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PARTICIPATION OF PERIPHERAL TRPV1, TRPV4, TRPA1 AND ASIC IN A MAGNESIUM SULFATE-INDUCED LOCAL PAIN MODEL IN RAT

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Abstract—We previously showed that magnesium sulfate (MS) has systemic antinociceptive and local peripheral pronociceptive effects. The role of transient receptor potential (TRP) channels and acid-sensing ion channels (ASICs) in the mechanism of action of MS has not been investigated in detail. The aim of this study was to explore the participation of TRP channels in the pronociceptive action of MS in rats after its intraplantar injection. The paw withdrawal threshold (PWT) to mechanical stimuli was measured by the electronic von Frey test. Drugs that were tested were either co-administered with an isotonic pH-unadjusted or pH-adjusted solution of MS intraplantarily, or to the contralateral paw to exclude systemic effects. We found that the subcutaneous administration of both pH-adjusted (7.4) and pH-unadjusted (about 6.0) isotonic (6.2% w/v in water) solutions of MS induce the pain at the injection site. The pH-unadjusted MS solution-induced mechanical hyperalgesia decreased in a dose-dependent manner as a consequence of co-injection of capsazepine, a selective TRPV1 antagonist (20, 100 and 500 pmol/paw), RN-1734, a selective TRPV4 antagonist (1.55, 3.1 and 6.2 μmol/paw), HC-030031, a selective TRPA1 antagonist (5.6, 28.1 and 140 nmol/paw), and amiloride hydrochloride, a non-selective ASIC inhibitor (0.83, 2.5 and 7.55 μmol/paw). In pH-adjusted MS-induced hyperalgesia, the highest doses of TRPV1, TRPV4 and TRPA1 antagonists displayed effects that were, respectively, either similar, less pronounced or delayed in comparison to the effect induced by administration of the pH-unadjusted MS solution; the ASIC antagonist did not have any effect. These results suggest that the MS-induced local peripheral mechanical hyperalgesia is mediated via modulation of the activity of peripheral TRPV1, TRPV4, TRPA1 and ASICs. Specific local inhibition of TRP channels represents a novel approach to treating local injection-related pain.

Key words: magnesium, pain at the site injection, TRPV1, TRPV4, TRPA1, ASIC.

INTRODUCTION

The magnesium ion is involved in different cellular processes, including regulation of metabolic pathways, signal transduction, cell proliferation, differentiation, apoptosis and angiogenesis (Wolf et al., 2007; Szewczyk et al., 2008; Gröber et al., 2015). It assumes important roles in controlling the activities of calcium, potassium and sodium ion channels, and in the release of neurotransmitters (acetylcholine, dopamine and glutamate) (Lin et al., 2002) at presynaptic terminals in the brain. Numerous preclinical and clinical studies have established that magnesium has beneficial effects in different medical conditions that are accompanied by pain and headache, as in dysmenorrhea, acute migraine and in certain neurological diseases (Alzheimer's and Parkinson's disease, stroke, traumatic brain injury, depression, etc.). In all these conditions, magnesium most probably participates with the central mechanism(s). However, little is known of its peripheral effect.

Although the analgesic effect of magnesium sulfate (MS) has been extensively studied in recent years, its pronociceptive effect remains to be elucidated. We have recently demonstrated that systemic MS exerts antihyperalgesia/antinociception in both somatic and visceral inflammatory pain (Srebro et al., 2014, 2016; Vuckovic et al., 2015) and that it has a pronociceptive effect in healthy animals when administered locally (Srebro et al., 2015).

The transient receptor potential (TRP) superfamily is comprised of a large number of cation channels that are mostly permeable to monovalent and divalent cations. TRPA1 (ankyrin type of TRP), TRPV1 and TRPV4 (vanilloid types of TRP) are found mainly in the nociceptive neurons of the peripheral nervous system, but also in the central nervous system, and are involved in the transmission and modulation of pain (Eid et al., 2008; Denadai-Souza et al., 2012; Uchytilova et al., 2014). TRPV4 is specifically activated under pathological conditions and does not contribute to baseline mechanical nociceptive thresholds. TRP melastatin (TRPM) types 6 and 7 are ion channels permeable to magnesium ions (Schlingmann et al., 2002; Monteilh-Zoller et al., 2003).

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Abbreviations: [d(g)], difference in pressures; AA%, analgesic activity; AITC, allyl isothiocyanate; ANOVA, analysis of variance; ASIC, acid-ion sensing channel; MS, magnesium sulfate; NMDA, *N*-methyl-D-aspartate receptors; NO, nitric oxide; PWT, paw withdrawal threshold; TRP, transient receptor potential; TRPA1, ankyrin 1; TRPM, TRP melastatin types 6 and 7; TRPV1, vanilloid 1; TRPV4, vanilloid 4.

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These channels are involved in magnesium reabsorption in the kidneys and intestine.

Acid-sensing ion channels (ASICs) are voltage-insensitive cationic channels that are activated only by extracellular changes in pH. ASICs are preferentially permeable to Na^+ but some are also permeable to cations such as Ca^{2+} and K^+. They are involved in the detection and transmission of mechanical pain in neuropathy, inflammation and other disease states (Ugawa et al., 2002; Jeong et al., 2013).

To determine the potential involvement of TRPV1, TRPV4, TRPA1 and ASIC in the modulation of the pronociceptive effect of MS, we tested the effects of capsazepine, a selective TRPV1 antagonist, RN-1734, a selective TRPV4 antagonist, HC-030031, a selective TRPA1 antagonist, and amiloride, a non-selective ASIC inhibitor, after the administration of pH-unadjusted and pH-adjusted isotonic solutions of MS.

EXPERIMENTAL PROCEDURES

Animals

All experimental procedures and animal care were conducted in accordance with the National Institutes of Health guidelines and were approved by the Ethics Committee for Animal Research and Welfare of the Faculty of Medicine, University of Belgrade (permit N° 5362/2) and approved by the Ethical Council for the Protection of Experimental Animals of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Experiments were designed to minimize the number of animals used and their discomfort.

The study was performed using two hundred and ten male Wistar rats (230–280 g; farm of the Military Medical Academy, Belgrade, Serbia) which were maintained at 22 ± 2 °C, on a 12/12-h light/dark cycle with food and water provided *ad libitum*. The animals were fed a standard rat pellet feed obtained from the Veterinary Institute Subotica, Serbia. The animals were housed in groups of three per cage (42.5 × 27 × 19 cm). Prior to each experiment the animals were habituated to handling and experimental procedures for at least three consecutive days. Rats were randomly divided into experimental groups consisting of six animals per group. The experiments were conducted by the same experimenter on consecutive days under constant laboratory conditions (temperature, light, humidity, quiet) and always at the same time of day (between 8:00 and 16:00 h), to avoid diurnal variations in the behavioral tests. Each animal was used only once and was killed at the end of the experiments with an intraperitoneal injection of sodium thiopental (200 mg/kg).

Induction of hyperalgesia

Peripheral hyperalgesia was induced after injection of 0.1 ml of isotonic MS (250 mmol/l or 6.2% w/v; 6.2 mg/paw) (Srebro et al., 2015) or capsaicin (30.5 μg/-paw) or allyl isothiocyanate (AITC) (50 μg/paw) into the plantar surface of the right hind paw.

Assessment of mechanical sensitivity

Mechanical sensitivity was assessed using von Frey filaments in the electronic pressure-meter test. The experimental procedure was based on the method described by Srebro et al. (2015). In brief, the rats were placed in individual Plexiglas boxes with a wire mesh platform. A 60-min period of acclimation was allowed before the test. Calibrated von Frey filaments (electronic von Frey Anesthesiometer, Model 2390, IITC Life Science, Woodland Hills, USA) were used to press upward on the mid plantar surface of the right hind paw for 5 s or until a withdrawal response occurred. The paw withdrawal threshold (PWT) to mechanical stimuli was measured at 0, 0.25, 0.5, 1, 2, 3, 4, 5 and 6 h after the intraplantar injection.

Calculations in the hyperalgesia

The intensity of hyperalgesia in each rat was quantified as the difference in pressures [d(g)] applied before (baseline) and after the injection of MS/capsaicin/AITC. Analgesic activity (AA%) for each rat was calculated according to the formula: AA% = (average d(g) in control group − d(g) of each rat in the test group)/(average d(g) in control group) × 100 (Srebro et al., 2014). The onset and cessation of the effect of the treatment were defined as the initial and last time points, respectively, when a statistically significant difference between the treated and control groups was observed for the PWT.

Drugs and administration

MS (Magnesio Solfato; S.A.L.F. Spa-Cenate Sotto-Bergamo, Italy), capsazepine (N-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carbothioamide; Sigma Aldrich, St. Louis, MO, USA), RN-1734 (2,4-dichloro-N-isopropyl-N-(2-isopropylaminoethyl) benzenesulfonamide; Tocris Bioscience, Bristol, UK), HC-030031 (2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl) acetamide; Sigma Aldrich, St. Louis, MO, USA), Amiloride hydrochloride hydrate (N-amidino-3,5-diamino-6-chloro pyrazinecarboxamide hydrochloride hydrate; Sigma Aldrich, St. Louis, MO, USA) dimethyl sulfoxide (DMSO; Tocris Bioscience, Bristol, Great Britain), AITC (Oil of mustard, Sigma Aldrich, St. Louis, MO, USA) and Capsaicin (Sigma Aldrich, St. Louis, MO, USA) were used. The purity of the chemicals was greater than 98%.

MS was dissolved in distilled water. Stock solutions of capsazepine, RN-1734, HC-030031, amiloride, AITC and capsaicin were made in DMSO. For all drugs, the final experimental dilutions were made in distilled water on the day of the experiment (the final concentration of DMSO was < 1%).

An isotonic MS solution (0.1 ml) that was pH-unadjusted (pH ∼ 6.0) and the same volume of a solution adjusted to pH = 7.4, were injected subcutaneously in the right hind paw of different rats. The antagonists were dissolved in the MS solutions and injected intraplantarly into the right hind paw in a final