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Magnesium ions and opioid agonists in vincristine-induced neuropathy

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Abstract:
Neuropathic pain is difficult to treat. Classic analgesics (i.e., opioid receptor agonists) usually possess low activity. Therefore other agents such as antidepressants, anticonvulsants, and corticosteroids are used. It is commonly known that NMDA antagonists increase analgesic activity of opioids. Unfortunately, clinical use of NMDA antagonists is limited because of the relatively frequent occurrence of adverse effects e.g., memory impairment, psychomimetic effects, ataxia and motor in-coordination. Magnesium ions (Mg^{2+}) are NMDA receptor blockers in physiological conditions. Therefore, in this study the effect of opioid receptor agonists and the influence of Mg^{2+} on the action of opioid agonists in vincristine-induced hyperalgesia were examined. Opioid agonists such as morphine (5 mg/kg, *ip*), and fentanyl (0.0625 mg/kg, *ip*), as well as the partial agonist buprenorphine (0.075 mg/kg, *ip*) administered alone on 5 consecutives days did not modify the hyperalgesia in vincristine rats. In contrast, pretreatment with a low dose of magnesium sulfate (30 mg/kg, *ip*) resulted in a progressive increase of the analgesic action of all three investigated opioids. After discontinuation of drug administration, the effect persisted for several days.

Key words:
neuropathy, hyperalgesia, magnesium ions, opioids, rats, vincristine, chemotherapy

Abbreviations: Mg^{2+} – magnesium ions, NMDA – N-methyl-D-aspartate

Introduction

Morphine and other opioid agonists are recommended as the drugs of choice in the management of patients with severe pain, particularly cancer pain.

However, under certain conditions (e.g., neuropathic pain), the effectiveness of opioids is much smaller than expected [2, 11]. In the previous study, we showed that opioid receptor agonists such as morphine and fentanyl, as well as the partial agonist buprenorphine, had little effect on streptozotocin-induced hyperalgesia in a diabetic neuropathy model in rats [7]. Insensitivity of diabetic neuropathic pain to opioid analgesics is difficult to explain. However, some authors have suggested that activation of N-methyl-D-aspartate (NMDA) receptors or the increase in the release of excitatory amino acids may lead to reduced sensitivity to morphine in some types of neuropathic pain [27].

It was demonstrated that NMDA receptor antagonists can alleviate pain in different experimental neuropathy models [5, 22]. Moreover, NMDA receptor antagonists in combination with opioids have been

shown to be more effective than those administered alone [5, 12, 13, 17, 18]. It was reported that spinal administration of the NMDA receptor channel blocker, MK-801, enhanced the ability of morphine to reverse hyperalgesia and allodynia in a nerve injury model [20, 29]. Christensen et al. [8] showed that concomitant systemic administration of the NMDA/glycine receptor antagonist, (+)-HA966, and morphine diminished the mechanical allodynia in the chronic constriction injury model. The nature of these interactions remains unclear. However, in electrophysiological and receptor binding studies, Yamakura et al. [28] suggested that the various opioid compounds (e.g., morphine, fentanyl, and codeine) might antagonize the activity of NMDA receptors. This inhibition is a result of channel-block mechanisms at the site, which partially overlaps with the inhibitory activity of magnesium ions (Mg^{2+}).

Under physiological conditions, Mg^{2+} are NMDA receptor blockers. Therefore, it has been suggested that Mg^{2+} could cause a similar effect as NMDA receptor antagonists [3, 10, 26, 27].

In the previous study we showed that a pretreatment with magnesium sulfate markedly enhanced the analgesia of morphine, fentanyl, and buprenorphine in a streptozotocin-induced diabetic neuropathy model in rats [7].

Since neuropathic pain accompanies anticancer chemotherapy treatment, in this study the experimental model of neuropathic pain caused by administration of the chemotherapeutic agent vincristine was used.

In the present paper, the influence of Mg^{2+} on the action of three opioids (morphine, fentanyl, and buprenorphine) in a vincristine-induced neuropathy model was investigated.

Materials and Methods

Animals

The study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw. The aforementioned Committee approved the experimental protocols. Male Wistar rats (260–320 g) were housed in a room maintained at a temperature of 20 ± 2°C, under 12 h–12 h light – dark cycles. Experimental groups consisted of six rats. The animals had free access to food and water.

Drugs

Vincristine sulfate was purchased from Sigma Chemical Co., USA; morphine sulfate, fentanyl, buprenorphine hydrochloride, magnesium sulfate were from Polfa, Warszawa, Poland.

Vincristine was dissolved in distilled water to a stock concentration of 1 mg/ml and stored at 4°C. Prior to administration, the stock was diluted in distilled water to a concentration of 70 μg/ml. This solution was administered into the tail vein (*iv*) at a dose of 70 μg/kg. Morphine, fentanyl, buprenorphine were dissolved in 0.9% saline immediately before injection and applied intraperitoneally (*ip*). Morphine was applied at a dose of 5.0 mg/kg, while fentanyl and buprenorphine were administered at doses that produced similar analgesic effects in naive rats (fentanyl – 0.0625 mg/kg, buprenorphine – 0.075 mg/kg). Magnesium sulfate was applied at a dose of 30 mg/kg (*ip*). Doses of analgesics were based on literature data [25] and were verified in our laboratory. Control animals were injected *ip* with 0.9% saline (control to morphine, fentanyl, buprenorphine, and magnesium sulfate) according to the same time schedule.

Chemotherapy (vincristine) – induced painful neuropathy

Vincristine neuropathy was induced in a protocol modified from Aley et al. [1], which was also used in our previous study [6]. Administration of vincristine was performed daily – Monday through Friday – for 10 days (this phase of the experiment lasted 12 days, no doses of drug were given on Saturdays and Sundays). The dosage calculations were based on daily body weight. Weight-matched control rats received injections of distilled water.

No weight gain was observed in rats receiving *iv* vincristine at a dose of 70 μg/kg (this means the weight failed to increase in comparison to the control group of animals). After cessation of drug administration, the animals regained weight.

Time schedule

In the chemotherapy induced neuropathy model, opioids were applied for five consecutive days (from

day 8 to 12 of the course of the experiment) 10 minutes before vincristine administration. Magnesium sulfate was administered 30 minutes before administration of morphine, fentanyl, or buprenorphine.

Measurement of the nociceptive threshold

The changes in pain thresholds were determined using mechanical stimuli – a modification of the classic paw withdrawal test described by Randall and Selitto [23]. To perform a mechanical stimulation, a progressively increased pressure was applied to the dorsal surface of the rat's paw using an analgesymeter (Ugo-Basile Biological Research Apparatus, Comerio – Varese, Italy). The instrument increased a force on the rat's paw at a rate of 32 grams per second. The nociceptive threshold was defined as the force in grams at which the rat attempted to withdraw its hind paw. Values of the pressure were recorded at this moment. The nociceptive threshold was measured in duplicate and its mean was determined for further calculations.

Measurement of the nociceptive threshold in rats with vincristine – induced painful neuropathy

The mean of nociceptive thresholds to mechanical stimuli measured on day 1 of a 23 day long study prior to administration of vincristine alone or vincristine with investigated drugs constituted the baseline pain threshold (A).

Measurements of prolonged activity of investigated drugs were performed on five consecutive days (for example measurement following days after administration of drugs and before consecutive drugs administration) from day 9 to day 13 of experiment (B) and after drug discontinuation (from day 15 to day 23 of the experiment).

Nociceptive thresholds were also determined at 15, 30, 45, 60, 90, 120, and 180 min after administration of morphine or Mg^{2+} + morphine on the first and last days of drug administration (day 8 and 12 of experiment, respectively) (Figs. 1, 2).

In all experimental sessions, obtained thresholds (B) were compared to the baseline (A).

Changes in pain threshold were calculated as percentage of the baseline value according to the following formula:

$$\% \text{ of analgesia} = (\frac{B}{A} \times 100\%) - 100\%;$$

A – pressure (in g), baseline pain threshold; *B* – pressure (in g) in consecutive measurements.

Percentage of analgesia values calculated as above for individual animals were subsequently used to calculate average values in specific experimental groups and for statistical analysis.

Statistical analysis

The results were expressed as mean values ± standard error of the mean (SEM). The statistical significance of differences between groups was evaluated by the *t*-Students test and the Newman-Keuls multiple range test; $p \leq 0.05$ was accepted as statistically significant. All statistical calculations were performed using computer software described by Tallarida and Murray [24].

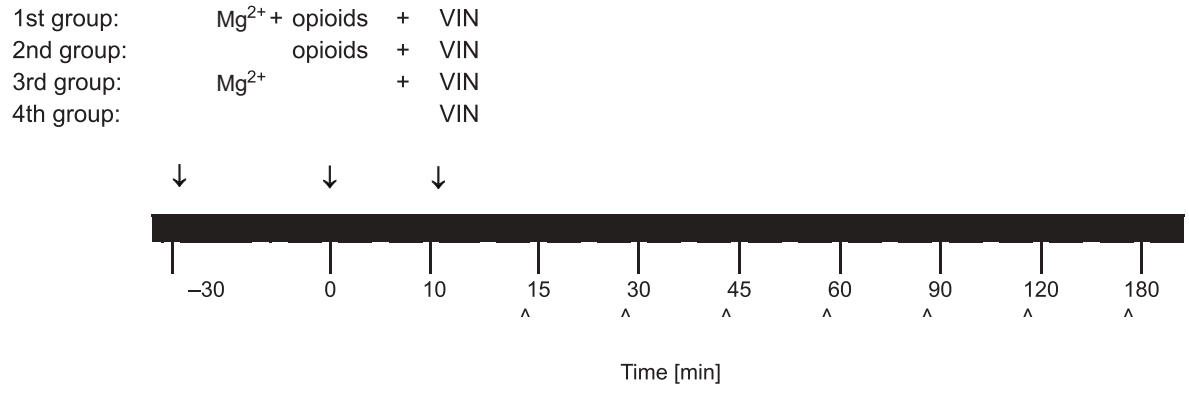

Fig. 1. Time schedule of acute experiments (measurements on day 1 and 5 of consecutive administration of investigated drugs; 8 and 12 days of experiment). ^ – measurements of acute activity of investigated drugs

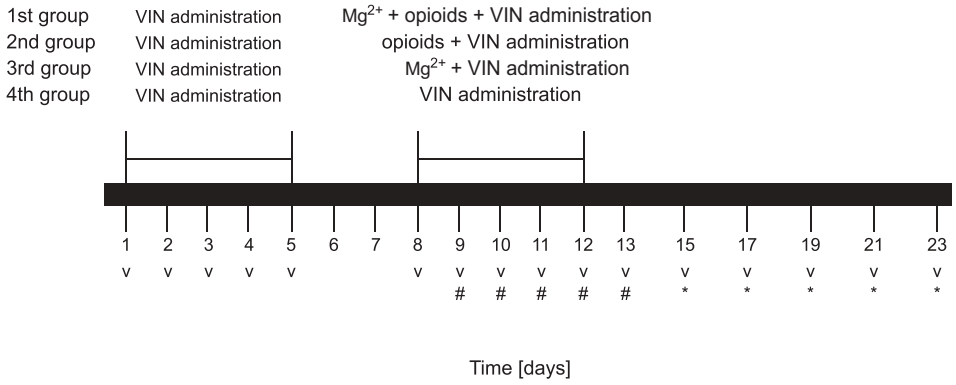

Fig. 2. Time schedule of prolonged experiments (measurements immediately before consecutive drugs administration). # - measurements of prolonged activity of investigated drugs, * – measurements after discontinuation of drugs administration, v – measurements of prolonged activity of vincristine (VIN)

Percentage of hyperalgesia was calculated as above for individual animals and was subsequently used to calculate average values in specific experimental groups and for statistical analysis.

Results

Effect of chronic administration of vincristine alone on nociceptive thresholds to mechanical stimuli

A statistically significant decrease of the nociceptive threshold was observed in vincristine treated animals starting from the day two. The decrease reached its nadir on day 9 (means of the first value in g = 150) and remained constant until day 12 (mean of the first value in g = 145). After discontinuation of vincristine administration at day 13, the nociceptive thresholds gradually increased, with the thresholds returning to baseline values by day 23 (mean of the first baseline value in g = 202.9) (Fig. 3).

Influence of magnesium ions on the antinociceptive activity of morphine, fentanyl, and buprenorphine in a vincristine-induced hyperalgesia model

Morphine, fentanyl, buprenorphine as well as Mg^{2+} administered alone for 5 consecutives days did not al-

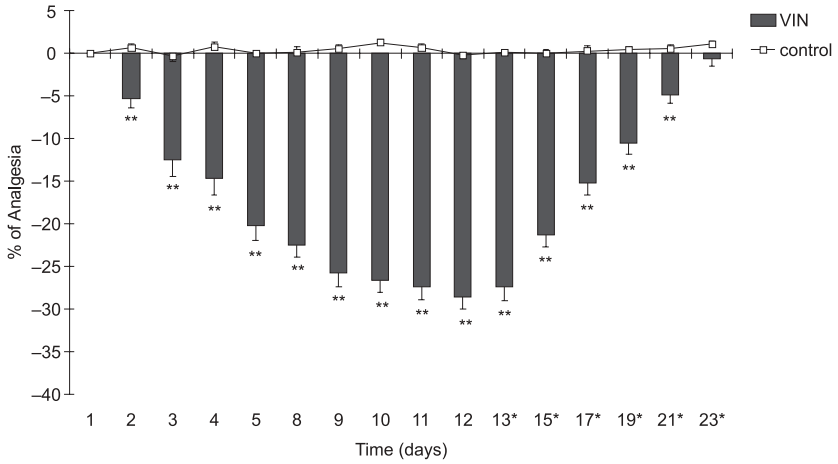

Fig. 3. Influence of vincristine (VIN) at a dose of 70 μg/kg, *iv* on the mechanical stimuli threshold (measurements immediately before consecutive VIN administration) and then after VIN discontinuation (from days 13*–23*). Values are means ± SEM. ** $p \leq 0.01$ VIN *vs.* control

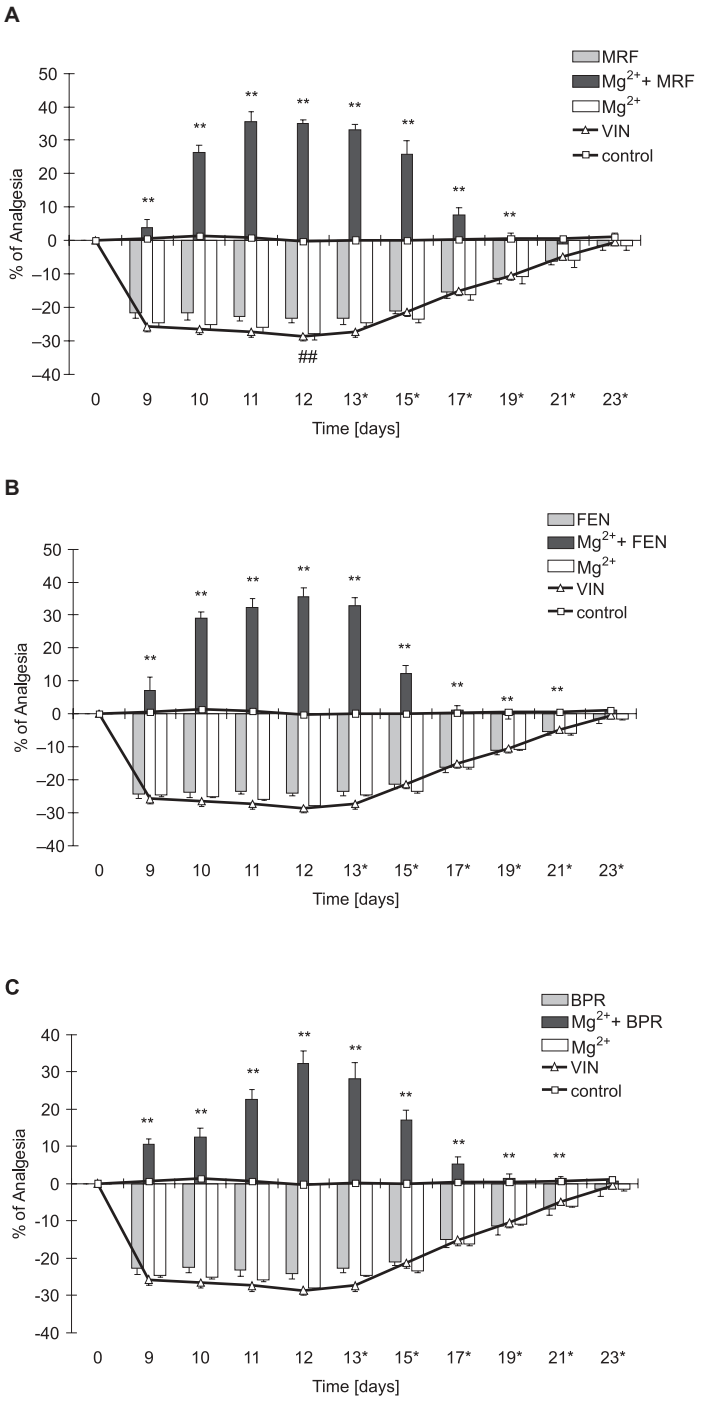

Fig. 4. Effect of magnesium (Mg^{2+}; 30 mg/kg, *ip*) on the analgesic activity of morphine (MRF; 5 mg/kg, *ip*) (**A**), fentanyl (FEN; 0.0625 mg/kg, *ip*) (**B**), and buprenorphine (BPR; 0.075 mg/kg, *ip*) (**C**), in VIN-treated rats. Drugs were administered for five consecutive days (from day 8 to 12), whereas measurements were performed from day 9 to 13* (24 hours after last administration but immediately before administration of the next drugs) and then after drug discontinuation (from day 15* to 23* of experiment). Values are means ± SEM. ** $p \leq 0.01$ MRF *vs.* Mg^{2+} + MRF; FEN *vs.* Mg^{2+} + FEN; BPR *vs.* Mg^{2+} + BPR; ## $p \leq 0.01$ MRF *vs.* VIN

ter the vincristine induced hyperalgesia (Fig. 4 A–C). However, pretreatment with Mg^{2+} resulted in a progressive increase in the analgesic action of all three opioids (Fig. 4 A–C). Cessation of drug administration resulted in a gradual return to baseline values.

On day one of acute morphine administration, premedication with magnesium not only abolished the vincristine-hyperalgesia but a clear analgesic effect occurred within 15 min to 60 min from the start of the experiment (Fig. 5A).

Interestingly, a significant analgesic effect was observed on day five of magnesium and morphine administration during the entire experiment time course (from 15 to 180 min). This effect appeared to be twofold greater than on day 1 of the observation (Fig. 5B).

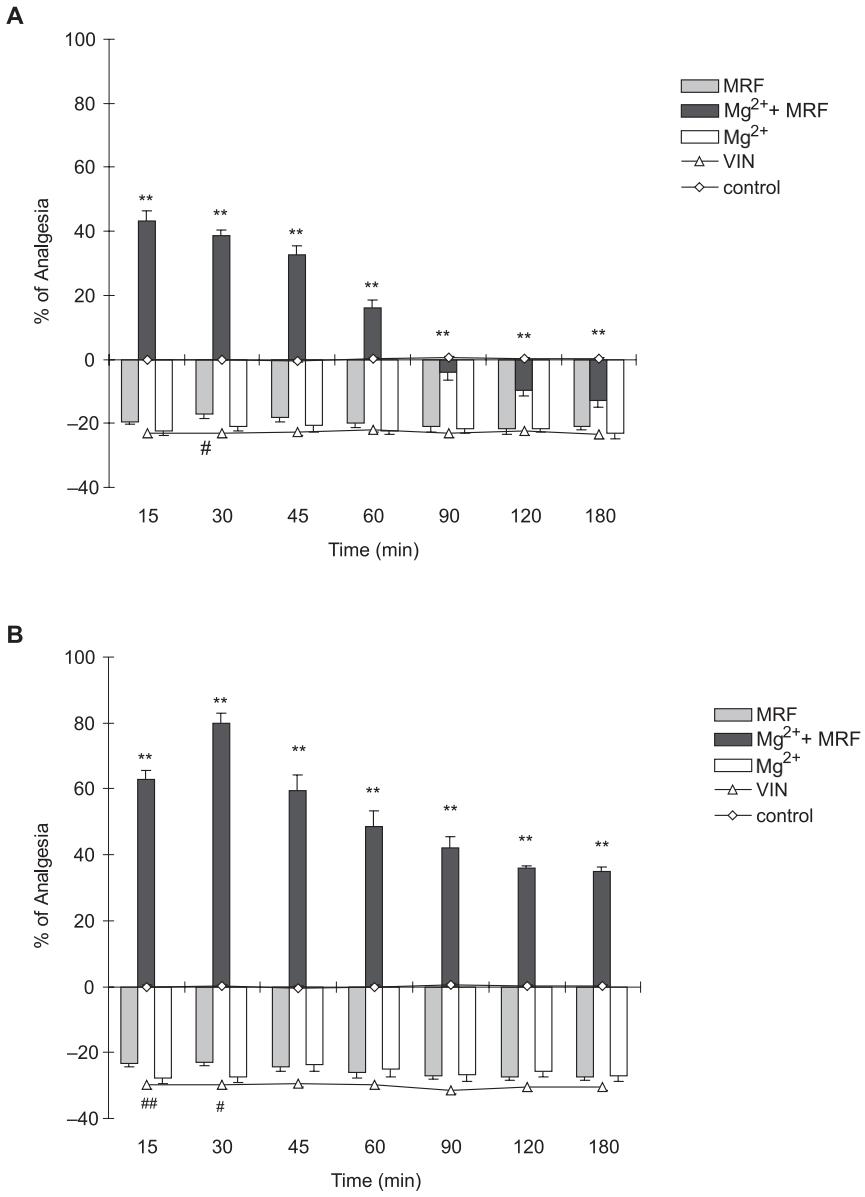

Fig. 5. Morphine (MRF) analgesia (5 mg/kg, *ip*) on day 1 (day 8 of experiment) (**A**) or 5 (day 12 of experiment) (**B**) of pretreatment with Mg^{2+} in VIN-treated rats. Values are means ± SEM. ** $p \leq 0.01$ MRF *vs.* Mg^{2+} + MRF; ## $p \leq 0.01$; # $p \leq 0.05$ MRF *vs.* VIN

Similar effects were also observed after fentanyl and buprenorphine administration on day 1 and 5 of magnesium pretreatment (not shown). However, the analgesic action of concomitant administration of magnesium and buprenorphine lasted longer on day 1 (up to 180 min).

Discussion

Morphine and other opioid agents belong to the classical analgesic drugs. However, the data concerning the effectiveness of opioids on neuropathic pain are controversial [2, 11]. One study reported that fentanyl (10–100 μg/kg, *ip*) produced a dose-related antinociceptive effect as related to reactions to electrical current, paw pressure and tail flick nociception in both neuropathic and non-neuropathic rats. However, in the same study higher doses of fentanyl were needed in order to alleviate the neuropathic pain [30]. In the diabetic neuropathy model, morphine induced antinociception, but at doses twice as high as those used in normal rats [9].

In the recent study, we demonstrated that opioid agonists such as morphine (5 mg/kg, *ip*) and fentanyl (0.0625 mg/kg, *ip*), as well as the partial agonist, bu-

prenorphine (0.075 mg/kg, *ip*), had a mild effect on streptozotocin-induced hyperalgesia in diabetic neuropathy [7].

The results in the present study showed that none of the three investigated opioids administered alone for five consecutive days modified the vincristine-induced hyperalgesia.

Insensitivity of neuropathic pain to opioid analgesic is difficult to explain. It has been shown that the loss of opioid receptors expressed on C-fibers, the activation of NMDA receptors, the increase in the levels of cholecystokinin and accumulation of morphine-3-glucuronide could lead to reduced sensitivity to morphine in neuropathic pain states [27].

NMDA receptors play a role in the etiology of neuropathic pain. Unfortunately, despite a well-established clinical efficacy, the use of NMDA antagonists is limited because of the relatively frequent occurrence of adverse effects (e.g., memory impairment, psychomimetic effects, ataxia and motor incoordination). In physiological conditions, the antagonists of the endogenous NMDA receptor are Mg^{2+}. The magnesium block is removed during the molecular sensitization process. The resulting activation of NMDA receptors causes calcium entry into the cell and triggers neuronal sensitization [4].

Reports have suggested that Mg^{2+} could cause a similar antinociceptive effect as NMDA antagonists. Feria et al. [14] showed that magnesium sulfate, administered at doses of 300 or 600 mg/kg *sc*, suppressed autotomy in peripherally deafferented rats. Ulugol et al. [27] found that Mg^{2+} exerted a significant anti-allodynic effect at higher doses (250 mg/kg) but not at low doses (125 mg/kg) in a sciatic nerve ligated model. Hyperalgesia was also reversed after administration of magnesium sulfate at cumulative doses of 90 mg/kg in a mononeuropathic and diabetic model but not in a formalin test [3]. On the other hand, magnesium sulfate administered intrathecally did not increase the nociceptive threshold on mechanical and thermal stimuli in an acute pain model and produced depression of pain responses only after the first 10 min in the formalin test [15].

In a previous study, magnesium sulfate administered for a few consecutive days at a very small dose did not modify streptozotocin-induced hyperalgesia in a diabetic neuropathy model in rats [7]. Since the administration of such a small dose of magnesium sulfate (30 mg/kg) did not cause any behavioral changes or change any vital sign during this study, we also decided to administer a low dose of 30 mg/kg. We demonstrated that magnesium sulfate administered daily for five successive days did not change vincristine hyperalgesia in chemotherapy-induced neuropathy model.

Data available in literature indicate that co-administration of Mg^{2+} and small opioid doses can be clinically useful [3, 27]. McCarthy et al. [19] demonstrated that intrathecal administration of magnesium sulfate increased the antinociceptive effect of morphine in a tail-flick test. Co-infusion of Mg^{2+} with morphine enhanced the nociceptive threshold to thermal stimuli in normal rats [16]. Begon et al. [3] showed that Mg^{2+} at a dose of 30 mg/kg or 90 mg/kg (3 × 30 mg/kg, *ip*), in combination with morphine, blocked the phase 2 of the formalin test and increased the mechanical pain threshold in diabetic and mononeuropathic rats. Some data indicate that co-administration of Mg^{2+} and opioids can be beneficial in pain relief for patients after surgery. Özalevli et al. [21] demonstrated that intrathecal addition of magnesium sulfate to spinal anesthesia induced by bupivacaine and fentanyl prolonged the period of analgesia without additional side effects.

The influence of Mg^{2+} on the analgesic activity of opioids in chronic, particularly neuropathic pain has not been sufficiently investigated. It was shown that the combination of subthreshold doses of morphine and magnesium sulfate attenuated allodynia to cold and mechanical stimuli in nerve-ligated rats [27]. Magnesium sulfate also potentiated morphine analgesia in a mechanical allodynia model after surgical incision [16].

We had previously found that Mg^{2+} markedly increased the activity not only of morphine, but also of other investigated opioids (e.g., fentanyl and buprenorphine) in a streptozotocin-induced diabetic neuropathy model [7].

Results from this study indicate that pretreatment with Mg^{2+} could significantly enhance opioid effects in rats with vincristine-induced hyperalgesia. Both on day 1 and 5 of acute morphine administration, premedication of Mg^{2+} not only abolished the vincristine-hyperalgesia but a clear analgesic effect occurred. This effect lasted 60 min on the first day of the experiment and for the duration of the experiment (180 min) on day 5 of the drugs' administration.

It is important to emphasize that the analgesic effect after the combination of Mg^{2+} and opioid treatment was prolonged and persisted for several days af-

ter discontinuance of the drug administration in a vincristine neuropathic model. It is of interest to note that an enhancement of opioid-induced analgesia after Mg^{2+} pretreatment in streptozotocin-treated rats persisted for a maximum 3 days in the previous study. The reason for the discrepancy in these results remains unknown.

Concluding, Mg^{2+} significantly increased the analgesia of morphine, fentanyl, and buprenorphine in vincristine-induced neuropathy. This effect is probably due to the antagonistic effect that Mg^{2+} has on NMDA receptors. However, more studies on NMDA receptors would be useful to make definitive conclusions on the interaction between opioids and Mg^{2+}. This problem is an objective of our future study. Assuming that Mg^{2+} will not increase opioid-induced respiratory depression, potentiation of opioid analgesia in neuropathic pain may be of clinical importance.

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