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MAGNESIUM SULFATE REDUCES SEIZURES INDUCED BY CENTRAL ADMINISTRATION OF THE EXCITATORY AMINO ACID *N*-METHYL-D-ASPARTATE IN RATS

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ABSTRACT

N-Methyl-D-aspartate (NMDA) has been implicated in a number of pathophysiologic conditions, including seizures. Magnesium is a physiologic blocker of the NMDA receptor. As magnesium sulfate ($MgSO_4$) is currently used as a treatment for eclamptic seizures in North America, we examined the anticonvulsant effects of $MgSO_4$ on central NMDA-induced seizures. Forty-one female rats were surgically anesthetized and a bipolar recording electrode was stereotaxically implanted into the dorsal hippocampus, while a cannula was implanted into the lateral cerebral ventricle for drug injection. Following 1-week recovery, baseline behavior and electrical activity were recorded. Two treatment protocols were examined: (*a*) chronic—intraperitoneal injection of 270 mg/kg $MgSO_4$, followed every 20 min with 27 mg/kg $MgSO_4$ for a total of 2 h; and (*b*) acute—intravenous injection of $MgSO_4$ (30, 60, or 90 mg/kg) in a volume of 1.5 mL/kg via the tail vein. Following either treatment protocol, rats received 1 μL of 20 mg/mL NMDA via the cannula, and seizure activity was assessed. Onset to the first seizure was significantly lengthened in rats receiv-

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ing both chronic ($P < 0.01$) and acute ($P < 0.01$) $MgSO_4$. Total seizure number was significantly reduced in the chronic $MgSO_4$ group ($P < 0.05$). Total seizure duration was significantly reduced in both the chronic ($P < 0.05$) and acute ($P < 0.05$) $MgSO_4$ groups. Mortality was 30% in the chronic control group, whereas none of the rats that received chronic $MgSO_4$ died. We conclude that magnesium sulfate reduces seizure activity induced by centrally administered NMDA. These results support our previous observations that magnesium does have central anticonvulsant effects.

Key Words: Eclampsia; Excitatory amino acids; Magnesium; Seizures.

INTRODUCTION

The *N*-methyl-D-aspartate (NMDA) receptor has been implicated as the final common pathway at the cellular level in the generation of seizure activity (1). This receptor is blocked by magnesium at physiological concentrations in a voltage-dependent manner (2). Upon neuronal depolarization, the magnesium block of the NMDA receptor ionophore is removed and glutamate binding to NMDA receptors opens channels that allow sodium and calcium to enter the cell. If the concentration of glutamate becomes too high, hyperexcitability and eventually a seizure can result.

Magnesium sulfate is currently used as an anticonvulsant agent in North America for the treatment and prevention of eclamptic seizures (3). The pathophysiology of eclamptic seizures is presently unknown. The present study was designed to determine whether magnesium could reduce seizure activity resulting from intraventricular administration of NMDA. Although it has not been established that eclamptic seizures are mediated through the NMDA system, this study may provide evidence which may indicate a possible mechanism for magnesium sulfate's anticonvulsant effects. This information may indirectly shed more light on the mechanism of eclamptic convulsions.

METHODS

Surgical Preparation

Female Long–Evans rats (Charles Rivers, Portage, MI, USA, 250–300 g) were individually housed in polyethylene cages in an environmentally controlled vivarium under 12-h light/dark cycles. Food and water were available ad libitum. The Wayne State University guide to the use and care of laboratory animals was adhered to in the present study. Forty-one rats were surgically anesthetized (45 mg/kg, sodium pentobarbital intraperitoneally) and a bipolar recording electrode (MS-303/2, Plastics One, Inc., Roanoke, VA) was stereotaxically implanted into the dorsal hippocampus. The coordinates for implantation were: 3.5 mm posterior to bregma, 2.0 mm lateral to midline, and 3.5 mm ventral to the surface of the skull

(4). Additionally, a 23-gauge stainless steel cannula was implanted into the lateral cerebral ventricle at the following coordinates: 2.0 mm anterior to bregma, 1.0 mm lateral to midline, and 5.4 mm ventral to the surface of the skull (4). A 30-gauge obturator plugged the cannula until the time of the injection. The electrode and cannula were anchored to the skull using cranioplastic and two stainless steel screws, with a third screw serving as ground. Animals were allowed 1 week to recover. A final group of 24 rats (250–300 g) was used to determine blood and cerebrospinal fluid magnesium levels under treatment and control conditions.

Experimental Design and Protocol

Experiment 1

In this experiment, the effects of chronic intraperitoneal treatment with $MgSO_4$ on NMDA-induced seizures was determined. Surgically prepared rats were placed into a Plexiglas recording chamber and leads from a polygraph (model 7, Grass Instruments Co., Quincy, MA) were attached to the implanted electrode. Baseline behavior and electroencephalographic (EEG) activity were recorded. Animals were then injected intraperitoneally with 270 mg/kg $MgSO_4$, followed every 20 min with 27 mg/kg $MgSO_4$ for 2 h (n = 7). Control rats received an equal volume of saline (n = 10). At 2 h, a 1-μL solution of 20 mg/mL NMDA was delivered through the indwelling cannula at a rate of 1 μL/min. Seizure activity was defined electrographically. Onset to seizure activity, total seizure duration, and total seizure number over a period of 20 min were recorded. Finally, the mortality rate for treated versus control rats was determined. The evaluator was blinded as to the treatment given.

Serum and cerebrospinal fluid (CSF) magnesium levels were determined after 2 h of either intraperitoneal saline or $MgSO_4$ injections in a group of 10 nonoperated rats. After 2 h of treatment, each rat was overdosed with 120 mg/kg sodium pentobarbital. A 0.5-mL blood sample was obtained from the heart, while a 0.2-mL sample of CSF was obtained through a puncture in the cisterna magna. Magnesium levels were analyzed colorimetrically (Eastman Kodak Co., Rochester, NY).

Experiment 2

The effects of intravenous injections of $MgSO_4$ at 30, 60, or 90 mg/kg on NMDA-induced seizures were determined. Twenty-four surgically prepared rats were placed into the Plexiglas recording chamber and baseline and EEG activity were recorded as described above. Animals were then placed into a small animal restrainer, with the tail exposed for intravenous drug injection. Rats received a tail vein injection of $MgSO_4$ [30 (n = 7), 60 (n = 5), or 90 (n = 6) mg/kg] or saline (n = 6) in a volume of 1.5 mL/kg. The injection was administered slowly over a period of 3 min. Rats were removed from the restrainer following treatment and placed back into the recording chamber, where EEG activity was continuously monitored. Fif-

teen minutes later, 1 μL of 20 mg/mL NMDA was delivered through the indwelling cannula at a rate of 1 μL/min, and seizure activity was assessed as described above. Again, the evaluator was blinded as to the treatment given.

In a separate group of 14 nonoperated rats, serum and CSF magnesium levels for the 90 mg/kg dose of $MgSO_4$ were compared to control injections in the same manner as described above.

Histology

Rats were allowed to survive for 4 days before being euthanized. Rats were injected with an overdose of sodium pentobarbital (120 mg/kg, intraperitoneally) and perfused transcardially with 0.9% buffered saline solution followed by 10% formalin solution. The brains were removed and sectioned on a freezing microtome at 40 μm. After placement of the tissue sections on microscopic slides, the sections were stained with Cresyl Violet. The location of the electrodes and cannulas were verified microscopically. In addition, neuronal damage was assessed using bright-field microscopy.

Statistics

Data were analyzed using one-way analysis of variance (ANOVA) and Student's *t* test. Post hoc testing was performed when appropriate using the Bonferroni test. Values are expressed as mean ± SEM. Data are reported as significant if $P < 0.05$.

RESULTS

Chronic Magnesium Sulfate

The initial intraperitoneal injection of $MgSO_4$ in rats produced a reduction in locomotor abilities and general ataxia. However, rats were observed to recover their locomotor skills over 1 h of chronic treatment with $MgSO_4$ and did not appear to be different from control rats at 2 h.

Histological analysis of the brains of the rats revealed that all electrodes were located within the hippocampus while all cannulae were located within the lateral cerebral ventricle. At the dose examined in this study, the chemical convulsant NMDA did not produce any severe morphological damage as evident by light microscopy. The hippocampus, dentate, cortex, thalamus, and basal ganglia all had intact neuronal cells, and the white matter also remained fully intact in the treatment and control groups.

Central NMDA-induced seizures were characterized by episodes of hypnotic staring, scratching, tonic–clonic activity, wild running, and jumping. Occasionally, seizures progressed to a state of status epilepticus. Periods of high-frequency/high-amplitude epileptiform discharges in the hippocampal EEG recording were also observed. These electrical seizures tended to occur with behavioral seizure activity,

although the electrical seizures could also occur without any behavioral manifestations.

The effects of chronic intraperitoneal $MgSO_2$ treatment on NMDA-induced seizures are shown in Table 1; it is seen that chronic $MgSO_4$ treatment delayed the onset to seizure activity ($P < 0.01$). In addition, total seizure duration ($P < 0.05$) and total number of seizures ($P < 0.05$) occurring over the 20-min recording period from the time of injection were also significantly reduced with chronic $MgSO_4$. Mortality was 30% in the saline-treated group (n = 10), while none of the rats that received $MgSO_4$ died (n = 7), although this did not reach statistical significance.

Serum magnesium levels were significantly elevated following 2 h of chronic intraperitoneal $MgSO_4$ treatment compared to control (control: 2.28 ± 0.09 mg/dL; $MgSO_4$: 7.12 ± 0.16 mg/dL, $P<0.01$). CSF magnesium levels were also elevated in the $MgSO_4$ treatment group compared to control (control: 1.56 ± 0.39 mg/dL; $MgSO_4$: 2.24 ± 0.04 mg/dL, $P < 0.01$).

Acute Magnesium Sulfate

Rats that received the lowest dose of $MgSO_4$ (i.e., 30 mg/kg) showed no overt behavioral effects, while rats that received 60 mg/kg $MgSO_4$ exhibited mild ataxia and locomotor difficulties lasting 1–2 min post-magnesium injection. The rats that received the highest dose of $MgSO_4$ (i.e., 90 mg/kg) were initially ataxic and showed labored breathing, but recovered rapidly.

Histological analysis of the brains of the rats revealed that all electrodes were located within the hippocampus while all cannulae were located within the lateral cerebral ventricles.

The effects of acute intravenous injection of $MgSO_4$ on central NMDA-induced seizures are shown in Figs. 1 and 2. Magnesium sulfate produced a dose-related lengthening of the time period from NMDA injection to seizure activity as recorded from the hippocampus (ANOVA, $P < 0.01$). In addition, the total seizure duration over 20 min was reduced in a dose-dependent manner by intravenous $MgSO_4$ (ANOVA, $P < 0.05$). The total number of seizures was not significantly affected (data not shown). Mortality was 33% in the saline-treated group (n = 6), while none of the rats that received 90 mg/kg $MgSO_4$ died (n = 6). This is an

Table 1. Summary of Effects of Chronic Intraperitoneal Injections of Magnesium Sulfate on NMDA-Induced Seizures (mean ± SEM)

GROUP	SEIZURE ONSET (sec)	TOTAL SEIZURE DURATION (sec)	TOTAL NUMBER OF SEIZURES
Saline (n = 10)	10 ± 6.0	344 ± 64.2	9.6 ± 1.2
$MgSO_4$ (n = 7)	102 ± 60.9**	179 ± 61.3*	5.9 ± 1.5*

**$P < 0.01$, *$P < 0.05$ compared to saline control group.

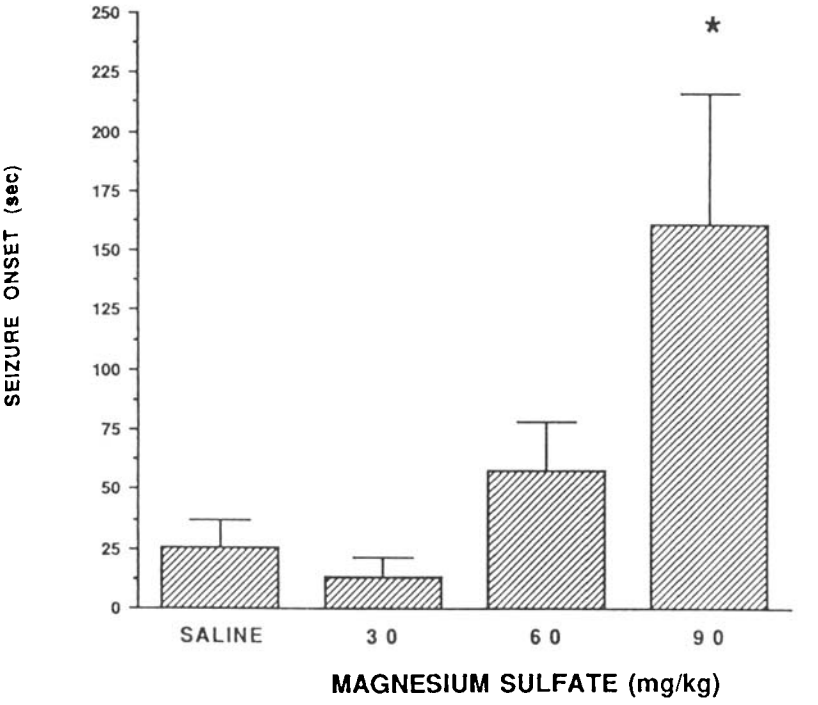

Figure 1. Seizure induction by central administration of 20 μg NMDA was significantly delayed by intravenous injection of magnesium sulfate. *$P < 0.05$ versus saline control injections.

interesting finding but did not reach statistical significance due to the small number of rats used in the study.

Serum magnesium levels were significantly elevated 15 min following intravenous injection of 90 mg/kg $MgSO_4$ compared to control (control: 3.19 ± 0.35 mg/dL; $MgSO_4$: 5.18 ± 0.21 mg/dL, $P < 0.01$). CSF magnesium levels were also elevated in the rats receiving 90 mg/kg $MgSO_4$ compared to control (control: 1.96 ± 0.04 mg/dL; $MgSO_4$: 2.42 ± 0.11 mg/dL, $P < 0.01$).

DISCUSSION

Our results led us to conclude that magnesium sulfate does have central effects against seizures induced by the excitatory amino acid *N*-methyl-D-aspartate (NMDA) in rats. This was evident as a delay in the time from injection of NMDA to the onset of seizure activity and a reduction in the total duration of NMDA-induced seizure activity by both chronic (intraperitoneal) and acute (intravenous) magnesium sulfate.

Previous studies from our laboratory have demonstrated that magnesium sulfate has anticonvulsant effects (5–7). We have shown that parenteral injection of

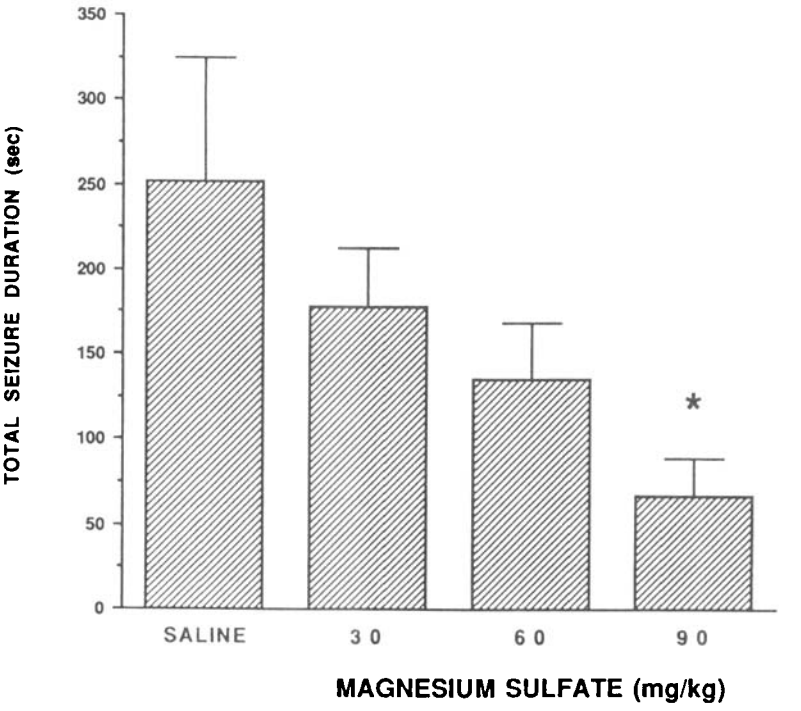

Figure 2. Total seizure duration over a period of 20 min following central administration of 20 μg NMDA was significantly reduced by pretreatment with intravenous magnesium sulfate. *$P < 0.05$ versus saline control injections.

magnesium sulfate reduces hippocampal seizure activity and increases seizure threshold following electrical stimulation (5, 6). However, in these studies, extremely high serum magnesium levels were required for an effect to be observed. In the present study, we did not measure brain levels, but we did observe a significant increase in blood and CSF magnesium levels in nonoperated rats that received intraperitoneal and intravenous $MgSO_4$. The doses of $MgSO_4$ used in the present study produced serum drug levels which were much closer to standard human therapeutic levels than those of previous studies (5, 6). The blood magnesium levels produced in this study are within the range of magnesium levels that have effectively reduced seizures in eclamptic patients (3).

In a previous study, direct hippocampal injection of NMDA was used as a seizure model to study effects of peripheral magnesium administration (7). In this study (7), rats were repeatedly injected with NMDA. As NMDA is a neurotoxin, multiple injections may have led to the variability in the results. In the present study, rats were injected intracerebroventricularly (ICV) with NMDA and tested only once. Sibai and colleagues (8) have reported diffuse abnormalities in the electroencephalogram of eclamptics. The hippocampus is involved in focal epilepsy. Thus, ICV administration of NMDA, allowing for a larger distribution, is a

more appropriate model for demonstrating the effect of magnesium sulfate. In fact, more efficacious seizure suppression was seen in the present study. Total seizure duration and total seizure number were reduced in the present study, while not reported previously (7).

Others have also demonstrated anticonvulsant effects of magnesium sulfate in penicillin-induced seizures (9) and seizures induced by hyperbaric oxygen in rats (10). Also, NMDA-induced neuronal brain injury is inhibited with parenteral magnesium (11). These studies also provide further evidence that magnesium sulfate has a central site of action.

The present study has examined different routes of administration (intravenous and intraperitoneal) and duration of therapy (acute and chronic). We tested acute magnesium treatment to see how fast an anticonvulsant effect would be observed and at what magnesium concentration. This is very relevant to the clinical setting, as seizures may occur prior to or upon arrival to the hospital in an eclamptic patient, and therapy would be needed immediately. The present results provide evidence that intravenous magnesium sulfate may rapidly reduce seizure activity. Future studies should be directed toward examining the transfer of magnesium to the brain with various routes of administration and at various time points.

A complete description of the mechanism of action of magnesium sulfate's anticonvulsant effect has yet to be determined. The anticonvulsant effect of magnesium sulfate may be mediated by one or more of the following: (*a*) antagonism of calcium transport (12); (*b*) reduction in surface charge screening (13), or (*c*) blockade of the NMDA receptor ionophore (2). The NMDA receptor has been previously implicated in the generation of epileptiform activity (1, 14). NMDA receptor antagonists have been shown to have anticonvulsant activity in a variety of seizure models (15–17). Our laboratory has recently demonstrated that magnesium sulfate treatment alters NMDA receptor binding in the rat brain (18). Although additional mechanisms may be involved, the possibility that magnesium may be acting to reduce seizure activity through inhibition of NMDA receptors should be directly investigated.

NMDA produces neuronal damage via excitotoxic mechanisms when injected directly into the brain. The excitotoxicity can lead to various brain injuries including hypoxia, ischemia, neurodegenerative diseases, and seizures (19). NMDA receptors are localized to specific areas in the brain, with the highest density found in the hippocampus (20). In the present study, intraventricular infusion of 20 μg NMDA did not produce any acute neuronal damage within the hippocampus or any other brain structure examined. Other authors have also reported similar results with intracerebroventricular injections of NMDA (21). Thus, the seizure model used in the present study appears to be an appropriate model for demonstrating anticonvulsant effects of magnesium sulfate, without the confounding factor of neuronal damage.

Clinically, magnesium sulfate has proven to be a safe and effective agent for treating and preventing seizures in preeclampsia–eclampsia. With the paucity of data that exists regarding magnesium sulfate's efficacy in other types of seizures, it

is understandable that there is controversy regarding its use in preeclampsia–eclampsia. It is possible that, given magnesium sulfate's efficacy in eclampsia, a clearer definition of its site and mechanism of action using a variety of seizure models may lead to a better understanding of the mechanism by which eclamptic convulsions occur.

We conclude that magnesium sulfate has anticonvulsant activity against central ICV NMDA-induced seizures when administered both acutely and chronically.

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