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Synergistic interaction between ketamine and magnesium in lowering body temperature in rats

Sonja M. Vučković [a,*], Katarina R. Savić Vujović [a], Dragana P. Srebro [a], Branislava M. Medić [a], Čedomir S. Vučetić [b], Milan Š. Prostran [c], Milica Š. Prostran [a]

[a] Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[b] Institute for Orthopaedic Surgery and Traumatology, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[c] Department of Anatomy, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

HIGHLIGHTS

- Magnesium sulfate shows no influence on body temperature in rats.
- Ketamine reduces body temperature in rats in a dose-dependent manner.
- When given in combination there is a maximum of 5.3 fold reduction in dosage of ketamine.
- There is a synergy between ketamine and magnesium sulfate.

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ABSTRACT

A large body of evidence supports the existence of an endogenous glutamate system that tonically modulates body temperature *via* *N*-methyl-D-aspartate (NMDA) receptors. Ketamine and magnesium, both NMDA receptor antagonists, are known for their anesthetic, analgesic and anti-shivering properties. This study is aimed at evaluating the effects of ketamine and magnesium sulfate on body temperature in rats, and to determine the type of interaction between them. The body temperature was measured by insertion of a thermometer probe 5 cm into the colon of unrestrained male Wistar rats (200–250 g). Magnesium sulfate (5 and 60 mg/kg, sc) showed influence neither on baseline, nor on morphine-evoked hyperthermic response. Subanesthetic doses of ketamine (5–30 mg/kg, ip) given alone, produced significant dose-dependent reduction in both baseline colonic temperature and morphine-induced hyperthermia. Analysis of the log dose–response curves for the effects of ketamine and ketamine–magnesium sulfate combination on the baseline body temperature revealed synergistic interaction, and about 5.3 fold reduction in dosage of ketamine when the drugs were applied in fixed ratio (1:1) combinations. In addition, fixed low dose of magnesium sulfate (5 mg/kg, sc) enhanced the temperature lowering effect of ketamine (1.25–10 mg/kg, ip) on baseline body temperature and morphine-induced hyperthermia by factors of about 2.5 and 5.3, respectively. This study is the first to demonstrate the synergistic interaction between magnesium sulfate and ketamine in a whole animal study and its statistical confirmation. It is possible that the synergy between ketamine and magnesium may have clinical relevance.

1. Introduction

N-methyl-D-aspartate (NMDA) receptors are ligand-gated receptor complexes that have been implicated in many processes, such as learning and memory, pain transmission, schizophrenia, neurodegenerative disorders, brain damage following a stroke, depression, and thermoregulation [19,27,32].

Most studies have demonstrated that NMDA receptor antagonists produce hypothermia [30,45]. NMDA receptor immunoreactivity and high glutamate levels are present in hypothalamic regions where cells express glutamate receptor mRNA [3,55]. These data suggest that NMDA receptors are associated closely with thermosensitive neurons in the hypothalamus and support the existence of an endogenous glutamate system that tonically modulates body temperature *via* NMDA receptors [19]. Also, several lines of evidence suggest that glutamate is a key factor in morphine-induced hyperthermia and pharmacological antagonism of NMDA receptors blocks the hyperthermia caused by systemically administered morphine [44]. Ketamine is the most potent NMDA-receptor-

* Corresponding author at: Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, Dr Subotića-starijeg 1, P.O. Box 38, 11129 Belgrade, Serbia. Tel.: +381 11 3643 400; +381 64 1562 341; fax: +381 11 3643 397.
E-mail addresses: svuckovic@med.bg.ac.rs, sonyav@sbb.rs (S.M. Vučković).

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channel blocker available for clinical use, binding to the phencyclidine site when the channels are in the open activated state [35,43]. It also may bind to a second membrane-associated site, which decreases the frequency of channel opening [34]. Magnesium is a physiological noncompetitive NMDA receptor antagonist that blocks ion channels in a voltage-dependent manner [9,12]. Both ketamine and magnesium are well known for their anesthetic, analgesic and anti-shivering properties [13,29]. There are several reports on the effect of ketamine on body temperature [8,39] but the effect of magnesium has not been evaluated before. Literature data suggest that ketamine and magnesium may interact in additive, synergistic and antagonistic manner in producing antinociceptive or anesthetic action [22,31,33,51] but there are no reports on the interaction between these drugs in generating effect on body temperature.

In the present study, we used a body temperature assay to test the effects of ketamine and magnesium sulfate, as NMDA receptor antagonists on the baseline body temperature and morphine-evoked hyperthermia in rats, and to determine the type of possible interaction between them.

2. Materials and methods

2.1. Animals

Prior to the start of the experiments, researchers asked for and obtained the permission from the Ethics Committee for Animal Research and Welfare of Faculty of Medicine, University of Belgrade (permission no. 5057/2). All 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 Animal Welfare Law of our country and National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) for the use of animals in research.

A total of one hundred and seventy-four Wistar rats (200–250 g) obtained from Military Farm (Belgrade, Serbia) were used in the entire study. The animals were housed in groups of three in home cages (42.5 × 27 × 19 cm) under standard conditions: temperature of 22 ± 1 °C, relative humidity of 60% and a 12/12 h light/dark cycle. Food and water were freely available, except during the experimental procedure. Prior to each experiment the animals were habituated to the handling and experimental procedures for at least three consecutive days.

2.2. Temperature measurement

Experiments were done under a combination of artificial light and daylight in a sound-proofed room maintained at a temperature of 22 ± 1 °C. Body temperature experiments were always started between 0800 and 0900 h to minimize the effects of circadian variation. The animals were allowed to acclimatize for 60 min before the first temperature reading was taken. They were unrestrained all the time, except during testing. Rats were individually removed from their housing for each temperature testing and returned immediately afterward. During measurements rats were restrained in Plexiglas holders for about 15 s. This provided similar level of restraint and distress for each animal. Experimental groups consisted of 6–8 rats. Each animal was used only once.

The temperature was measured by the insertion of a thermometer probe (ALMEMO, Holzkirchen, Germany), about 5 cm into the colon. The colonic temperature of the rats was measured several times, and when the temperature was observed to be stable for approximately 2 h, drugs and/or 0.9% NaCl were injected intraperitoneally or subcutaneously [48]. The temperature was measured at 30, 60, 90, 120, 150, 180 and 210 min after the injection.

2.3. Drug administration

In the present study, three series of experiments were performed. The evaluation of the effects of morphine, magnesium sulfate and their combination (Fig. 1a) was followed by testing the influence of ketamine and the co-administration of ketamine and magnesium sulfate (Fig. 1b) on body temperature in rats. The third group of experiments tested the effect of ketamine with/without magnesium sulfate on morphine-evoked hyperthermia in rats (Fig. 1c), as well as the antagonism with naloxone (Fig. 1d).

Morphine hydrochloride (Alkaloid, Skopje, Macedonia), ketamine (InresaArzneimittel GmbH, Freiburg, Germany), magnesium sulfate (Zorka, Šabac, Serbia) and naloxone hydrochloride (Sigma-Aldrich Chemical Co., St Louis, Mo., USA) were dissolved in 0.9% NaCl and injected intraperitoneally (morphine and ketamine) or subcutaneously (magnesium sulfate and naloxone hydrochloride) at a final volume of 2 ml/kg. In order to test whether 0.9% NaCl injection has any effect on body temperature, the same volume of 0.9% NaCl was administrated in a control group of rats.

2.4. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) of colonic temperature for every measured time. Temperature values were statistically analyzed using analysis of variance (one-way ANOVA) followed by Tukey's HSD test. At the same time, in order to test the interaction between drugs, the magnitude of the temperature response as a mean ± SEM of peak change in body temperature (Δ·T·°C) relative to the control (0.9% NaCl) measured at any time [46] was calculated and then two log dose–response curves were constructed and compared by test for parallelism and relative potency test [54]. Potency ratio was considered statistically significant when 95% confidence limits (CL) did not overlap 1.0 ($p < 0.05$). The significance level in all tests was taken to be $p < 0.05$.

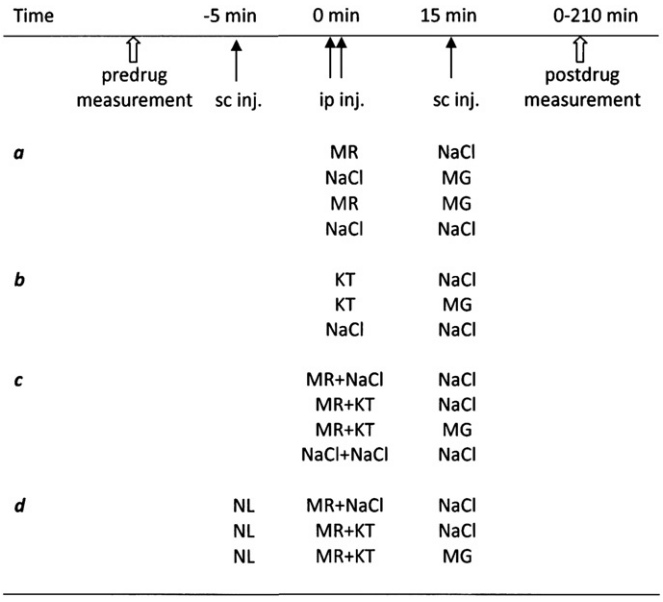

Fig. 1. Experimental protocol used in the evaluation of the effects of morphine (MR), magnesium sulfate (MG), and their combination (a), ketamine (KT) and ketamine–magnesium sulfate combination (b) morphine–ketamine and morphine–ketamine–magnesium sulfate combination (c) and the effect of naloxone (NL) on the effects of morphine, morphine–ketamine and morphine–ketamine–magnesium sulfate combination (d) on body temperature in rats. Control animals received the corresponding injections of 0.9% NaCl (NaCl) instead of test compounds. Ip, intraperitoneal. Sc, subcutaneous. Inj., injection.