Short communication

Magnesium and MK-801 have a similar effect in two experimental models of neuropathic pain

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Abstract

Considering that magnesium and non-competitive NMDA receptor antagonists inhibit the opening of the channel linked to the NMDA receptor, we assessed their effects on mechanical hyperalgesia in two animal models of neuropathic pain (rats with a sciatic nerve ligation and diabetic rats). Our data show that magnesium reverses the hyperalgesia, as does MK-801. These results suggest that magnesium could be an alternative for the treatment of neuropathic pain in patients. © 2000 Elsevier Science B.V. All rights reserved.

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Current hypotheses about the pathophysiology of neuropathic pain highlight the role of the N-methyl-D-aspartate (NMDA) receptor channel in the sensitization of the spinal cord neurons which leads to chronic pain [8,26]. As a matter of fact, many experimental studies as well as some clinical trials reported the analgesic efficacy of non-competitive NMDA antagonists such as dizocilpine maleate (MK-801) or ketamine in neuropathic pain [5]. However, with these drugs, a lot of adverse events, described in patients [20], limit the therapeutic use of NMDA antagonists. Electrophysiological and binding studies have shown that MK-801 is able to block the NMDA receptor channel [24] and furthermore, that the opening of this channel depends on the magnesium (Mg2+) ion [18]. Therefore, we hypothesized that Mg2+ could have similar anti-hyperalgesic properties as non-competitive NMDA antagonists with less adverse events. The aim of this study was to compare the anti-hyperalgesic effect of repeated dosages of Mg2+ and MK-801 in two experimental models of neuropathic pain, the diabetic rat and the mononeuropathic rat.

These studies were conducted in accordance with the IASP guidelines for animal experiments [28]. For the experiment carried out in diabetic rats, Sprague–Dawley male rats (Charles River, France) were injected intraperitoneally (i.p.) with streptozocin (STZ) (75 mg/kg) (Zanosar, Upjohn). From the 21st day onwards, several troubles of nociception have been described [6], particularly a mechanical hyperalgesia. The other experiment used a model of peripheral mononeuropathy in rat. Sprague–Dawley male rats (Charles River, France) were anesthetized and a chronic constrictive injury (CCI) of their right common sciatic nerve was performed according to the method described by Bennett and Xie [3].

The CCI model develops a mechanical hyperalgesia from day 12 after surgery [1]. The contralateral limb remained unoperated.

In these two experiments, the changes in mechanical nociceptive thresholds were assessed by the paw pressure test [21]. An increasing mechanical pressure was applied by an analgesymeter (Aplex type 003920, Ugo Basil, Italy) on a hind paw, until a vocalization was elicited (vocalization threshold expressed in grams). For the CCI model, the measurement was carried out on the ipsilateral to the ligature and the contralateral paws. For each experiment, the vocalization threshold was assessed before the induction of the neuropathic hyperalgesia (baseline

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values), before the induction of treatment, when the hyperalgesia was developed (control values) and then twice daily (performed before each injection, at 8:00 am and 8:00 pm) throughout the period of treatment. No significant difference was observed between these two daily values of the vocalization thresholds; thus they have been averaged for each day.

For the two experiments, the treatments were given twice daily for 5 days. One group of animals (n=8) received MK-801, dizocilpine maleate (RBI/Sigma–Aldrich, France) (0.1 mg/kg, i.p., b.i.d.). For the control group (n=8) animals were injected with saline (2 ml/kg, i.p., b.i.d.). A third group (n=8) was treated with magnesium sulfate (MgSO₄) (Sigma–Aldrich Chimie, France). The diabetic rats were given a slightly higher dosage of Mg²⁺ (200 mg/kg MgSO₄, i.e. 40 mg/kg Mg²⁺, i.p., b.i.d.) in comparison with the dosage used in mononeuropathic rats (150 mg/kg MgSO₄, i.e. 30 mg/kg of Mg²⁺, i.p., b.i.d.). That choice was guided by published data [10] reporting a Mg²⁺ depletion in diabetic rats due to the polyuria. All the treatments were performed blind.

The data analysis was performed by a two-way ANOVA, followed by a PLS Fisher’s test to compare the treated groups with Mg²⁺ or MK-801 versus the control saline-treated group (Statview 4.1 software). The area under the time-course curve (AUC) of the vocalization threshold was also determined throughout the treatment period. That parameter was analyzed by a Student’s t-test to compare the treated groups with Mg²⁺ or MK-801 versus the control saline-treated group.

In the model of diabetic neuropathy, the animals developed a mechanical hyperalgesia according to the time schedule described by Courteix et al. [6]. Three weeks after the induction of the diabetes, the decrease of the vocalization threshold (40.8±3.9, 41.8±2.9 and 45.0±1.6% in MgSO₄, MK-801 and saline-treated groups, respectively), as well as the baseline values (319.3±10.8, 319.3±16.2 and 312.9±13.0 g for MgSO₄, MK-801 and saline-treated groups, respectively) were very similar in each group. The treatment with MgSO₄ (200 mg/kg, i.p., b.i.d.) increased progressively the vocalization threshold (Fig. 1A). After 3 days of treatment, this threshold was significantly higher (P<0.001) in the Mg²⁺-treated group (354.2±37.4 g) than in the control saline-treated group (192.6±10.2 g). Thereafter, the vocalization threshold was leveled off until the end of the experiment without significant differences with the baseline values. With the NMDA antagonist (0.1 mg/kg, i.p., b.i.d.), the mechanical hyperalgesia was also totally reversed so that the vocalization threshold returned to the baseline values (Fig. 1A). In the MK-801-treated group the anti-hyperalgesic effect appeared more rapidly than in the Mg²⁺-treated group. On day 2, a significant difference (P<0.05) was observed between the treated and the control group (305.9±33.1 versus 211.8±9.7 g, respectively).

The overall effects over the 5 days of treatment, assessed by the AUC values, show that Mg significantly reversed (P<0.01) the mechanical hyperalgesia in the diabetic rats compared to the control saline-treated group, with the same efficacy as does the NMDA antagonist treatment (Fig. 1B).

In the experiment with the CCI model, a mechanical hyperalgesia occurred 12 days after the sciatic nerve ligature (Fig. 2A). In comparison to the baseline values (272.3±12.0, 257.8±9.3 and 263.4±12.1 g for MgSO₄, MK-801 and control saline-treated groups, respectively) before surgery, the vocalization threshold fell to 47.9±2.3, 50.1±2.3 and 48.2±2.4%, respectively, for each group of animals. Systemic injection of MgSO₄ (150 mg/kg, i.p., b.i.d.) reduced the mechanical hyperalgesia in mononeuropathic rats. In comparison with the control saline-treated group, the vocalization threshold became significant (P<0.05) on the first day of treatment with MgSO₄ (138.1±5.5 versus 201.5±19.0 g, respectively), then remained significant throughout all the experiment. The mechanical hyperalgesia was also significantly reduced (P<0.05) in MK-801-treated rats from day 4 (200.0±13.7 g) compared to the control saline-treated group (155.6±7.8 g). None of the tested drugs induced changes in the vocalization threshold for the contralateral paw in the CCI.
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Malcangio et al. [17] also found a significant anti-hyperalgesic effect which occurs 2 days after starting a similar treatment as the one we used. These authors found no effect of repeated dosages of MK-801 in normal rats.

In mononeuropathic rats, we showed curative effect of MK-801 (0.1 mg/kg, i.p., b.i.d.) on the induced hyperalgesia. Similarly, Birch et al. [4] reported, as we did, such a curative effect in the CCI model. Nevertheless, most studies investigated a preemptive treatment with MK-801 by using fairly high dosages of MK-801 (0.5, 0.6 or 1 mg/kg/day, s.c. or i.p.) [7,15,22]. In these conditions MK-801 reduced mechanical and thermal hyperalgesia more rapidly and with a larger magnitude as observed in our experiments. On the counterpart, at these dosages, MK-801 often induces stereotypic behavior (head weaving and circling, jerky movements), loss of balance and ataxia [13,22,23] that we did not observe in our experiments.

The results obtained in our study show for the first time, that repeated dosages of Mg totally or partially reverse the mechanical hyperalgesia both in diabetic rats and in CCI model. Similarly, after repeated systemic injection of Mg, Xiao et al. [27] showed a prolonged effect on the heat-hyperalgesia in the CCI model.

Hallack et al. [12] showed in rat, an increase in Mg level in both cerebrospinal fluid and different brain regions, after i.p. injections of MgSO4. That is in agreement with the possibility of Mg to cross the blood brain barrier. Significant changes in Mg concentration were observed in the spinal cord, after a systemic injection, by Feria et al. [9]. On the other hand, Mg is able to modulate the opening of the Na+/Ca2+ channel of the NMDA receptor [18]. Several authors [4,17] give evidence that this type of receptor channel is involved in neuropathy-induced central sensitization. Thus, the anti-hyperalgesic effect of Mg in these two models of neuropathy, could result from a direct interaction of this ion with the activated NMDA receptor channel.

As for the MK-801 treatment, we took care to avoid some adverse events due to the Mg2+ injection, which could lead to a misinterpretation of the behavioral tests. Ishizaki et al. [14] after intrathecal administration of MgSO4, described the occurrence of motor paralysis or ataxia. As a consequence, we used fairly low dosages of MgSO4 in order to avoid these phenomena. Xiao et al. [27] reported, as we did, that low dosages of Mg2+ (30 and 40 mg/kg) did not induce any adverse effects. In ours studies, the fact that both tested drugs did not modify the vocalization threshold for the contralateral paw in the CCI model, gives evidence that neither MK-801 nor Mg2+ disturbed the behavioral assessment of the nociceptive sensitivity in rats. On the other hand, abdominal constrictions were reported in mice, after an i.p. injection of MgSO4 (120 mg/kg, i.e. 25 mg/kg Mg2+), that persisted 20 min following injection [11], as well as in our experiment. This could induce a counter-irritation phenomenon and interfere

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**Fig. 2.** Effect of a twice daily treatment, for 5 days, with MgSO4 (150 mg/kg, i.p.) or MK-801 (0.1 mg/kg, i.p.) or saline in rats with a chronic constrictive injury of the sciatic nerve (n=8/group). The vocalization threshold was assessed on the ipsilateral (A) and the contralateral (B) paws. The two daily measurements of the vocalization threshold were averaged for each day. Data are presented as means±S.E.M., **P<0.01, *P<0.05, versus the corresponding values of the control saline-treated group. AUC was calculated throughout 5 days of treatment (C), ***P<0.001, **P<0.01, versus the control saline-treated group.

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model (Fig. 2B). AUC values (Fig. 2C), integrating the effect of both compounds throughout the whole period of treatment, confirm the efficacy of Mg2+ and MK-801 treatment on the hyperalgesia compared to the control saline-treated group (P<0.001 and P<0.01, respectively).

The effects we observed with MK-801, taken as a reference NMDA antagonist, are fully in agreement with published data collected with similar models. As a matter of fact, our study demonstrates that a 5-day treatment with MK-801 totally reverses the mechanical hyperalgesia in diabetic rats.
with the assessment of pain threshold. That is why we deliberately assessed the vocalization threshold far from the injection time to avoid them.

In these two models of experimental neuropathic pain, we showed that both MK-801 and Mg$^{2+}$, which share some blocking properties on NMDA receptor channel, are able to reverse the induced hyperalgesia with a very similar degree of efficacy. The two treatments did not induce any changes in the non-ligated paw in the CCI model. This suggests an action only in conditions of persistent pain and this is in line with recently published data emphasizing the role of NMDA receptors on the sensitization of dorsal horn neurons observed in similar conditions [25]. Therefore, much attention has been placed on NMDA receptor antagonists as a treatment for chronic pain [5]. In patients suffering from neuropathic pain the response to these treatments is often satisfactory in terms of pain relief [16,19], but its clinical use is strongly limited by frequent and very uncomfortable adverse events [2]. Our results suggest that it would be interesting to undertake clinical trials on Mg$^{2+}$ therapy in patients suffering from painful peripheral neuropathies.

References


