsions induced by NMDA or non-NMDA mediating agents in normal mice. We then explored the effect of elevation of the plasma Mg^{2+} level on several pharmacological and behavioral tests in which the activity of NMDA antagonists has been documented. Results indicate that increasing Mg^{2+} in biological fluids has psychopharmacological consequences resembling those achieved after MK-801 administration.

METHODS

Animals

OF1 mice (20–25 g) of both sexes (Centre d'Elevage Depre, Doulcharel, France) were kept on a normal day–night cycle at 22 ± 1°C, with free access to food and water. In the case of oral drug administration, animals fasted for 12 h before testing.

All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drug Administration

Drugs and vehicle were administered per os (PO), intraperitoneally (IP), or subcutaneously (SC) in a constant volume of 10 ml·kg^{-1} body weight. (+)-MK-801 hydrogen maleate was purchased from Research Biochemical, Inc., and diazepam (Valium®) came from Laboratoires Roche (France). NMDA, pentylenetetrazol, strychnine, bicuculline, carbamazepine, imipramine hydrochloride, yohimbine hydrochloride, reserpine, L-aspartic acid, and DL-pyroglutamic acid were purchased from Sigma (France). α,β-L-Magnesium aspartate (Megamag® Mg^{2+} content 12%) was obtained from Laboratoires Mayoly Spindler, magnesium L-lactate hydrate (Mg^{2+} content 12%) came from Fluka Chemica, and magnesium pyroglutamate (Mg^{2+} content 8%) came from UCIB (France). Mg^{2+} salts were suspended in distilled water for PO administration. All doses of magnesium salts were calculated and are expressed as the quantity of magnesium element. Doses of aspartic acid and pyroglutamic acid are expressed as amounts corresponding to those present in the Mg^{2+} salt doses used. Bicuculline and reserpine were dissolved in three to four drops of glacial acetic acid before dilution in distilled water. Carbamazepine was dissolved in distilled water containing 0.2% Tween 80. All other drugs were dissolved in distilled water.

Seizure Induction

We examined the protective effect of magnesium salts against seizures induced by NMDA, pentylenetetrazol, bicuculline, and strychnine in naive animals. NMDA was administered at 150 mg·kg^{-1} and bicuculline at 4 mg·kg^{-1} by the IP route; these doses induced convulsions in 80–90% of mice. Pentylenetetrazol at 100 mg·kg^{-1} IP and strychnine at 0.75 mg·kg^{-1} IP produced convulsions in 100% and 60% of mice, respectively. Anticonvulsant compounds and magnesium salts were given IP and PO, respectively, 30 min before administration of convulsant. Immediately after administration of the convulsant drug, animals were individually placed in plastic cages. Seizure incidence was noted for 30 min following injection of convulsants. A full seizure was recorded when clonic movements of the limbs were observed, accompanied by loss of posture. The presence or absence of convulsions, time to the first convulsive period (latency), and lethality during the observation period were noted. Each experimental group consisted of at least 10 animals. Control animals received vehicle.

Determination of Plasma Magnesium Level

Blood samples were collected from mice at different times after oral administration of magnesium salts. Plasma aliquots were stored at −20°C until analysis. Magnesium level was determined using the magnesium xylidile reagent kit (Merck-Biotrol, France) according to the manufacturer's instructions.

Forced Swimming Test

The swimming test was performed according to Porsolt et al. (37) in glass cylinders (18 cm in diameter and 40 cm high) containing 10-cm-deep water at 25°C. The total immobility time was assessed throughout a 6-min observation period. The test was started 1 h after drug administration. Each experimental group consisted of at least 10 animals. Control mice received vehicle.

Locomotor Activity

Locomotor activity was measured in photoresistor actometers (two light beams) in which the animals were placed individually 1 h after administration of the test compounds. Activity counts were recorded for 15 min. Each group consisted of 15 animals.

Motor Coordination

One hour after administration of the test compound, animals were placed for 180 s on a rotarod apparatus revolving at 16 rpm. The frequency with which mice fell from the rod during this period was scored. Each group consisted of 15 animals.

Potentiation of Yohimbine Lethality

Potentiation of yohimbine lethality was studied according to the procedure described by Quinton (39). Drugs were administered 30 min before yohimbine (25 mg·kg^{-1} IP). The percentage of lethality in each group was recorded 24 h after treatment. Each experimental group consisted of at least 10 mice. Control animals received vehicle.

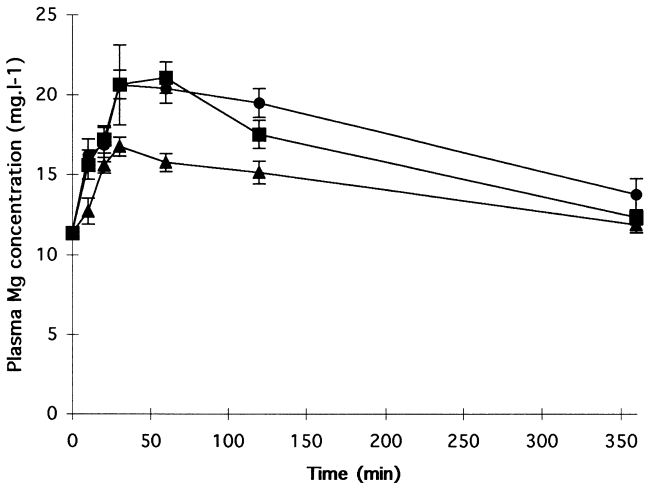

FIG. 1. Kinetics of magnesium concentration in plasma after a single administration of 300 mg·kg^{-1} of magnesium element. ■, Magnesium aspartate; ▲, magnesium lactate; ●, magnesium pidolate. Results are shown as the mean ± SE of at least 10 animals.

Reserpine-induced Hypothermia

Antagonism of reserpine-induced hypothermia was studied by the procedure described by Puech et al. (38). Magnesium salts were given orally and imipramine and MK-801 were administered IP 30 min before IP injection of reserpine (2.5 mg·kg^{-1}). Rectal temperature was measured with an electrothermal probe before drug administration and 1, 2, and 3 h after reserpine injection. Each experimental group consisted of 10 animals. Control animals received vehicle.

Statistical Analysis

Data from seizure latencies, absorption, forced swimming, and reserpine tests were analyzed by Student's *t*-test. Seizure frequencies and mortality were compared by the κhgr;[2] Yates probability test.

RESULTS

Effects of Oral Treatment with Magnesium Salts on Plasma Mg^{2+} Levels in Naive Mice

Before testing the pharmacological effects of oral treatment with magnesium, we first evaluated the kinetic variation of magnesemia in animals after single administration of magnesium aspartate, lactate, and pyroglutamate at doses corresponding to 300 mg·kg^{-1} Mg^{2+}. At this dose (Fig. 1), a rapid increase in plasma Mg^{2+} level was observed as early as 30 min after treatment. This hypermagnesemia persisted over the next 2 h and declined after 6 h. No lethal effect was observed at this dose even after 24 h. With magnesium aspartate and pyroglutamate, the highest level achieved corresponded to a doubling of the normal Mg^{2+} plasma concentration from 0.472 ± 0.01 to 0.867 ± 0.004 mM. With magnesium lactate, the increase in magnesemia was limited to 50%, suggesting that this salt has a slightly different absorption rate or bioavailability. At 100 mg·kg^{-1}, the same differential effect was observed after 30 min between magnesium aspartate and lactate, and the maximum increase was limited to 30% of basal level (data not shown). Based on these results, Mg^{2+} salts were administered 30–60 min before other pharmacological agents in all subsequent experiments.

Anticonvulsant Effects of Mg^{2+} Salts in Naive Mice

Animals were treated with magnesium aspartate, pyroglutamate, and lactate PO before IP administration of 150 mg·kg^{-1} NMDA in saline. Seizure incidence and latency were then followed for the next 30 min (Table 1). A significant delay in appearance of seizures was observed in mice treated with magnesium aspartate doses corresponding to 100 mg·kg^{-1} ($p < 0.05$) or 300 mg·kg^{-1} ($p < 0.001$) Mg^{2+} or with a magnesium pyroglutamate dose corresponding to 300 mg·kg^{-1} Mg^{2+}. No effect was observed with magnesium lactate at the same Mg^{2+} concentration. This delay was accounted for by magnesium, because no effect on the seizure latency was observed after treatment of mice with aspartic acid or pyroglutamic acid at doses corresponding to the amount present in the Mg^{2+} salts used. Neither Mg^{2+} salts nor MK-801 (0.3 mg·kg^{-1}) had a significant effect on the incidence of convul-

TABLE 1

ANTICONVULSANT EFFECT OF MAGNESIUM AGAINST NMDA-INDUCED SEIZURES

Treatment (mg·kg^{-1}) + NMDA (150)	Seizure Incidence	Seizure Latency (s)	Fatality Incidence	Fatality Latency (s)
—	26/33	384.7 ± 27.7	22/33	552.1 ± 29.1
MK-801 (0.3)	6/12	438.2 ± 70.8	0/12††	—
Imipramine (25)	8/10	327.0 ± 49.2	7/10	764.1 ± 138.1
Magnesium aspartate (100)	10/14	557.0 ± 74.5*	8/14	770.3 ± 93.1
Magnesium aspartate (300)	12/17	614.1 ± 59.6***	10/17	808.7 ± 69.1**
Magnesium lactate (300)	10/10	382.6 ± 143.1	8/10	557.0 ± 86.5
Magnesium pidolate (300)	10/10	601.0 ± 106.2*	8/10	613.8 ± 57.2
Aspartic acid (100)	3/6	236.3 ± 85.1	3/6	446.3 ± 120.2
Aspartic acid (300)	5/8	295.6 ± 49.4	4/8	525.5 ± 117.4
Pyroglutamic acid (300)	6/10	296.2 ± 36.7	6/10	468.3 ± 106.5

Compounds were given PO 30 min before IP administration of NMDA. Animals not convulsing within the 30-min observation period were considered protected. Latencies are expressed as the mean ± SE of at least 10 animals. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. the control group (Student's *t*-test); ††$p < 0.01$ vs. the control group (χ^2, Yates test).

TABLE 2

EFFECT OF MAGNESIUM ON PICROTOXIN-, BICUCULLINE-, PENTYLENETETRAZOL-, AND STRYCHNINE-INDUCED SEIZURES

Treatment ($mg \cdot kg^{-1}$)	Seizure Incidence	Seizure Latency (s)	Fatality Incidence	Fatality Latency (s)
Pentylenetetrazol (100)	10/10	91.0 ± 8.0	7/10	571.0 ± 170.0
+ Carbamazepine (75.5)	10/10	255.0 ± 47.0**	3/10	709.0 ± 69.0
+ Magnesium aspartate (300)	10/10	92.0 ± 6.0	5/10	990.0 ± 257.0
Picrotoxin (5)	7/10	738.0 ± 29.8	0/10	—
+ Diazepam (1)	4/10	1,034.0 ± 28.8**	0/10	—
+ Magnesium aspartate (300)	9/10	628.0 ± 43.2	1/10	1,290.0 ± 45.0
Bicuculline (4)	8/10	175.0 ± 51.0	7/10	247.0 ± 45.0
+ Diazepam (1)	0/10††	—	0/10††	—
+ Magnesium aspartate (300)	8/10	94.0 ± 18.0	8/10	106.0 ± 16.0
Strychnine (0.75)	6/10	340.0 ± 75.0	5/10	264.0 ± 8.0
+ Diazepam (1)	0/10†	—	0/10†	—
+ Magnesium aspartate (300)	4/10	653 ± 75*	3/10	1,020 ± 233*

Compounds were given PO (magnesium aspartate) or IP (diazepam and carbazepine) 30 min before IP administration of convulsants. Animals not convulsing within the 30-min observation period were considered protected. Latencies are expressed as the mean ± SE of 10 animals. *$p < 0.05$ and **$p < 0.01$ vs. the control group (Student's t-test); †$p < 0.05$ and ††$p < 0.01$ vs. the control group (χ^2, Yates test).

sions. However, MK-801 protected mice against NMDA-induced fatality, in contrast to magnesium treatment. These results suggested a moderate but significant antagonist effect of Mg^{2+} on NMDA receptors in normal mice.

To evaluate the specificity of the anticonvulsant property of Mg^{2+}, we then tested the antagonist effect of magnesium aspartate on convulsions induced by pentylenetetrazol (100 $mg \cdot kg^{-1}$) (a nonspecific convulsant), by picrotoxin (5 $mg \cdot kg^{-1}$) and bicuculline (4 $mg \cdot kg^{-1}$) (GABA-A antagonists), and by strychnine (0.75 $mg \cdot kg^{-1}$) (a glycine-receptor blocker). As shown in Table 2, diazepam (1 $mg \cdot kg^{-1}$) had a highly significant antagonist effect on both latency and fatality incidence of convulsions induced by bicuculline, picrotoxin, and strychnine. The latency of seizures induced by pentylenetetrazol was increased by treatment with 75.5 $mg \cdot kg^{-1}$ carbamazepine ($p < 0.001$). No effect of Mg^{2+} at 300 $mg \cdot kg^{-1}$ was observed except in the case of strychnine-induced convulsions, where a significant ($p < 0.05$) delay in seizures and fatality was detected. These observations indicated that Mg^{2+} treatment selectively antagonizes convulsions induced by NMDA and, to a lesser extent, by strychnine.

Effects of Mg^{2+} in the Forced Swimming and Locomotor Activity Tests

Imipramine and MK-801 given at single doses of 25 and 0.3 $mg \cdot kg^{-1}$, respectively, greatly shortened the immobility time of normal mice in the forced swimming test ($p < 0.001$) (Fig. 2). Magnesium aspartate had no effect at 10 $mg \cdot kg^{-1}$. Magnesium aspartate and magnesium lactate, however, significantly shortened the immobility time at doses corresponding to 30 $mg \cdot kg^{-1}$ Mg^{2+}. Increasing the dose of Mg^{2+} did not lead to a reduction in the immobility time. On the other hand, increasing the dose of Mg^{2+} to 300 $mg \cdot kg^{-1}$ with magnesium aspartate or 100 $mg \cdot kg^{-1}$ with magnesium lactate drove the immobility time back to the control value. The effect of magnesium organic salts in the forced swimming test was due to Mg^{2+}. Similar results were obtained after treatment with magnesium chloride, suggesting that the anion was ineffective. Moreover, the immobility time, which fell from 160.4 to 110.4 s ($p < 0.01$) 1 h after administration of magnesium aspartate corresponding to 100 $mg \cdot kg^{-1}$ Mg^{2+}, was back to 154.6 s after 6 h. The Mg^{2+} plasma level increased from 16.3 to 19.8 $mg \cdot L^{-1}$ after 1 h but returned to 16.6 $mg \cdot L^{-1}$ after 6 h, indicating a good correlation between the Mg^{2+} level in body fluids and the score in the forced swimming test.

Unique oral doses of magnesium aspartate ranging from 20 to 300 $mg \cdot kg^{-1}$ had no significant effect on locomotor activity after 30 min, as measured in a photoresistor actometer (data not shown). However, in the rotarod test, magnesium aspartate treatment increased the frequency of falls at an ED_50 corresponding to 73.4 $mg \cdot kg^{-1}$ Mg^{2+}, with lower and upper 95% confidence limits of 41.17 and 130.95 $mg \cdot kg^{-1}$ Mg^{2+}, respectively. These results indicate that magnesium has an influence on motor coordination that can be attributed to muscular hypotony or ataxia.

Effect of Mg^{2+} on Yohimbine Toxicity

Administration of yohimbine at 25 $mg \cdot kg^{-1}$ IP did not cause fatal toxicity in mice. Coadministration of increasing doses of Mg^{2+} salts 30 min before yohimbine resulted in the induction of fatal toxicity (Table 3). Magnesium pyroglutamate at 300 $mg \cdot kg^{-1}$ Mg^{2+} and magnesium aspartate at 350 $mg \cdot kg^{-1}$ Mg^{2+}, in combination with yohimbine killed 90% of mice. Magnesium lactate was less efficient, because only 60% of mice died after administration of yohimbine and magnesium lactate corresponding to 400 $mg \cdot kg^{-1}$ Mg^{2+}. The synergic toxic effect of Mg^{2+} organic salts with yohimbine was caused by magnesium. Administration of aspartic acid at doses corresponding to those provided by magnesium aspartate treatment did not induce fatal toxicity. The differential effects of Mg^{2+} salts in the test could be attributed to lower bioavailability of magnesium lactate (see Fig. 1). The higher

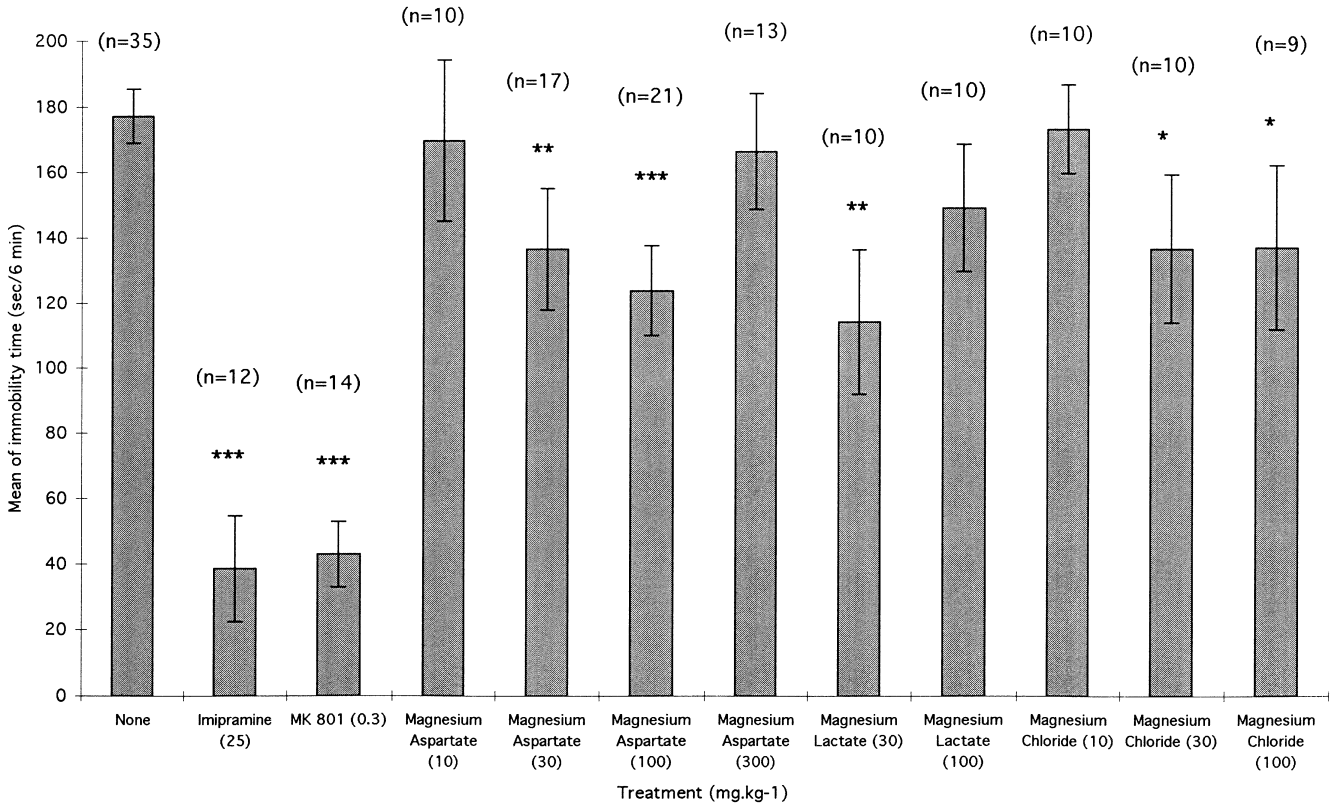

FIG. 2. Effects of magnesium on the total duration of immobility score in the forced swimming test. Compounds were administrated 24 h and 1 h before the 6-min test. Results are shown as the mean ± SE of at least 10 animals. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. the control group (Student's *t*-test).

fatality observed at intermediate doses with magnesium pyroglutamate as compared with aspartate (Table 3) was due to the pharmacological effect of the pyroglutamate anion. Administration of pyroglutamic acid at doses equivalent to those of magnesium pyroglutamate induced fatality in combination with yohimbine in 10% of mice. Fatality of 90% was obtained in mice treated with yohimbine and 25 $mg \cdot kg^{-1}$ imipramine. MK-801 was inefficient at increasing yohimbine toxicity: only 10% fatality could be achieved at an MK-801 dose of 0.1 $mg \cdot kg^{-1}$. MK-801 was not effective at higher doses, as reported by Panconi et al. (34).

Effect of Magnesium on Eeserpine-induced Hypotermia

Pretreatment of animals with Mg^{2+} aspartate or Mg^{2+} lactate corresponding to 300 $mg \cdot kg^{-1}$ Mg^{2+} did not antagonize hypothermia (Table 4) or ptosis (data not shown) induced by reserpine. MK-801 given at a dose of 0.3 $mg \cdot kg^{-1}$, which blocks NMDA-induced convulsions (Table 1), did not inhibit reserpine-induced hypothermia. This result is in agreement with observations reported by Panconi et al. (34). Reserpine-induced hypothermia was consistently antagonized significantly by imipramine given at 15 $mg \cdot kg^{-1}$.

DISCUSSION

The results reported herein demonstrate that oral treatment with a single dose of magnesium organic salts leads to a transient increase of plasma Mg^{2+} level to, at most, twice the normal physiological concentration. However, this change is sufficient to reveal significant pharmacological and behavioral effects in normal mice that can be compared qualitatively to those obtained with MK-801, a noncompetitive ion-channel blocker of the NMDA receptor complex. Mg^{2+} treatment selectively antagonizes NMDA-induced seizures and reduces immobility time in the forced swimming test, as previously described with MK-801 (47,49). Quantitatively, Mg^{2+} treatment has a more limited, although significant, effect on these tests than does MK-801. Several elements can account for this situation. First, experiments were performed in naive animals in which the Mg^{2+} level in the CSF is normal and close to 0.5 mM, a concentration thought to block the voltage-dependent NMDA ion-channel (17). Data to be published indicate that Mg^{2+} treatment has a more potent effect on these tests in magnesium-deficient animals. Then, if a rapid increase in plasma Mg^{2+} concentration could be demonstrated, neither the kinetics nor the Mg^{2+} level reached in CSF could be easily assessed in mice. It is thus possible that the concentrations reached in CSF and brain were lower than those seen in plasma 30 min to 1 h after Mg^{2+} administration (32). This, however, seems to conflict with the spectacular potentiating effect of Mg^{2+} salts on yohimbine-induced lethality.

The anticonvulsant effect of magnesium on convulsions induced by NMDA did not affect the incidence of seizures or of fatality. However, its effect on the latency of both events was highly significant and could be compared with that obtained after treatment with low doses of NMDA antagonists (40). Mg^{2+} administration did not antagonize convulsions induced by bicuculline and picrotoxin GABA-A antagonists or by

TABLE 3
POTENTIATION OF YOHIMBINE-INDUCED LETHALITY

Treatment (mg·kg^{-1}) + Yohimbine (25)	Lethality (%)	Fatality Incidence
—	0	0/25
Magnesium aspartate (200)	10	1/10
Magnesium aspartate (250)	40	4/10
Magnesium aspartate (300)	41	11/27
Magnesium aspartate (350)	89	8/9
Magnesium aspartate (400)	89	17/19
Magnesium lactate (200)	20	2/10
Magnesium lactate (300)	30	3/10
Magnesium lactate (400)	60	6/10
Magnesium pidolate (100)	50	5/10
Magnesium pidolate (300)	90	9/10
Aspartic acid (200)	0	0/10
Aspartic acid (250)	0	0/10
Aspartic acid (300)	0	0/10
Aspartic acid (350)	0	0/10
Aspartic acid (400)	0	0/10
Pyroglutamic acid (100)	10	1/10
Pyroglutamic acid (300)	10	1/10
MK-801 (0.01)	0	0/10
MK-801 (0.03)	10	1/10
MK-801 (0.1)	10	1/10
MK-801 (0.3)	0	0/10
Imipramine (25)	90	18/20

Compounds were given PO (magnesium salts, aspartic acid, and pyroglutamic acid) or IP (MK-801 and imipramine) 30 min before IP administration of yohimbine. Lethality was calculated 24 h after drug administration.

pentylenetetrazol, but a significant effect was observed on convulsions induced by strychnine. The effect of magnesium on strychnine-induced convulsions might be explained by stimulation of serine-hydroxymethyl-transferase, which converts serine into glycine. Mg^{2+}-induced glycine production might competitively antagonize the binding of strychnine to strychnine-sensitive glycine receptors. In the meantime, Mg^{2+} would block the co-agonist effect of glycine on the NMDA receptor complex. The fact that magnesium treatment was inactive on convulsions induced by bicuculline, picrotoxin, and pentylenetetrazol is not fully consistent with results indicating that MK-801 has anticonvulsant properties in these models (4). However, other groups have reported that MK-801 (23) or CGP39551 and CGP37849 competitive NMDA antagonists (40) do not inhibit seizures induced by bicuculline. Again, this discrepancy could be explained by a limited variation of Mg^{2+} concentration in CSF and brain after a single oral administration of magnesium salts. Moreover, NMDA agonists have been shown to affect GABAergic transmission (45), suggesting that noncompetitive NMDA antagonists may interfere with GABAergic transmission. It is not known whether Mg^{2+} also participates in this interaction, which is not fully understood (46). It should be kept in mind that antiepileptic drugs currently used in patients do not affect NMDA-mediated excitation (23). It is also worth noting that imipramine had no effect on NMDA-induced convulsions, although this compound is known to have a proconvulsant side effect in patients.

Treatment of normal mice with magnesium also reveals a significant effect in the forced swimming test, which is commonly used for evaluation of antidepressant activity (37). This effect can be compared with that observed with imipramine or MK-801 (47), although the antidepressant activity of MK-801 has been subject to debate because of its ability to induce locomotor hyperactivity in various animal models (16,24–27,34). MK-801, however, is efficient in the forced swimming test at doses below those producing motor-stimulatory action. Moreover, several competitive and noncompetitive NMDA antagonists (including memantine, which has a lower affinity) share antidepressant activities with MK-801 (19,33,35,42). The effect of magnesium in the forced swimming test was observed in a relatively narrow range of concentrations. No significant effect was observed below 10 mg·kg^{-1} or above 100–300 mg·kg^{-1}, depending on the salt used. Single administration of magnesium had no effect on locomotor activity tested with an actometer, but a moderate and significant myorelaxant effect was observed in the rotarod test. Hence, it can be postulated that the antidepressant-like activity of Mg^{2+} in the forced swimming test is masked by its myorelaxant effect at high concentrations. Consistently, data to be published from our laboratory indicate that magnesium-deficient rats, which exhibit locomotor hyperactivity, respond to magnesium administration with a shortened immobility time in the forced swimming

TABLE 4
EFFECT OF MAGNESIUM ON RESERPINE-INDUCED HYPOTHERMIA

Treatment (mg·kg^{-1}) + Reserpine (2.5)	0 h	1 h	2 h	3 h
Experiment 1				
—	38.5 ± 0.1	31.2 ± 0.3	29.5 ± 0.6	27.4 ± 0.6
Magnesium aspartate (300)	38.6 ± 0.1	32.3 ± 1.0	29.3 ± 0.8	28.0 ± 1.0
Magnesium lactate (300)	38.5 ± 0.1	30.5 ± 0.4	28.1 ± 0.5	26.7 ± 0.4
Imipramine (15)	38.7 ± 0.1	33.4 ± 0.7**	35.4 ± 0.5***	34.1 ± 0.5***
Experiment 2				
—	38.1 ± 0.2	36.4 ± 0.2	33.9 ± 0.8	31.4 ± 1.1
MK-801 (0.3)	38.0 ± 0.2	37.9 ± 0.1	33.3 ± 0.3	33.3 ± 0.6
Imipramine (15)	38.1 ± 0.2	37.7 ± 0.3	37.3 ± 0.4*	35.2 ± 0.2**

Values are the mean ± SEM of body temperature (°C) at 0, 1, 2, and 3 h after reserpine administration. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. the control group (Student's *t*-test).

test. It is interesting that the myorelaxant effect of Mg^{2+} may parallel ataxia induced by MK-801 or PCP (14).

Potentiation of yohimbine-induced lethality is another test commonly used to assess antidepressant activity of drugs (10,39). In this test, a single administration of magnesium had a dramatic and significant effect comparable to that obtained with imipramine. A slight differential effect of salts was observed, which, in the case of magnesium pyroglutamate, can be attributed to the pharmacological effect of the pyroglutamate anion (30), because pyroglutamic acid alone potentiated yohimbine toxicity. In agreement with observations reported by Panconi et al. (34), MK-801 was inefficient at potentiating yohimbine lethality. This could be anticipated from experiments indicating that MK-801, like other NMDA antagonists such as phencyclidine, blocks yohimbine-induced seizures (9). It has been suggested that yohimbine, in addition to its α2-antagonist activity and negative effect on GABAergic transmission (29), nonspecifically increases the release of endogenous EAA, such as glutamate or aspartate and glycine (9). This would explain the antagonist effect of MK-801 and PCP on yohimbine-induced seizures. However, the affinity of these compounds for their NMDA receptor binding site is influenced not only by glutamate and glycine but also by Ca^{2+}, H^{+}, and Mg^{2+} cations (20,21). A moderate potentiating effect of MK-801 on yohimbine toxicity was seen at low doses in our own experiments and in those reported by Panconi et al. (34). Increasing the dose of MK-801 was without effect on induction of lethality, suggesting that a partial blockade of the NMDA ion channel is required for potentiating yohimbine toxicity. This would be achieved by administration of magnesium or by low doses of MK-801, whose affinity would be reduced by EAA release induced by the high yohimbine concentration (25 $mg \cdot kg^{-1}$) used to assess toxicity. Because the EAA system is functionally heterogeneous at the physiological response level (7), it is possible that neurons participating in seizure induction and lethality might be different. Alternatively, it is possible that the effect of Mg^{2+} salts on yohimbine-induced toxicity results from the cardiac effect of Mg^{2+}, which might potentiate the sympathicolytic property of yohimbine (50). Finally, antagonism of reserpine-induced hypothermia is one of the pharmacological tests traditionally used to assess the activity of antidepressant drugs (38). In this test, Mg^{2+} treatment, like MK-801 treatment, was inactive, as reported by Panconi et al. (34), reinforcing the idea that Mg^{2+}, like other NMDA inhibitors, represents a new class of antidepressant agents. It would be of interest to test whether chronic magnesium treatment can downregulate cortical β-adrenoceptors. This phenomenon is consistently observed with all antidepressant compounds (13), including non-NMDA antagonists such as imipramine as well as MK-801 and other NMDA inhibitors (36).

In conclusion, our results indicate that under normal physiological conditions, NMDA receptor complexes remain sensitive to EAA stimulation, as previously shown by Hornfeldt et al. (15), despite a high Mg^{2+} concentration in the CSF, which is known to block the ion channel in vitro (17). More importantly, our results are all consistent with the hypothesis that the NMDA receptor complexes respond to an increase in Mg^{2+} concentration in biological fluids with pharmacological effects resembling the antidepressant-like effects of MK-801. Although predictable, these observations are of particular interest because variations in magnesium concentration have been observed in patients suffering from epileptic seizures or depression (18,28,44). Personality change and depression are also the major symptoms of hypomagnesemia (11,12). This has led several authors to propose magnesium supplementation in the treatment of mild psychiatric disorders (3,41). This perspective should motivate the development of appropriate experimental models to evaluate further the pharmacological properties of magnesium, both alone and in conjunction with other antidepressant drugs.

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