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Inhibition of neuronal and inducible nitric oxide synthase does not affect the analgesic effects of NMDA antagonists in visceral inflammatory pain

Dragana Srebro*, Sonja Vučković, and Milica Prostran

Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia,
** Email: srebrodragana1@gmail.com*

Previously we described the antinociceptive effect of magnesium sulfate and dizocilpine (MK-801) in the visceral and somatic rat models of pain. In the somatic model of pain, we established the influence of selective inhibitors of neuronal and inducible nitric oxide synthase on the antihyperalgesic effects of magnesium sulfate and dizocilpine. Therefore, the objective of the present study was to determine in the rat model of visceral pain whether same mechanisms are involved in the antinociceptive action of magnesium sulfate and dizocilpine. Analgesic activity was assessed using the acetic acid-induced writhing test in rats. Subcutaneous injection of either magnesium sulfate (15 mg/kg) or dizocilpine (0.01 mg/kg) decreased the number of writhes by about 60 and 70%, respectively. The role of nitric oxide on the effects of magnesium sulfate and dizocilpine was evaluated using selective inhibitor of neuronal [N-ω-Propyl-L-arginine hydrochloride (L-NPA)] and inducible [S-methylisothiourea (SMT)] nitric oxide synthase, which *per se* did not affect the number of writhes. We observed that the antinociceptive effect of magnesium sulfate or dizocilpine did not change in the presence of L-NPA (2 and 10 mg/kg, i.p.) and SMT (0.015 and 10 mg/kg, i.p.). We conclude that, nitric oxide produced by neuronal and inducible nitric oxide synthase does not modulate the effects of magnesium sulfate and dizocilpine in the visceral inflammatory model of pain in the rat.

Key words: magnesium, dizocilpine, nitric oxide, acetic acid, writhing test, rat

INTRODUCTION

Intraperitoneal injection of acetic acid serves an animal model for acute and tonic visceral pain in the rat (Mogil et al. 1996). Acetic acid induces inflammation in the peritoneum, causing a writhing response. Writhing includes a stereotypic response pattern characterized by constrictions composed of abdominal contractions, twisting and turning of the trunk and extension of the hind limbs. A complex network of signaling molecules mediates the perception of visceral pain. The intraperitoneal injection of acetic acid is followed by the activation of chemosensitive nociceptor or irritation of the visceral surface, which leads to the release of histamine, bradykinin, prostaglandins and serotonin, which (García et al. 2004) produce the sensation of pain. The role of *N*-methyl-D-aspartate (NMDA) receptors and nitric oxide in the modulation of visceral pain has also been shown (Li et al. 2004, Willert et al. 2004, Strigo et al. 2005).

The writhing assay is a nonspecific but sensitive method widely used as a screening test to assess the analgesic or anti-inflammatory properties of new compounds (Le Bars et al. 2001). This test is sensitive to opioid and nonsteroidal anti-inflammatory drugs (NSAIDs) that act through a central and peripheral mechanisms. Given that the use of these drugs is accompanied by side effects, it is necessary to develop novel analgesics with less side effects. Previous experiments conducted in our laboratory demonstrated the analgesic effect of magnesium sulfate and dizocilpine (MK-801) in different models of nociception, such as the carrageenan-induced somatic (Srebro et al. 2014a, 2014b) and acetic acid-induced visceral inflammatory pain models (Vučković et al. 2015). However, the mechanisms responsible for the effects of magnesium and dizocilpine remain unknown. Nitric oxide is a controversial molecule with dual effects, pronociceptive and antinociceptive (Zhuo et al. 1993, Abacioğlu et al. 2000). There is evidence that inflammatory somatic and visceral pain is associated with nitric oxide (Zhuo et al. 1993, Abacioğlu et al. 2000). We proposed that nitric oxide and cyclic guanosine monophosphate (cGMP)/adenosine triphosphate (ATP)-sensitive K^+ channel (K^+ATP) pathways play an important role in the modulation of antihyperalgesic/antinociceptive effects of magnesium sulfate and dizocilpine in somatic and visceral models of pain. Using a model of visceral inflammatory pain in the rat,

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Correspondence should be addressed to D. Srebro
Email: srebrodragana1@gmail.com

our aim was to determine whether a nitric oxide-dependent mechanism is responsible for in the antinociceptive action of magnesium sulfate and dizocilpine in this model of pain in the rat. To that end, we used the selective inhibitor of neuronal [N-ω-Propyl-L-arginine hydrochloride (L-NPA)] and inducible [S-methylisothiourea (SMT)] nitric oxide synthase.

MATERIALS AND METHODS

Subjects

The experiments were carried out on 120 adult male Wistar rats (250–300 g) obtained from the Military Medical Academy (Belgrade, Serbia). The animals were housed in groups of three *per* cage (42.5×27×19 cm) under standard conditions of temperature (22±1°C), relative humidity (60%) and light/darkness (12 h, respectively) cycle. Food and water were available *ad libitum,* except during the experimental procedure. The animals were fed with standard rat pellet feed obtained from the Subotica Veterinary Institute, Serbia. All experiments were performed at the same time of day (between 8:00 and 16:00 h) to avoid diurnal variations in the behavioral tests. All experimental groups were comprised of 6 rats. Each animal was used only once and was killed at the end of the experiments with an i.p. injection of sodium thiopental (200 mg/kg).

Ethical statement

The research was approved by the Ethics Committee for Animal Research and Welfare of the Faculty of Medicine, University of Belgrade (permission N° 4946/2 and 5362/2). All experiments were 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, which operates in accordance with the Animal Welfare Law of Serbia and the European Community Council Directive for the Care and Use of Laboratory Animals from November 24, 1986 (86/609/EEC).

Experimental procedure

Experimental model of visceral nociception

The acetic acid-induced writhing test in rats served as the model for visceral nociception. The experimental procedure was performed as described previously (Al-Mamun et al. 2011, Vučković et al. 2015). Briefly, before the induction of writhing, the animals were placed in individual cages and acclimatized to the laboratory conditions. The writhing response was elicited by an intraperitoneal (i.p.) injection of 0.7% acetic acid solution (10 ml/kg body weight). The number of writhes was counted during a 20-min period, starting 5 min after administration of the acetic acid. The reduction in the number of abdominal contractions compared to the control was considered as an analgesic response.

Calculations used in the experimental model of visceral pain

The number of writhes in each treated group was compared with a control (saline-treated group); the treatments that produced a significant decrease in the number of writhing events were considered to be antinociceptive. Analgesic activity (AA%), expressed as the percentage decrease in the number of writhes in each rat was calculated according to the formula (Vučković et al. 2015): $\%AA=[(W_c-W_t)/W_c]\times100$, where W_c is the average number of abdominal contractions in the control group and W_t is the number of abdominal contractions observed in each rat in the tested group.

Drugs and their administration

Magnesium sulfate (Magnesio Solfato; S.A.L.F. Spa-Cenate Sotto-Bergamo, Italy), MK-801 [(+)-MK-801 hydrogen maleate, dizocilpine maleate; Sigma-Aldrich, St. Louis, MO, USA], N-ω-Propyl-L-arginine hydrochloride [L-NPA; Tocris Bioscience, Bristol, UK), S-methylisothiourea (SMT, Tocris Bioscience, Bristol, Great Britain) and acetic acid (pure refined 80% acetic acid; MSK Kikinda, Serbia) were dissolved in saline. All drugs used were injected at a final volume of 2 ml/kg (or 10 ml/kg for acetic acid].

The following protocol was used for drug administration: magnesium sulfate or MK-801 was administrated subcutaneously (s.c.) 15 min before the injection of acetic acid. L-NPA or SMT were administrated (i.p.) 10 min before the magnesium sulfate/dizocilpine. To test whether the injection of L-NPA or SMT had any effect on the number of writhes (analgesic activity) in the acetic acid-treated rats, the same volumes of these compounds were administered to a separate group of rats. Control rats in all groups received the corresponding volume of the vehicle.

Statistical analysis

The data are presented as mean difference in number of writhing ±standard error of the mean (SEM) obtained by testing in six rats. Differences between corresponding means were verified by using Kruskal-Wallis test, followed by Mann-Whitney (U) test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

The influence of L-NPA on the effect of magnesium sulfate in the acetic acid-induced writhing test

Magnesium sulfate administered subcutaneously (s.c.) at a dose of 15 mg/kg significantly (p=0.002) decreased the number of writhes movements by 59.9±7%. Doses of L-NPA (2 and 10 mg/kg, i.p.), which did not *per se* affect writhing, also did not affect (p>0.05) the antinociceptive effect of magnesium sulfate (15 mg/kg, s.c.) (Fig. 1).

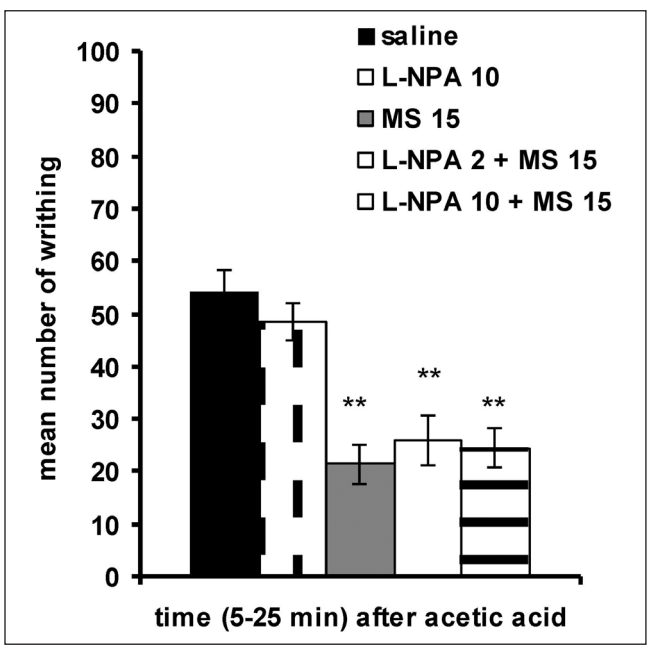

Fig. 1. Effect of L-NPA (2 and 10 mg/kg, i.p.) on the antinociceptive effect of magnesium sulfate (MS 15 mg/kg, s.c.) in acetic acid-induced writhing test. Each bar represents the mean ±SEM for a group of 6 rats, analyzed by Kruskal-Wallis test, followed by Mann-Whitney (U) test. **p<0.01 *vs*. saline group. s.c., subcutaneous, i.p., intraperitoneal.

The influence of SMT on the effect of magnesium sulfate in the acetic acid-induced writhing test

There was no statistical significance (p>0.05) between the antinociceptive effects of magnesium sulfate (15 mg/kg, s.c.) and magnesium sulfate in combination with SMT (0.015 and 10 mg/kg, i.p.) (Fig. 2). When compared with saline, magnesium sulfate given alone, or in combination with either 0.015 or 10 mg/kg SMT (i.p.) – significantly (p=0.002) decreased the number of writhes by 67.9±6.0, 61.1±7.0 and 62.1±6.9%, respectively. The same doses of SMT (0.015 and 10 mg/kg, i.p.) administered alone did not affect the number of writhes. SMT at a dose as high as 25 mg/kg (i.p.) significantly (p=0.021) reduced the number of writhes in rats by 52.5±10.9% (but it is not here).

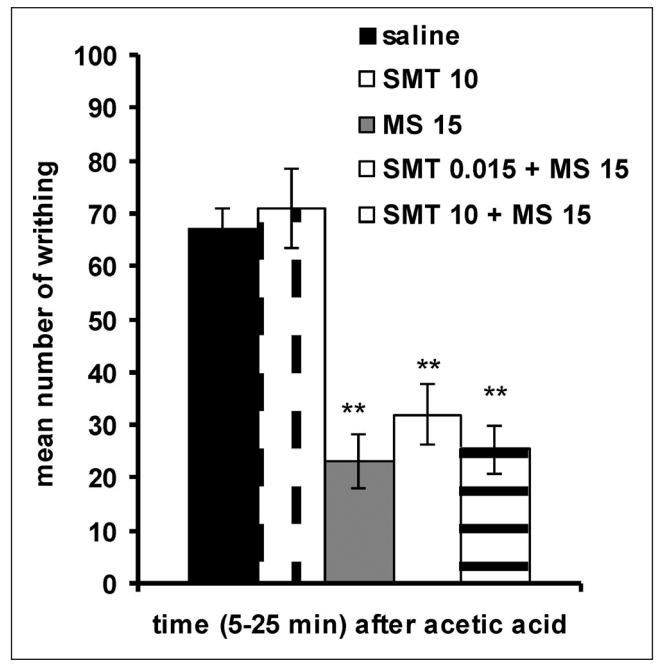

Fig. 2. Effect of SMT (0.015 and 10 mg/kg, i.p.) on the antinociceptive effect of magnesium sulfate (MS 15 mg/kg, s.c.) in acetic acid-induced writhing test. Each bar represents the mean ±SEM for a group of 6 rats, analyzed by Kruskal-Wallis test, followed by Mann-Whitney (U) test. **p<0.01 *vs*. saline group. s.c., subcutaneous, i.p., intraperitoneal.

The influence of L-NPA on the effect of dizocilpine in the acetic acid-induced writhing test

Dizocilpine, given alone or in combination with L-NPA, significantly (p=0.002) decreased the number of writhing in rats (Fig. 3). The antinociceptive effect of dizocilpine (0.01 mg/kg, s.c) did not change with L-NPA (2 and 10 mg/kg, i.p.), which did not *per se* cause any writhing. The antinociceptive effects in this combination were manifested as decreases in the number of writhes to 73.2±6 and 68.8±5.2%, respectively.

The influence of SMT on the effect of dizocilpine in the acetic acid-induced writhing test

As shown in Fig. 4, dizocilpine (0.01 mg/kg, s.c) significantly (p=0.002) decreased the number of writhes in rats by 71.8±5.8%. Dizocilpine, in combination with the same doses of SMT, had similar and significant (p=0.002) antinociceptive effects, manifested as decreases in the number of writhes to 70.9±6.8 and 75.7±4.3%. In control rats, SMT given alone at doses of 0.015 and 10 mg/kg – i.p. did not influence (p>0.05) the writhing episodes after injection of acetic acid in rats.

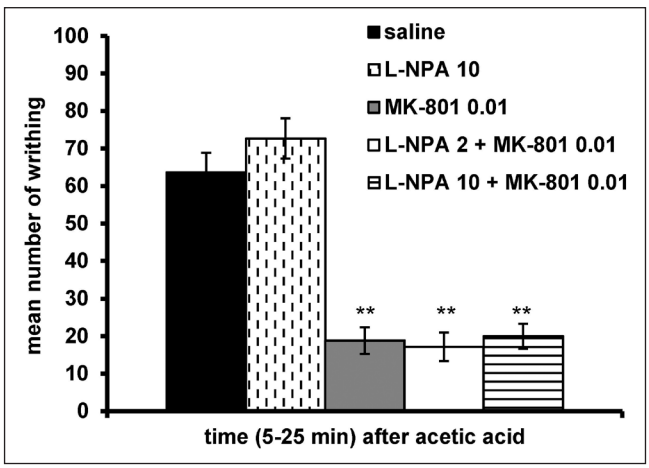

Fig. 3. Effect of L-NPA (2 and 10 mg/kg, i.p.) on the antinociceptive effect of dizocilpine (MK-801, 0.01 mg/kg, s.c.) in acetic acid-induced writhing test. Each bar represents the mean ±SEM for a group of 6 rats, analyzed by Kruskal-Wallis test, followed by Mann-Whitney (U) test. **p<0.01 *vs*. saline group. s.c., subcutaneous, i.p., intraperitoneal.

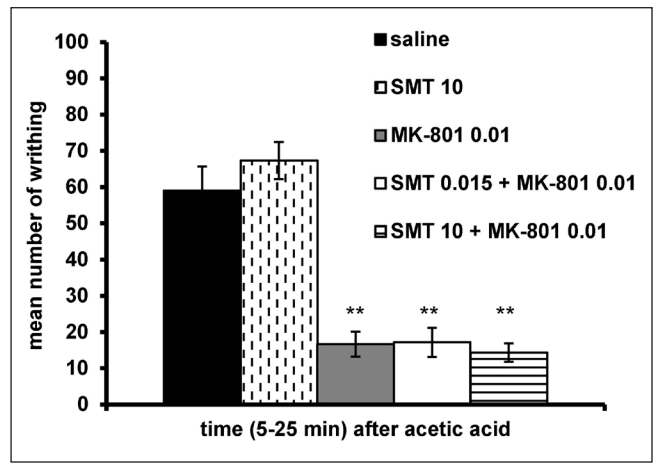

Fig. 4. Effect of SMT (0.015 and 10 mg/kg, i.p.) on the antinociceptive effect of dizocilpine (MK-801, 0.01 mg/kg, s.c.) in acetic acid-induced writhing test. Each bar represents the mean ±SEM for a group of 6 rats, analyzed by Kruskal-Wallis test, followed by Mann-Whitney (U) test. **p<0.01 *vs*. saline group. s.c., subcutaneous, i.p., intraperitoneal.

DISCUSSION

In this study, we observed that the antinociceptive effects of magnesium sulfate and dizocilpine in the visceral inflammatory model of pain were not reverted by the inhibition of neuronal and inducible nitric oxide synthase. This result is contrary to the finding we obtained in the somatic inflammatory model of pain.

The writhing test serves as model of acute visceral pain and has been suggested as a clinically relevant model for intestinal pain in humans (Reichert et al. 2001). We previously reported that both magnesium sulfate and dizocilpine produce an analgesic effect in the writhing test, without inducing central nervous system (CNS) side effects. Magnesium sulfate and dizocilpine displayed biphasic and linear dose-response patterns, respectively (Vučković et al. 2015). Dizocilpine is a non-competitive NMDA receptor antagonist and blocks NMDA-induced currents in a voltage-dependent manner, preventing the influx of calcium ions through the channel (Kovacic and Somanathan 2010). Magnesium, in addition to exerting other effects, is an endogenous, noncompetitive, voltage-dependent NMDA receptor-channel blocker (Fawcett et al. 1999, Herroeder et al. 2011). It has been documented that magnesium in different formulations (magnesium sulfate, magnesium oxide and magnesium oxide nanoparticles) can either produce or not produce an antinociceptive effect in the acetic acid-induced writhing test (Assi 2001, Jahangiri et al. 2013). Literature data suggest that both dizocilpine (Suardíaz et al. 2007) and ketamine (Bulutcu et al. 2002, Mohammad et al. 2012) decrease the number of writhes in a dose-dependent manner. Ketamine acts as a noncompetitive NMDA receptor antagonist (Øey 1998). We propose that the NMDA-dependent mechanism is involved in the antinociceptive effects of dizocilpine, and in part, of magnesium sulfate. This is in agreement with the view that NMDA receptors are peripherally and centrally expressed and involved in nociceptive visceral input processing (Li et al. 2004, Willert et al. 2004, Strigo et al. 2005). It has been shown that glutamate receptors are present in the stomach, duodenum and descending colon in the rat (Burns et al. 1994) and, that they play a role in intestinal motility and inflammation (Varga et al. 2010). Also, it was suggested that in hypothyroid rats changes in the distribution of NMDA receptors in the hippocampal neurons contributed to DNA damage and apoptosis and this effect is prevented by the dizocilpine (Alva-Sanchez et al. 2014). Since dizocilpine and magnesium block NMDA receptor activation by distinct mechanisms of action, and as dizocilpine is a NMDA antagonist, while magnesium has other mechanisms of action, it is expected that their effects are to a certain extent different (Salińska and Lazarewicz 1995). In the *in vivo* animal model of pain, we showed that magnesium activates the transient receptor potential ankyrin 1 (TRPA1) channels at the local peripheral level (Srebro et al. 2015). An electrophysiological study has shown that the activation the TRPA1 receptors in the spinal dorsal horn has an antinociceptive effect (Yamanaka et al. 2015). In accordance with this, we speculate that magnesium could activate TRPA1 receptors in the CNS, contributing to pain reduction of pain, while at the periphery it contributes to local hyperalgesia (Srebro et al. 2015). TRPA1 receptors are involved in the development of both somatic and visceral inflammatory pain (Eid et al. 2008, Pereira et al. 2013). HC-030031, a selective TRPA1 receptor antagonist, attenuated visceral nociception by

a mechanism independent of the modulation of nitric oxide and opioid pathways (Pereira et al. 2013).

It is known that nitric oxide is involved in somatic and visceral pain modulation, both in the CNS and peripheral nervous systems, in somatic and visceral pain (Bulutcu et al. 2002, Freire et al. 2009, Srebro et al. 2014a, 2014b). Nitric oxide plays both nociceptive and antinociceptive roles. Because the synthesis of nitric oxide by constitutive nitric oxide synthase enzymes is calcium- and calmodulin-dependent and NMDA receptors are permeable for calcium ions, it is possible that nitric oxide is activated through the NMDA receptors. In accordance with this preconditioning of the cerebellar granule cells in primary culture by 30-minute exposure to dizocilpine or memantine, NMDA receptor antagonists, might induce tolerance to event associate with glutamate-induced increase in intracellular calcium levels (Słomka et al. 2014). Previously, we found that nitric oxide modulates the antihyperalgesic effects of magnesium sulfate and dizocilpine in somatic pain in a different manner. Only at the highest tested doses did we observe a decrease of the antihyperalgesic effect of magnesium sulfate with the selective inhibitors of neuronal (L-NPA) and inducible (SMT) nitric oxide synthase (Srebro et al. 2014a). With inhibitor of neuronal nitric oxide synthase, we observed an increase in the antihyperalgesic effect of dizocilpine, whereas SMT, a selective inhibitor of the inducible nitric oxide synthase, lowered the antihyperalgesic effect of dizocilpine (Srebro et al. 2014b). In the present study, we examined how the same and higher doses of nitric oxide synthase inhibitors affect the antinociceptive effects of magnesium sulfate and dizocilpine in the visceral model of pain. Contrary to our expectations, the selective inhibitors of nitric oxide synthase had no influence on the antinociceptive effects of magnesium sulfate and dizocilpine. A possible explanation is that different mechanisms contribute to the antinociceptive effects of both magnesium sulfate and dizocilpine in somatic and visceral pain. Also, visceral and somatic pain exhibit differences in the neurobiological mechanisms that mediate the sensory process. Something can be similar because visceral pain can be "true" visceral pain and referred visceral pain which is "convergence-projection" combination of viscero-somatic neurons (Cervero 1985). Bearing in mind the differences between somatic and visceral pain models, we used higher doses of antagonists to examine the involvement of nitric oxide in the analgesic action of magnesium sulfate and dizocilpine. With a five-fold higher dose of L-NPA we did not observe a significant influence on the antinociceptive effects of either magnesium sulfate or dizocilpine. L-NPA is highly a selective and potent inhibitor of neuronal nitric oxide synthase, displaying 3158- and 149-fold higher selectivity on inducible and endothelial nitric oxide synthases, respectively. Also, the higher dose of SMT (10 mg/kg, i.p.) did not change the antinociceptive effects of magnesium sulfate and dizocilpine. In our previous study, SMT, an inducible nitric oxide synthase inhibitor, was effective at a later time-point than L-NPA (Srebro et al. 2014a, 2014b). It is possible that the short duration of the observation period is the reason why we obtained different results those reported in our previous study. In addition, it was shown that the selective inhibitor of inducible nitric oxide synthase produces an antinociceptive effect in the acetic-acid writhing test, suggestions that the nitric oxide produced by the inducible nitric oxide synthase plays a role in pain processing (LaBuda et al. 2006). It was shown that SMT has a dose-dependent analgesic effect in the formalin-induced pain model as soon as 15–30 min after formalin injection (Bhat et al. 2008). Moreover, the dose of SMT (25 mg/kg, i.p.) that did not reduce the late phase (15–30 min) of formalin-induced pain behavior (Bhat et al. 2008), induced a 50% reduction in the number of writhes in the model used herein.

We previously reported that L-NAME, a non-selective inhibitor of nitric oxide synthase, does not reduce the antinociceptive effect of magnesium sulfate in the same visceral pain model (Vučković et al. 2015). However, L-NAME eliminates the antihyperalgesic effect of dizocilpine (Vučković et al. 2015). In light of the presented finding, the most likely explanation for the inhibitory role of L-NAME on the antinociceptive effect of dizocilpine, as, reported in our previous study (Vučković et al. 2015), is that L-NAME decreased the delivery of dizocilpine to the brain, perhaps as a result of the effect of L-NAME-induced alterations in blood flow. Mueller and Hunt (1998) reported that L-NAME, due to pharmacokinetic interaction, decreased the delivery of ketamine into rat brain and reduced behavioral response to ketamine. In addition, an *in vitro* study demonstrated that ketamine had no direct effect on nitric oxide synthase activity (Tobin et al. 1994). As regards magnesium sulfate, it was reported that it increases the velocity of cerebral blood flow (Ludbrook et al. 1999) and significantly reduces L-NAME-induced hypertension in pregnant rats (Standley et al. 2006).

The present study shows that in an acetic acid-induced visceral pain model in the rat, the analgesic effects of magnesium, a non-typical NMDA antagonist, and dizocilpine, a referent NMDA antagonist, do not depend on nitric oxide production.

CONCLUSIONS

Research efforts have focused on investigations of analgesic drugs that may serve as be alternatives to NSAIDs and opiates which produce unwanted side effects. Magnesium sulfate and dizocilpine could be used as adjuvant analgesics. Dizocilpine is not used clinically

because of its side effects. It is used as a reference drug in the investigations of the NMDA receptors. The exact mechanisms involved in the antinociceptive effect of magnesium sulfate or dizocilpine in the visceral model of inflammatory pain are not known. Here, we have shown that nitric oxide produced by neuronal and inducible nitric oxide synthase does not modulate their effects in the visceral inflammatory model of pain. More experiments are needed to clarify their mechanisms.

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REFERENCES

Abacioğlu N, Tunçtan B, Akbulut E, Cakici I (2000) Participation of the components of L-arginine/nitric oxide/cGMP cascade by chemically-induced abdominal constriction in the mouse. Life Sci 67: 1127–1137.

Al-Mamun Mst, Hajera khatun Md, Rafikul Islam, Laizuman Nahar K, M Shams-ud-doha, Farhana Alam Ripa (2011) Evaluation of CNS depressant and analgesic activities of methanol extract of Piper longum Linn. Leaves. Int J Pharm Sci Res 2: 2874–2879.

Alva-Sanchez C, Rodriguez A, Villanueva I, Anguiano B, Aceves C, Pacheco-Rosado J (2014) The NMDA receptor antagonist MK-801 abolishes the increase in both p53 and Bax/Bcl2 index induced by adult onset hypothyroidism in rat. Acta Neurobiol Exp (Wars) 74: 111–117.

Assi AA (2001) The influence of divalent cations on the analgesic effect of opioid and non-opioid drugs. Pharmacol Res 43: 521–529.

Bhat AS, Tandan SK, Kumar D, Krishna V, Prakash VR (2008) The interaction between inhibitors of nitric oxide synthase and cyclooxygenase in formalin-induced pain in mice: an isobolographic study. Anesth Analg 106: 978–984.

Bulutcu F, Dogrul A, Güç MO (2002) The involvement of nitric oxide in the analgesic effects of ketamine. Life Sci 71: 841–853.

Burns GA, Stephens KE, Benson JA (1994) Expression of mRNA for the N-methyl-D-aspartate (NMDAR1) receptor by the enteric neurons of the rat. Neurosci Lett 170: 87–90.

Cervero F (1985) Visceral nociception: peripheral and central aspects of visceral nociceptive systems. Philos Trans R Soc Lond B Biol Sci 308: 325–337.

Eid SR, Crown ED, Moore EL, Liang HA, Choong KC, Dima S, Henze DA, Kane SA, Urban MO (2008) HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol Pain 4: 48.

Fawcett WJ, Haxby EJ, Male DA (1999) Magnesium: physiology and pharmacology. Br J Anaesth 83: 302–320.

Freire MA, Guimarães JS, Leal WG, Pereira A (2009) Pain modulation by nitric oxide in the spinal cord. Front Neurosci 3: 175–181.

García MD, Fernández MA, Alvarez A, Saenz MT (2004) Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of Pimenta racemosa var. ozua (Mirtaceae). J Ethnopharmacol 91: 69–73.

Herroeder S, Schönherr ME, De Hert SG, Hollmann MW (2011) Magnesium--essentials for anesthesiologists. Anesthesiology 114: 971–993.

Jahangiri L, Kesmati M, Najafzadeh H (2013) Evaluation of analgesic and anti-inflammatory effect of nanoparticles of magnesium oxide in mice with and without ketamine. Eur Rev Med Pharmacol Sci 17: 2706–2710.

Kovacic P, Somanathan R (2010) Clinical physiology and mechanism of dizocilpine (MK-801): electron transfer, radicals, redox metabolites and bioactivity. Oxid Med Cell Longev 3: 13–22.

LaBuda CJ, Koblish M, Tuthill P, Dolle RE, Little PJ (2006) Antinociceptive activity of the selective iNOS inhibitor AR-C102222 in rodent models of inflammatory, neuropathic and post-operative pain. Eur J Pain 10: 505–512.

Le Bars D, Gozariu M, Cadden SW (2001) Animal models of nociception. Pharmacol Rev 53: 597–652.

Li J, McRoberts JA, Nie J, Ennes HS, Mayer EA (2004) Electrophysiological characterization of N-methyl-D-aspartate receptors in rat dorsal root ganglia neurons. Pain 109: 443–452.

Ludbrook GL, James MF, Upton RN (1999) The effect of magnesium sulfate on cerebral blood flow velocity, cardiovascular variables, and arterial carbon dioxide tension in awake sheep. J Neurosurg Anesthesiol 11: 96–101.

Mogil JS, Kest B, Sadowski B, Belknap JK (1996) Differential genetic mediation of sensitivity to morphine in genetic models of opiate antinociception: influence of nociceptive assay. J Pharmacol Exp Ther 276: 532–544.

Mohammad FK, Al-Baggou BKh, Naser AS (2012) Antinociception by metoclopramide, ketamine and their combinations in mice. Pharmacol Rep 64(2): 299–304.

Mueller RA, Hunt R (1998) Antagonism of ketamine-induced anesthesia by an inhibitor of nitric oxide synthesis: a pharmacokinetic explanation. Pharmacol Biochem Behav 60: 15–22.

Øey I (1998) Ketamine analgesia, NMDA receptors and the gates of perception. Acta Anaesthesiol Scand 42: 747–749.

Pereira LM, Lima-Júnior RC, Bem AX, Teixeira CG, Grassi LS, Medeiros RP, Marques-Neto RD, Callado RB, Aragão KS, Wong DV, Vale ML, Brito GA, Ribeiro RA (2013) Blockade of TRPA1 with HC-030031 attenuates visceral nociception by a mechanism independent of inflammatory resident cells, nitric oxide and the opioid system. Eur J Pain 17: 223–233.

Reichert JA, Daughters RS, Rivard R, Simone DA (2001) Peripheral and preemptive opioid antinociception in a mouse visceral pain model. Pain 89: 221–227.

Salińska E, Lazarewicz JW (1995) Modulation of NMDA receptor-mediated release of [3H]arachidonate in hippocampal slices of immature rats. Acta Neurobiol Exp (Wars) 55: 11–21.

Słomka M, Kuszczyk M, Łazarewicz JW, Makarewicz D (2014) NMDA receptor antagonists MK-801 and memantine induce tolerance to oxygen and glucose deprivation in primary cultures of rat cerebellar granule cells. Acta Neurobiol Exp (Wars) 74: 396–404.

Srebro DP, Vučković SM, Savić Vujović KR, Prostran MS (2014b) Nitric oxide synthase modulates the antihyperalgesic effect of the NMDA receptor antagonist MK-801 on Carrageenan-induced inflammatory pain in rats. Tohoku J Exp Med 234: 287–293.

Srebro DP, Vučković SM, Savić Vujović KR, Prostran MŠ (2015) TRPA1, NMDA receptors and nitric oxide mediate mechanical hyperalgesia induced by local injection of magnesium sulfate into the rat hind paw. Physiol Behav 139: 267–273.

Srebro DP, Vučković S, Vujović KS, Prostran M (2014a) Anti-hyperalgesic effect of systemic magnesium sulfate in carrageenan-induced inflammatory pain in rats: influence of the nitric oxide pathway. Magnes Res 27: 77–85.

Standley CA, Batia L, Yueh G (2006) Magnesium sulfate effectively reduces blood pressure in an animal model of preeclampsia. J Matern Fetal Neonatal Med 19: 171–176.

Strigo IA, Duncan GH, Bushnell MC, Boivin M, Wainer I, Rodriguez Rosas ME, Persson J (2005) The effects of racemic ketamine on painful stimulation of skin and viscera in human subjects. Pain 113: 255–264.

Suardíaz M, Estivill-Torrús G, Goicoechea C, Bilbao A, Rodríguez de Fonseca F (2007) Analgesic properties of oleoylethanolamide (OEA) in visceral and inflammatory pain. Pain 133: 99–110.

Tobin JR, Martin LD, Breslow MJ, Traystman RJ (1994) Selective anesthetic inhibition of brain nitric oxide synthase. Anesthesiology 81: 1264–1269.

Varga G, Erces D, Fazekas B, Fülöp M, Kovács T, Kaszaki J, Fülöp F, Vécsei L, Boros M (2010) N-Methyl-D-aspartate receptor antagonism decreases motility and inflammatory activation in the early phase of acute experimental colitis in the rat. Neurogastroenterol Motil 22: 217–225.

Vučković S, Srebro D, Savić Vujovic K, Prostran M (2015) The antinociceptive effects of magnesium sulfate and MK-801 in visceral inflammatory pain model: The role of NO/cGMP/K(+)ATP pathway. Pharm Biol 53: 1621–1627.

Willert RP, Woolf CJ, Hobson AR, Delaney C, Thompson DG, Aziz Q (2004) The development and maintenance of human visceral pain hypersensitivity is dependent on the N-methyl-D-aspartate receptor. Gastroenterology 126: 683–692.

Yamanaka M, Taniguchi W, Nishio N, Hashizume H, Yamada H, Yoshida M, Nakatsuka T (2015) In vivo patch-clamp analysis of the antinociceptive actions of TRPA1 activation in the spinal dorsal horn. Mol Pain 21: 11–20.

Zhuo M, Meller ST, Gebhart GF (1993) Endogenous nitric oxide is required for tonic cholinergic inhibition of spinal mechanical transmission. Pain 54: 71–78.