

A synergistic interaction between magnesium sulphate and ketamine on the inhibition of acute nociception in rats

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Abstract. – OBJECTIVE: Magnesium is an endogenous voltage-dependent NMDA receptor-channel blocker and ketamine is a non-competitive NMDA receptor antagonist. Magnesium may potentiate the effect of ketamine in analgesia and anaesthesia, but may also interact in an opposing manner. This study aimed at evaluating type of the interaction between magnesium sulphate and ketamine administered systemically in rats with an acute nociceptive pain (tail-immersion test).

MATERIALS AND METHODS: Analgesic activity was assessed by tail-immersion test in male Wistar rats (200-250 g). The distal 5 cm of the tail was immersed in a warm water bath ($55 \pm 0.5^\circ\text{C}$) and the time for tail-withdrawal was measured as response latency.

RESULTS: Magnesium sulphate (2.5-30 mg/kg, s.c.) and ketamine (2.5-30 mg/kg, i.p.) administered alone did not produce any effect. However, significant antinociception (synergistic interaction) was revealed at the following doses of ketamine: magnesium sulphate of 5:5 mg/kg, 2.5:5 mg/kg and 10:5 mg/kg. The effect was not dose-dependent, and a greater response was obtained when ketamine was administered before magnesium sulphate.

CONCLUSIONS: This study revealed that (1) magnesium sulphate and ketamine given alone were not effective against acute nociceptive pain in rats, but (2) a combination of both drugs resulted in synergistically inhibited nociception, (3) which occurred only at selected low doses and proportions of the medications in a combination and (4) suggested the importance of the order of drug administration.

Key Words:

Magnesium, Ketamine, Antinociception, Rats, Tail-immersion.

Introduction

Magnesium is an endogenous voltage-dependent NMDA receptor-channel blocker^{1,2}. As a sole

drug magnesium demonstrated analgesic efficacy in different models of pain in animals^{3,4} and humans^{5,6}. Magnesium has also been demonstrated to enhance the effects of general^{7,8} and local anaesthetics^{9,10} in both preclinical and clinical studies.

Ketamine is a non-competitive NMDA receptor antagonist that binds to the phencyclidine site when the channels are in an open activated state¹¹ and may also bind to a second membrane-associated site, which decreases the frequency of channel opening¹². Moreover, ketamine is a dissociative anaesthetic that has analgesic properties in subanaesthetic doses¹³. Because of its low risk for cardiorespiratory depression, ketamine is an ideal medication for procedure-related pain and trauma¹⁴. However, ketamine is rarely used as a sole analgesic agent for postoperative pain control because it produces adverse psychotomimetic reactions, sedation and motor impairment¹⁴. In most instances, ketamine is used in combination with other injectable agents, like opioids^{15,16}.

According to the literature, magnesium may potentiate the effect of ketamine in analgesia^{17,18} and anaesthesia¹⁹, but may also interact in an opposing manner²⁰⁻²². Synergistic interaction between magnesium and ketamine has been demonstrated in an *in vitro* study which examined the inhibitory effect of ketamine-magnesium combination on the responses of recombinantly expressed NR1/NR2A and NR1/NR2B glutamate receptors²³. Up to today, synergistic interaction between magnesium and ketamine has not been documented in a whole-animal model of pain. Because ketamine and magnesium block NMDA receptor activation by distinct mechanisms of action, we hypothesized that in acute nociception, a combination of ketamine and magnesium might be more effective than ketamine alone. Therefore, the objective of the present study was to de-

termine the type of the interaction between magnesium sulphate and ketamine administered systemically in rats with an acute nociceptive pain and to determine the importance of the order of drugs administration.

Materials and Methods

Animals

The study was performed using 70 male Wistar rats (Military Farm, Belgrade, Serbia) weighting 200-250 g. Before the start of the experiments, the researchers requested and obtained permission from the Ethics Committee for Animal Research and Welfare of Faculty of Medicine, University of Belgrade (permit N° 5057/2). All of the experiments were approved by the Ethical Council for Protection of Experimental Animals of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, which operates in accordance with the Animal Welfare Law of the Republic of Serbia and the IASP (International Association for the Study of Pain) Guidelines for the Use of Animals in Research. The animals were housed in groups of 3 in Plexiglas cages (42.5 × 27 × 19 cm) under standard conditions: temperature of $22 \pm 1^\circ\text{C}$ and a 12/12 h light/dark cycle, with lights on at 08:00. Food and water were freely available, except during the experimental procedures. The animals were fed with standard rat pellets obtained from the Veterinary Institute Subotica, Serbia.

Before each experiment, the animals were habituated to the handling and experimental procedures for at least three consecutive days. The rats were allocated to experimental groups consisting of 6-8 animals per group. The experiments were conducted by the same experimenter on consecutive days, always at the same time of the day, between 08:00 and 14:00 to avoid diurnal variation in behavioural tests. The animals were unrestrained throughout, except during testing. The rats were individually removed from their housing for each test and were returned immediately afterward. During the measurements, the rats were restrained in Plexiglas holders for approximately 15 s. Each animal was used only once and was killed at the end of the experiments using an intraperitoneal injection of sodium thiopental (200 mg/kg).

Administration of Medications

The medications were administered intraperitoneally and subcutaneously as shown in Figure 1. We combined ketamine and magnesium sulphate and made the combinations of different doses and different proportions.

Ketamine (Inresa Arzneimittel GmbH, Freiburg, Germany) and magnesium sulphate (Zorka, Šabac, Serbia) were dissolved in 0.9% NaCl and injected intraperitoneally (ketamine) or subcutaneously (magnesium sulphate) at a final volume of 2 ml/kg. To test whether the 0.9% NaCl injection has any effect on antinociception, the same volume of 0.9% NaCl was administered to a control group of rats.

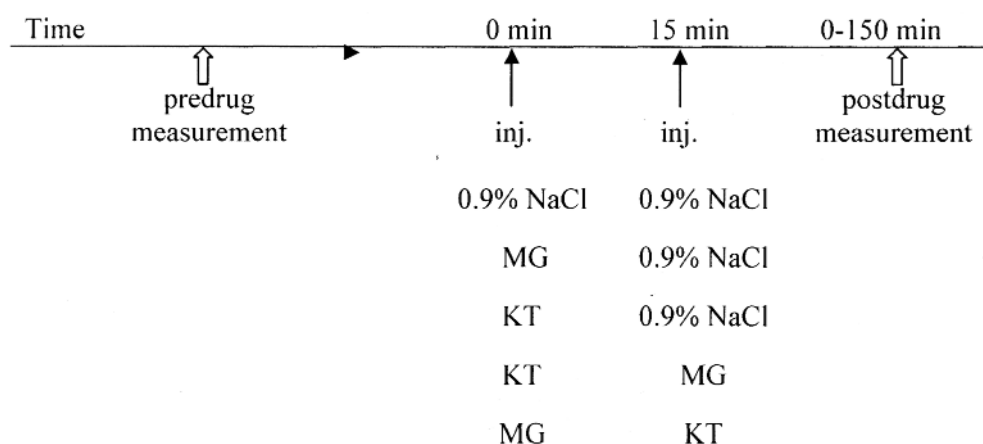


Figure 1. Experimental protocol. The effects of magnesium sulphate, ketamine and combinations of ketamine and magnesium sulphate on tail-withdrawal latencies in rats were evaluated. The control animals received the corresponding injections of 0.9% NaCl (NaCl) instead of test compounds. I.p., intraperitoneal; S.c., subcutaneous; Inj., injection.

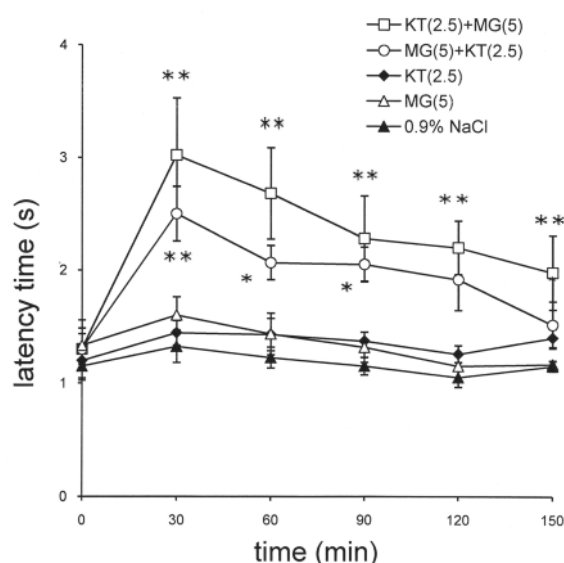


Figure 2. Time-response curves for the effects of combinations of ketamine (2.5 mg/kg) and magnesium sulphate (5 mg/kg) (0.5:1). Each point represents the mean $\pm$ SEM of the antinociceptive latency time in seconds (s) obtained in 6-8 rats. Statistical significance was found between the ketamine-magnesium sulphate combination and 0.9% NaCl and the magnesium sulphate-ketamine combination and 0.9% NaCl (** $p < 0.01$; * $p < 0.05$).

Tail-immersion Test in Rats

In the experiments, the antinociceptive activity was determined using a tail-immersion test^{24,25}. The rat was placed in a hemicylindrical Plexiglas cage with its tail hanging freely outside the cage. The distal 5 cm of the tail was immersed in a warm water bath ($55 \pm 0.5^\circ\text{C}$), and the time for tail-withdrawal was measured to the nearest 0.1 s. The animals that showed a tail-withdrawal response within 1-2 s were selected for the study. To minimise tissue damage by repeated testing, a cut-off time of 10 s was adopted. The pre-medication latency was determined from an average of two pre-medication determinations obtained with a 30-min interval. Post-medication latency was measured after the intraperitoneal (i.p.) injection of ketamine and/or magnesium sulphate (or the 0.9% NaCl in the control group) at 30, 60, 90, 120 and 150 min.

Statistical Analysis

The differences between the corresponding means in tail-withdrawal latency were verified using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test. A $p < 0.05$ was considered to be statistically significant.

Results

Synergistic Interaction Between Ketamine and Magnesium Sulphate in the Tail-Immersion Test in Rats

Administered alone, ketamine (2.5 mg/kg, 5 mg/kg, 10 mg/kg and 30 mg/kg) and magnesium sulphate (2.5 mg/kg, 5 mg/kg, 10 mg/kg and 30 mg/kg) did not produce any effect. The rats showed head weaving and circling behavior only at a dose of 30 mg/kg of ketamine.

Different doses of ketamine (2.5 mg/kg, 5 mg/kg, and 10 mg/kg) and magnesium sulphate (2.5 mg/kg, 5 mg/kg, and 10 mg/kg) and different proportions of these medications in combination (0.5:1, 1:1 and 2:1) were tested. Significant antinociception (synergistic interaction) was revealed at the following doses of ketamine: magnesium sulphate of 5:5 mg/kg, 2.5:5 mg/kg and 10:5 mg/kg (Figures 2 to 4). The combinations did not show a dose-dependent effect. Significant antinociception (synergistic interaction) was not obtained at the following doses of ketamine: magnesium sulphate of 2.5:2.5 mg/kg, 5:2.5 mg/kg, 5:10 mg/kg and 10:10 mg/kg (not shown).

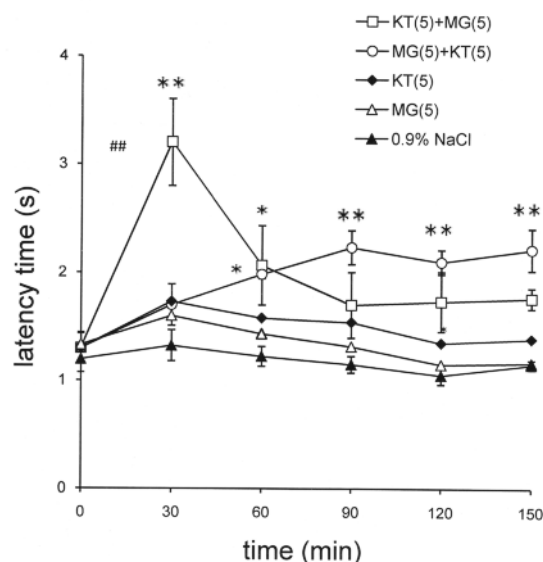


Figure 3. Time-response curves for the effects of combinations of ketamine (5 mg/kg) and magnesium sulphate (5 mg/kg) (1:1). Each point represents the mean $\pm$ SEM of the antinociceptive latency time in seconds (s) obtained in 6-8 rats. Statistical significance was found between the ketamine-magnesium sulphate combination and 0.9% NaCl and the magnesium sulphate-ketamine combination and 0.9% NaCl (** $p < 0.01$; * $p < 0.05$). Statistical significance was observed between combinations where ketamine was administered before and after magnesium sulphate (## $p < 0.01$).

Combinations of Ketamine (2.5 mg/kg) and Magnesium sulphate (5 mg/kg) (0.5:1)

The combinations produced significant antinociception, no matter whether ketamine (2.5 mg/kg) was administered before or after magnesium sulphate (5 mg/kg) (Figure 2). The order of medication administration had no significant influence on the magnitude of the response.

The effect of the ketamine-magnesium sulphate combination was significantly higher compared with ketamine alone and magnesium sulphate alone at time points of 30 min, 60 min, 90 min and 120 min ($p < 0.01$) and at time points of 30 min, 60 min, 90 min and 120 min ($p < 0.01$), respectively (not shown).

The effect of the magnesium sulphate-ketamine combination was significantly higher compared with ketamine alone and magnesium sulphate alone at time points of 30 min, 90 min, and 120 min ($p < 0.05$) and at time points of 90 min and 120 min ($p < 0.05$), respectively (not shown).

Combinations of Ketamine (5 mg/kg) and Magnesium Sulphate (5 mg/kg) (1:1)

Whether ketamine was given before or after magnesium sulphate, significant antinociception was achieved (Figure 3). However, significantly higher ($p < 0.01$) antinociception was obtained when ketamine (5 mg/kg, i.p.) was administered before rather than after magnesium sulphate (5 mg/kg, s.c.).

The antinociceptive effect of the ketamine-magnesium sulphate combination was significantly higher compared with ketamine magnesium sulphate alone. Statistical significance was found at a time point of 30 min ($p < 0.01$) (between ketamine-magnesium sulphate combination and ketamine) and at time points of 30 min and 150 min ($p < 0.01$) (between ketamine-magnesium sulphate combination and magnesium sulphate) (not shown).

The effect of the magnesium sulphate-ketamine combination was significantly higher compared with ketamine alone and magnesium sulphate alone at time points of 90 min ($p < 0.05$), 120 min and 150 min ($p < 0.01$) and at time points of 90 min, 120 min and 150 min ($p < 0.01$), respectively (not shown).

Combinations of Ketamine (10 mg/kg) and Magnesium Sulphate (5 mg/kg) (2:1)

There was no difference in maximum antinociception between the ketamine-magnesium sulphate and magnesium sulphate-ketamine combination

(Figure 4). However, both combinations produced a significant effect in comparison with 0.9% NaCl ($p < 0.05$).

The effect of the ketamine-magnesium sulphate combination was significantly higher than ketamine alone and magnesium sulphate alone at time points of 30 min, 60 min, 90 min ($p < 0.01$) and 120 min ($p < 0.05$) and at time points of 30 min, 60 min, 90 min ($p < 0.01$), 120 min and 150 min ($p < 0.05$), respectively (not shown).

Statistical significance was observed between magnesium sulphate-ketamine combination and ketamine/magnesium sulphate alone at time points of 60 min and 90 min ($p < 0.05$) and at time points of 60 min, 120 min and 150 min ($p < 0.05$), respectively (not shown).

There was no difference in basal tail-withdrawal latency between the tested groups ($p > 0.05$) (Figures 2 to 4).

Discussion

The major finding in the present study is a synergistic interaction between two NMDA antagonists, ketamine and magnesium sulphate, in an animal model of acute thermal nociception. At the doses employed, each of these agents is ineffective when given alone in the model of pain. However, their combinations yielded a signifi-

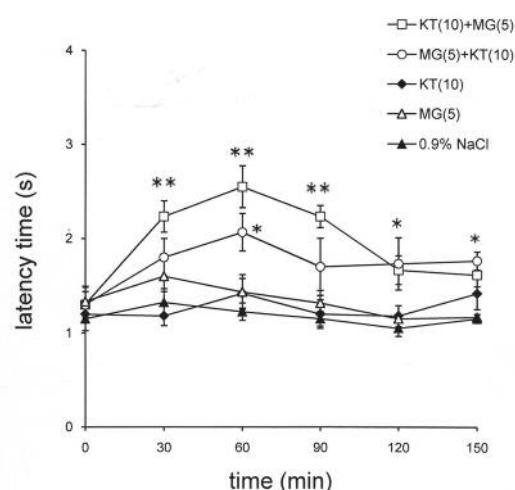


Figure 4. Time-response curves for the effects of ketamine (10 mg/kg) and magnesium sulphate (5 mg/kg) (2:1). Each point represents the mean $\pm$ SEM of the antinociceptive latency time in seconds (s) obtained in 6-8 rats. Statistical significance was found between the ketamine-magnesium sulphate combination and 0.9% NaCl and the magnesium sulphate-ketamine combination and 0.9% NaCl (** $p < 0.01$; * $p < 0.05$).

cant prolongation of tail-immersion latency, which suggested a synergistic interaction (we have consulted with Dr. Ronald J. Tallarida). For the first time, the efficacy of the ketamine-magnesium combination is reported to be influenced by the order of medication administration; a higher level of activity is demonstrated when ketamine is administered before magnesium sulphate.

Contrary to our findings, a study conducted by Gupta²⁶ reported that magnesium sulphate (5 mg/kg), administered alone, produced a transient increase in tail-flick latency measured by a radiant heat method in mice. The reason for this discrepancy may be attributed to the difference in thermal nociception test (tail-flick *vs.* tail immersion), as well as the species used. Consistent with our results, several previous studies showed that ketamine at doses up to 30 mg/kg produced no *per se* antinociception in rat tail-flick test and its excitatory effects limited the further increase of the dose^{15,27}.

Magnesium is recognised to block calcium influx and antagonise NMDA receptor channels²⁸, whereas ketamine binds to the phencyclidine binding site of NMDA receptors²⁹ and modifies them *via* allosteric mechanisms¹². Because ketamine and magnesium block the NMDA receptor activation by distinct mechanisms of action, it is not surprising that a synergistic pharmacodynamic interaction between the two agents exists. Beside NMDA blocking activity, both magnesium and ketamine possess several other mechanisms of action that may be responsible for the interaction too^{14,4}. Ketamine interacts with calcium and sodium channels, dopamine receptors, cholinergic transmission, noradrenergic and serotonergic re-uptake, together with opioid-related and anti-inflammatory effects¹⁴. Magnesium has been shown to reduce the activity of other presynaptic and postsynaptic calcium channels and to modulate the release of neurotransmitters. Magnesium also exhibits modulator effects on sodium and potassium currents, thus, influencing membrane potentials^{1,4}. Because ketamine and magnesium sulphate administered alone have no effects, isobolographic analysis could not be performed. Different doses of ketamine (2.5 mg, 5 mg, and 10 mg) and magnesium (2.5 mg, 5 mg, and 10 mg) and different proportions (0.5:1, 1:1 and 2:1) of medications in combination were tested. The activity of the combination was revealed only at doses of ketamine: magnesium sulphate of 2.5:5 mg, 5:5 mg, and 10:5 mg/kg, which indicated a

synergistic interaction; however, the effect did not show dose-dependence. We have recently reported that combinations containing magnesium sulphate/ketamine equal to or greater than 1:1 exert a synergistic lowering effect on body temperature in rats and that the combination effect is dose-dependent³⁰. Although the dose of ketamine:magnesium sulphate of 10:10 mg/kg produced a significant hypothermic response in a previous work, it lacked the effect on nociception in the present study, which suggested that tail-withdrawal latency is not significantly influenced by body temperature changes.

The reason that magnesium sulphate was effective in combination with ketamine at a dose of 5 mg/kg only has not been established. The interaction may be influenced by the doses of magnesium sulphate but not by the doses of ketamine or the relative proportion of the medications in the combination (0.5:1, 1:1, 2:1).

A pharmacokinetic interaction between ketamine and magnesium sulphate seems to be less likely because no pharmacokinetic interactions between systemic magnesium and other drugs have been reported¹.

Several whole-animal studies of ketamine-magnesium interaction have been conducted, with opposing results. In mice, magnesium injected intraperitoneally simultaneously potentiated ketamine anaesthesia (increases in the percentage of loss of the righting reflex, the sleeping time and the recovery time)¹⁹. In another study²⁰, young growing rats kept on a magnesium-deficient diet showed significantly longer sleeping times than the control rats. Similar to this study, Orser et al²¹ found that hypomagnesaemia in rats was associated with an increased sensitivity to ketamine as indicated by a decrease in the time to loss of righting reflex and a prolongation in the latency time to withdrawal to toe pinch. These two findings are consistent, in part, with our observation that the effect of ketamine-magnesium sulphate combination is stronger when ketamine is followed by magnesium sulphate, and weaker when magnesium sulphate is given before ketamine. It may be possible that magnesium ions given first keep channels blocked for a while, preventing the binding of ketamine. However, when ketamine is bound to phencyclidine binding site first, the increment of magnesium concentration may enhance the block. Considering the drug administration protocol used in this study, our data suggest that magne-

sium enhances the phencyclidine's site blockade promoted by ketamine. This possible mechanism could explain the fact that ketamine followed by magnesium administration was more effective than magnesium followed by ketamine administration. We did not measure the serum magnesium concentration, and are therefore unable to correlate it with our findings. However, in our study it depends on the dose of magnesium whether there would be interaction between ketamine and magnesium sulphate.

Similar to these results regarding analgesia, DeRossi et al¹⁷ showed that in sheep, the duration of analgesia using the lumbosacral epidural ketamine-magnesium sulphate combination was much longer than that obtained using ketamine or magnesium sulphate alone.

It should be pointed out that ketamine-magnesium sulphate combination for postoperative analgesia was also investigated in humans. A randomized, double-blind, placebo-controlled study³¹ did not demonstrate a decrease in pain or analgesic consumption in children undergoing tonsillectomy when pretreated with a small dose of ketamine and/or magnesium sulphate intravenously prior to the start of surgery. Recently, in a double-blind randomized controlled study²², a trial group of patients treated during induction of general anesthesia with a combination of magnesium sulphate and S(+)-ketamine showed a trend towards more opioid piritramide use postoperatively *via* patient controlled analgesia (PCA) device compared with ketamine alone.

The present report raised the question whether discrepancy in results from previous studies occurred because of differences in doses and the order of ketamine and magnesium sulphate administration as well. These results might at least partially explain why previous studies which tested the combination of ketamine and magnesium provided inconsistent results.

Conclusions

The present study demonstrated for the first time (1) a synergy between magnesium sulphate and ketamine against acute nociceptive pain, which (2) occurred only at selected low doses and proportions of the medications in a combination and (3) suggested the importance of the order of drug administration with higher activity when ketamine was given before magnesium sulphate.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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