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Magnesium sulfate and sodium valproate block methylphenidate-induced hyperlocomotion, an animal model of mania

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Abstract:
Magnesium sulfate ($MgSO_4$) is used to treat and prevent eclamptic seizures, and several anticonvulsant drugs (e.g., sodium valproate) are clinically effective antimanic drugs. Psychostimulant-induced hyperlocomotion has been proposed as an animal model for the study of antimanic drugs. The present study evaluated the effects of $MgSO_4$ and sodium valproate (as a positive control) on hyperlocomotion induced by methylphenidate in mice. Acute $MgSO_4$ (300–400 mg/kg), but not sodium valproate (100–300 mg/kg), prevented the increase in locomotor activity induced by methylphenidate (5.0 mg/kg). In contrast, repeated treatment (14 days) with valproate (300 mg/kg), but not $MgSO_4$ (400 mg/kg), blocked methylphenidate-induced hyperlocomotion. Thus, acute $MgSO_4$ exerted antimanic-like effects in this animal model.

Key words:
anticonvulsant, locomotor activity, mania, psychostimulant, magnesium sulfate, valproate

Abbreviations: $MgCl_2$ – magnesium chloride, $MgSO_4$ – magnesium sulfate, NMDA – *N*-methyl-D-aspartate, PKC – protein kinase C

Introduction

Magnesium, an inorganic cation, has been associated with changes in monoaminergic neurotransmission, is a noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist, and alters protein kinase C activity (PKC) [6, 21, 30]. These targets are associated with the mechanism of action of some drug treatments for mood disorders, such as the PKC inhibitors lithium, valproate, and tamoxifen, and the anticonvulsant lamotrigine that reduces glutamate release and alters serotonin neurotransmission [5, 8, 32, 33]. One important clinical use of magnesium is for the prevention and treatment of eclamptic seizures, although its anticonvulsant mechanism of action is unclear [14]. Interestingly, some anticonvulsant drugs (e.g., carbamazepine

and oxcarbazepine) exert clinical antimanic effects [5] and antidepressant-like effects in animal models [3, 4]. Moreover, mood stabilizers, such as lithium, valproate and carbamazepine, have been shown to increase Mg^{2+} in erythrocytes in bipolar patients [22].

Consistent with these findings, a few reports suggest that magnesium sulfate ($MgSO_4$) can exert clinical antimanic effects [7, 16, 18]. Heiden et al. [18] found that intravenous $MgSO_4$ increased the clinical efficacy of antimanic drugs (lithium, haloperidol, and clonazepam) in an open-label study in severely agitated manic patients. Moreover, Giannini et al. [16] found that the addition of magnesium oxide augmented the efficacy of verapamil for maintenance therapy of mania in a small-sample size double-blind study. Chouinard et al. [7] carried out the only study that we are aware of using magnesium monotherapy to treat mania in an open-label design. They found that oral magnesium aspartate reduced symptoms in rapid cycling patients. Moreover, magnesium also exerted antidepressant-like effects in animal models, such as the forced swim test and tail suspension test [6, 9, 23–26, 30], similar to some anticonvulsant drugs [3, 4]. The antidepressant-like effects of magnesium can be reversed by D-serine administration [24]. Magnesium is a noncompetitive NMDA receptor antagonist, and D-serine acts as an agonist at the glycine site in the NMDA receptor complex. Therefore, the antidepressant and antimanic effects of magnesium were suggested to involve a glutamatergic mechanism of action *via* NMDA receptors [9, 24, 26, 27, 30]. Consistent with this possibility, an intra-nucleus accumbens injection of magnesium chloride blocked the increase in locomotion induced by *N*-methyl-aspartic acid [10]. The antimanic effects of Mg^{2+} have also been suggested to be related to a reduction in the actions of calcium (Ca^{2+}) [16, 18].

Recently, some animal models of acute manic episodes have been proposed, such as hyperlocomotion induced by psychostimulants or paradoxical sleep deprivation and hedonic-like behaviors induced by genetic manipulations [12]. Psychostimulant-induced hyperlocomotion was blocked by the clinically effective antimanic drugs lithium and sodium valproate [11, 12, 15, 17, 28], conferring predictive validity to the model. This model was further used to study the new antimanic agent tamoxifen [13, 28].

The objective of the present study was to evaluate the effects of $MgSO_4$ in an animal model of mania (methylphenidate-induced hyperlocomotion), with sodium valproate as a positive control.

Material and Methods

Animals

The animals used in this study were male adult Swiss albino mice (60–90 days old) from our own breeding stock. The mice were maintained in groups of five in polypropylene cages with wood shaving bedding under a 12-h/12-h light/dark cycle (lights on at 7:00 a.m.) and controlled temperature (22 ± 2°C). They had free access to water and food throughout the experiment. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments of the Biological Sciences Sector, Universidade Federal do Paraná (protocol number 306).

Drugs

Methylphenidate (Novartis, Brazil) was suspended in two drops of Tween 80 and saline and administered subcutaneously (*sc*) at a dose of 5.0 mg/kg. Sodium valproate solution (Sanofi-Aventis, Suzano, SP, Brazil) was dissolved in saline and administered intraperitoneally (*ip*) at doses of 100, 200, and 300 mg/kg. $MgSO_4$ (Sigma, St. Louis, MO, USA) was dissolved in saline and administered *ip* at doses of 100, 200, 300, and 400 mg/kg. All drugs and vehicles were administered at a constant volume of 10 ml/kg. The drug doses were calculated from salt form, and administration schedules were based on those used previously [1, 2, 6, 24, 27].

Locomotor activity in the open-field test

Locomotor activity was measured in the open-field test, which consisted of a circular arena (diameter 40 cm; height 28 cm) painted white. The open-field floor area was divided by black lines into 25 spaces arranged in three concentric circles (inner circle, diameter of 12 cm; middle circle, diameter of 26 cm; outer circle, diameter delimited by the wall of the arena, 40 cm). The number of spaces in the inner, middle, and outer circles were 1, 8, and 16, respectively. The level of illumination on the floor of the apparatus was 110 lux. The number of lines crossed was cumulatively recorded over a five-minute period. Peripheral locomotion was recorded when the mouse moved within the

spaces of the outer circle, and central locomotion was recorded when the mouse moved within the spaces of the inner and middle circles. The animals were tested in the open-field 15 min after methylphenidate treatment. The open field arena was wiped with a water-alcohol (10%) solution before each behavioral test to avoid possible bias caused by odors or residues left by the previously tested mouse.

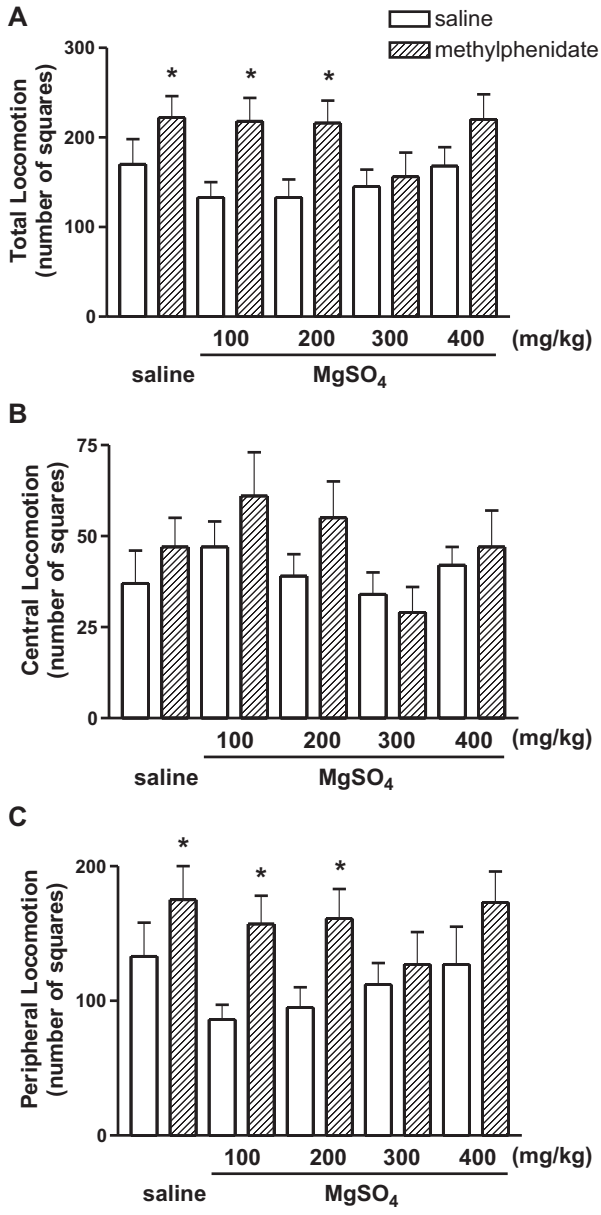

Fig. 1. Effects of acute magnesium sulfate administration ($MgSO_4$, 100–400 mg/kg, *ip*) on hyperlocomotion induced by methylphenidate (5.0 mg/kg, *sc*) in the open-field test in mice. Data are expressed as the mean ± SEM (n = 6–15/group) of (**A**) total locomotion, (**B**) central locomotion, and (**C**) peripheral locomotion. * $p < 0.05$, compared with respective saline group (+ saline or $MgSO_4$)

Experimental procedure

To determine whether the drugs have effects on methylphenidate-induced hyperlocomotion, we administered the test drugs combined with methylphenidate and compared locomotor activity with the control groups. Forty-five minutes before the test, mice were treated with either vehicle (*ip*) or a test drug ($MgSO_4$ or sodium valproate, both *ip*). Thirty minutes later, saline or methylphenidate 5.0 mg/kg was administered (both *sc*). Fifteen minutes after methylphenidate (or saline) administration, the mice were tested in the open-field for 5 min.

Statistical analysis

Because the data showed heterogeneity of variance (heteroscedasticity), the raw data were transformed to natural logarithmic forms. The logarithmic data, which have homoscedastic variance and normal distribution, were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test. For practical convenience and to avoid the influence of circadian rhythm, the mice were randomly distributed into groups, and the experiments were conducted on different days. These results were combined as one data set because no significant differences were observed between the control groups (vehicle + saline) for each experimental day. Statistical significance was set at $p < 0.05$.

Results

Acute $MgSO_4$ administration

Figure 1 shows the effects of methylphenidate and acute $MgSO_4$ administration on open-field activity. An ANOVA indicated a significant effect of treatment on total locomotion ($F_{9,91} = 2.193$, $p < 0.05$) and peripheral locomotion ($F_{9,91} = 2.245$, $p < 0.05$), but not on central locomotion ($F_{9,91} = 1.002$, $p > 0.05$). Methylphenidate increased total locomotion in groups pretreated with saline and two lower doses of $MgSO_4$ (100 and 200 mg/kg, all $p < 0.05$), whereas methylphenidate did not alter locomotion in mice treated with higher $MgSO_4$ doses (300 and 400 mg/kg). Similar results were observed with peripheral locomotion.

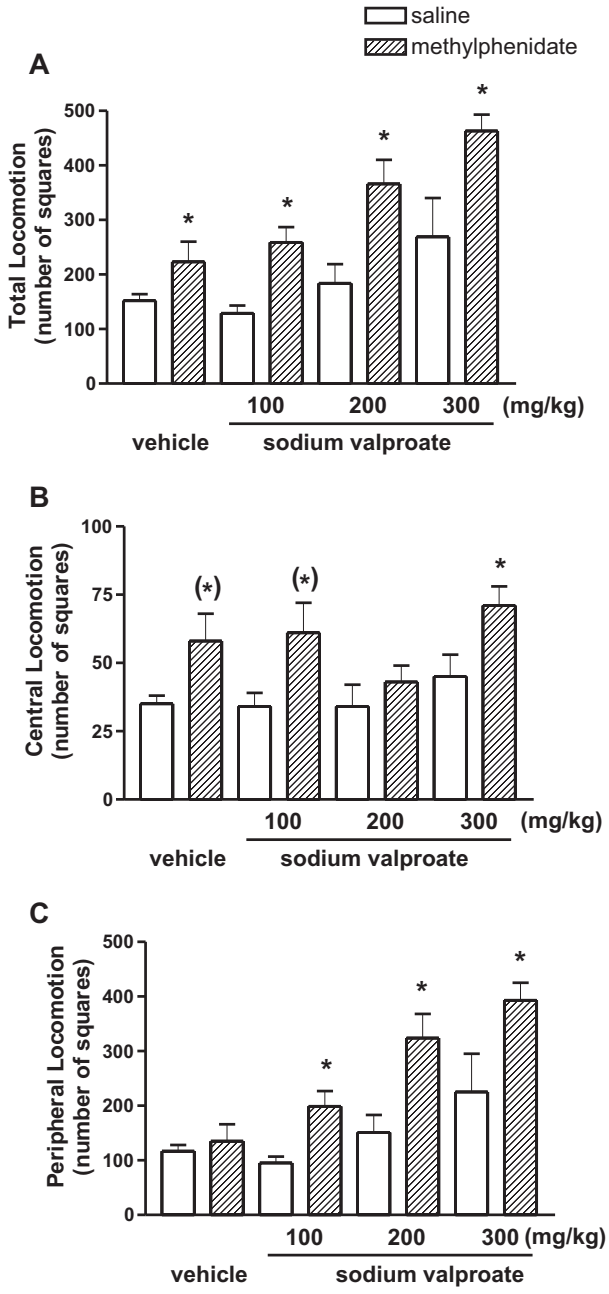

Fig. 2. Effects of acute sodium valproate (100–300 mg/kg, *ip*) on hyperlocomotion induced by methylphenidate (5.0 mg/kg, *sc*) in the open-field test in mice. Data are expressed as the mean ± SEM (n = 5–14/group) of (**A**) total locomotion, (**B**) central locomotion, and (**C**) peripheral locomotion. * $p < 0.05$, compared with respective saline group (+ saline or valproate); (*) $0.10 > p > 0.05$, compared with respective saline group (+ saline or valproate)

Acute sodium valproate administration

Figure 2 shows the effect of methylphenidate and acute sodium valproate on locomotor activity in the open-field. An ANOVA indicated a significant effect on total locomotion ($F_{7,61} = 7.667$, $p < 0.0001$), central locomotion ($F_{7,61} = 2.447$, $p < 0.05$), and peripheral locomotion ($F_{7,61} = 6.045$, $p < 0.0001$). Methylphenidate increased the total locomotion in all groups (all $p < 0.05$), independent of pretreatments. Methylphenidate significantly increased central locomotion in mice pretreated with valproate 300 mg/kg and trended towards an increase in central locomotion ($0.10 > p > 0.05$) in mice pretreated with saline and valproate 100 mg/kg. No significant difference was observed in mice treated with methylphenidate + sodium valproate 200 mg/kg compared with saline + sodium valproate 200 mg/kg. A significant increase in peripheral locomotion was observed in mice treated with methylphenidate + valproate (all $p < 0.05$).

Effects of repeated treatment with $MgSO_4$ and sodium valproate on methylphenidate-induced hyperlocomotion

Figure 3 shows the effects of repeated administration (14 days) of $MgSO_4$ (400 mg/kg) and sodium valproate (200 mg/kg) on methylphenidate-induced hyperlocomotion. An ANOVA indicated a significant effect of this treatment on total locomotion ($F_{5,27} = 5.554$, $p < 0.01$), central locomotion ($F_{5,27} = 6.189$, $p < 0.001$), and peripheral locomotion ($F_{5,27} = 4.985$, $p < 0.01$). Methylphenidate increased the total locomotion in saline- and $MgSO_4$-treated mice (both $p < 0.05$), an effect that was prevented by valproate administration. Methylphenidate also increased central locomotion in saline- and $MgSO_4$-treated mice (both $p < 0.05$), but not in valproate-treated mice. The only significant difference observed on peripheral locomotion was an increase in spaces crossed in the $MgSO_4$ + methylphenidate group compared with the $MgSO_4$ + saline group ($p < 0.01$).

Discussion

The main finding of the present study was that acute $MgSO_4$ blocked the methylphenidate-induced increase in locomotor activity in the open-field test in mice. This effect was observed at a dose of $MgSO_4$ that alone did not alter locomotor activity ($MgSO_4$ + saline group compared with saline + saline group).

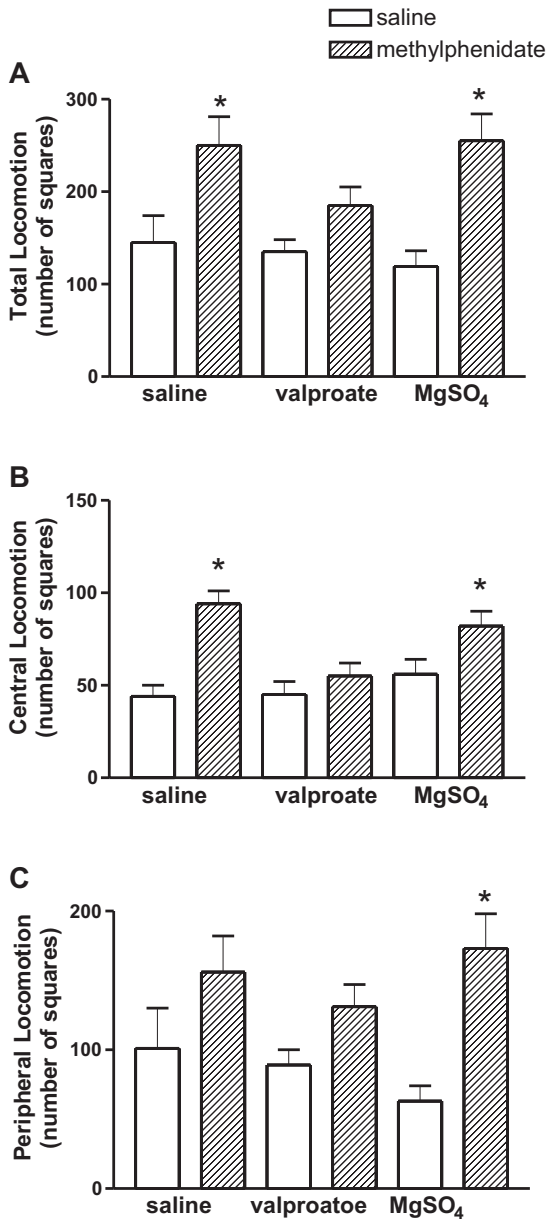

Fig. 3. Effects of repeated administration (14 days) of sodium valproate (200 mg/kg, *ip*) or magnesium sulfate ($MgSO_4$, 400 mg/kg, *ip*) on hyperlocomotion induced by methylphenidate (5.0 mg/kg, *sc*) in the open-field test in mice. Data are expressed as the mean ± SEM (n = 5–7/group) of (**A**) total locomotion, (**B**) central locomotion, and (**C**) peripheral locomotion. * $p < 0.05$, compared with respective saline group (+ saline, valproate or $MgSO_4$)

This profile suggests a putative antimanic-like effect, which is consistent with clinical studies that employed an add-on schedule and small sample size [16, 18]. However, the locomotor effects were not seen after repeated $MgSO_4$ treatment. The reduction of antimanic-like effects with repeated administration could be attributable to tolerance to the antimanic-like effect or to the specific experimental conditions of this model. Supporting the tolerance hypothesis, Poleszak et al. [27] observed tolerance to the antidepressant-like effects in the forced swim test in rats, although this effect was not seen in mice [23]. The use of a model of mania that does not employ psychostimulant treatment could help clarify this issue. Nonetheless, the results of acute treatment in the present study are consistent with the clinical data.

In contrast to the present results, Kantak and Adlerstein [20] found increased apomorphine-induced stereotypy and L-amphetamine-induced hyperlocomotion, effects seen with Mg^{2+} doses (anhydrous $MgCl_2$ 30–125 mg/kg and $MgSO_4$ 300–400 mg/kg) comparable to the present study. Mg^{2+} was previously shown to exert biphasic effects on aggressive behavior in mice in a resident-intruder paradigm (increase at low dose and decrease in high dose) [19]. This discrepancy may be attributable to the different sensitivities of the mouse lines or to the experimental procedures (e.g., time schedule or experimental chamber dimensions). Another possibility raised by Kantak and Adlerstein [20] is that reduced activity could be attributable to stereotypy induced by Mg^{2+} + methylphenidate. However, although not formally recorded in the present study, no overt stereotypic behavior in either the homecage or open-field was seen in mice in any group.

Several candidates may mediate the effects of Mg^{2+} on methylphenidate-induced hyperlocomotion, such as PKC, NMDA glutamate receptors, and Ca^{2+}. An interesting approach may be the one suggested by Szabo et al. [29], who showed that NMDA receptors may be a substrate for PKC in the pathophysiology of mania and that both may be targets of antimanic drugs in an amphetamine-induced hyperlocomotion model of mania. Thus, both PKC and NMDA may be the molecular targets for the $MgSO_4$ effects observed in the present study. Further behavioral studies using other pharmacological tools, such as D-serine, may clarify this issue.

Valproate, in contrast to $MgSO_4$, blocked the methylphenidate-induced increase in open-field activity only after repeated treatment. This result is consistent with previous studies that did not find any effects of acute valproate administration (75–300 mg/kg) on D-amphetamine-induced hyperlocomotion in mice [1] but a significant effect of valproate (200 mg/kg) after repeated treatment on the hyperlocomotion induced by repeated amphetamine administration in rats [15]. Eckerman et al. [11] found that sodium valproate (50

and 200 mg/kg) blocked the acute effects of methylphenidate (2.5 mg/kg). In an acute manic state, the onset of the clinical efficacy of valproate is seen only after 2–3 days of treatment [5]. Furthermore, lithium blocked the increased locomotor activity induced by methylphenidate [31]. Altogether, these data support the predictive validity of psychostimulant-induced hyperlocomotion as an animal model of mania [12]. In this model, increased activity in open areas (or in unprotected areas) induced by psychostimulants may be an index of increased risk-taking or poor judgment [12], a psychopathology frequently seen in mania. In the present study, however, the increase in central activity in the open-field induced by methylphenidate was found in the repeated $MgSO_4$ and valproate experiment, was marginal in the acute $MgSO_4$ experiment, and was absent in the acute valproate experiment. The inconsistent effects of methylphenidate on central activity could be attributable to the threshold dose used in the present study, suggesting that a full dose-response study may be necessary to clarify this issue. Nonetheless, in the repeated $MgSO_4$ or valproate administration experiment, methylphenidate increased central locomotion, an effect that was prevented by valproate treatment, supporting the hypothesis that locomotion in unprotected areas may be an index of risk-taking behavior.

In conclusion, the present results indicate that acute $MgSO_4$ exerts an antimanic-like effect on methylphenidate-induced hyperlocomotion and support future investigations of $MgSO_4$ as a potential treatment for acute mania.

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