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Magnesium Increases Morphine Analgesic Effect in Different Experimental Models of Pain

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Background: **An excess of excitatory pathway activation *via N*-methyl-D-aspartate (NMDA) receptors has been described in neuropathic pain that responds poorly to morphine. However, in this situation, several published data sets show that coadministration of NMDA receptor antagonists restores the efficacy of opioids. Considering that magnesium behaves like an NMDA receptor antagonist, we investigated the effect of the combination of magnesium and morphine in experimental models of chronic and tonic pain.**

Methods: **Mechanical hyperalgesia was assessed with the paw-pressure test in mononeuropathic (chronic constrictive injury model) and diabetic rats. Behavioral reactions were scored in a model of inflammation induced by formalin. The animals were assigned to one of three groups according to the intraperitoneal pretreatment: magnesium (30 mg/kg × 3), magnesium (30 mg/kg), and saline. Before testing, morphine was injected intravenously in mononeuropathic (0.3 mg/kg) and diabetic rats (1 mg/kg) and by the subcutaneous route in rats with the formalin test (1.5 mg/kg).**

Results: **Magnesium alone induced a significant antihyperalgesic effect in mononeuropathic and diabetic rats after a cumulative dose of 90 mg/kg. Furthermore, it significantly increased morphine analgesia, regardless of the loading dose used (30 or 90 mg/kg) in the two models of neuropathic pain. In the formalin test, magnesium alone did not have a significant effect. However, in combination with morphine, it revealed the analgesic effect of this opiate.**

Conclusions: **These data show that magnesium amplifies the analgesic effect of low-dose morphine in conditions of sustained pain. Considering the good tolerability of magnesium, these findings may have clinical applications in neuropathic and persistent pain.**

N-METHYL-D-ASPARTATE (NMDA) receptors are involved in persistent pain[1] and in the generation and maintenance of a spinal hypersensitivity phenomenon leading to chronic pain.[2] Hence, many authors give evidence of the efficacy of NMDA receptor antagonists in different experimental models of neuropathic[3,4] or inflammatory pain.[5] However, despite a well-established clinical efficacy,[6,7] their use is limited because of the high occurrence of adverse effects.[8] Depending on their etiology, some clinical[9–11] and experimental[12] studies emphasize a limited efficacy of morphine to relieve neuropathic pain. To overcome this phenomenon, a combination of NMDA receptor antagonists with opioids has shown proof of efficiency in the treatment of neuropathic pain.[13] Here again, this therapeutic approach is unfortunately counterbalanced by a high incidence of adverse effects.[14]

It is known that magnesium is able to modulate NMDA receptor activation by blocking the receptor channel.[15] Recent studies have stressed the antinociceptive effects of intrathecal magnesium in experimental models of neuropathic pain[16] and in the formalin test.[17,18] Furthermore, we showed that systemic treatment with multiple doses of magnesium in diabetic and mononeuropathic rats could reverse mechanical hyperalgesia.[19]

The purpose of this study was to investigate whether systemic magnesium might amplify the antinociceptive effect of a low dose of morphine in two models of neuropathic pain and in one model of tonic pain induced by paw injection of formalin.

Materials and Methods

These experiments were conducted according to the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals as issued by the International Association for the Study of Pain.[20]

Animals

Male Sprague-Dawley rats (CD1 Charles River; IFFA-CREDO, L'Arbresle, France), initially weighing 200–250 g, were used. Animals (n = 4 per cage) were housed in standard laboratory conditions with food and water *ad libitum*, 1 week before the experiments.

Induction of Peripheral Mononeuropathy

After brief anesthesia (sodium pentobarbital, 40 mg/kg, intraperitoneal), a chronic constrictive injury (CCI) of the right common sciatic nerve was performed, according to the method described by Bennett and Xie.[21] The contralateral limb remained unoperated. Mechanical hyperalgesia developed in the CCI model from day 12 after surgery.[22]

Induction of Diabetes

Animals were injected with streptozocin (75 mg/kg, intraperitoneal, Zanosar®; Upjohn, Paris, France), and 1 week later, diabetes was confirmed by measurement of tail vein blood glucose concentration (> 14 mM) with Glucotide test strips and a reflectance colorimeter (Glucometer 4; Ames Division, Bayer Laboratories, Puteaux,

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France). Mechanical hyperalgesia has been described[23] from the 21st day onward.

Paw-pressure Test

The antinociceptive effect of the tested compounds was assessed by the paw-pressure test.[24] Increasing mechanical pressure was applied by an analgesimeter (Apelex type 003920; Ugo Basil, Bioseb, Chaville, France) on the right and left hind paws, until vocalization was elicited (threshold expressed in grams). In the neuropathic pain models, the nociceptive threshold was assessed before induction of hyperalgesia (baseline values) and then reassessed before the induction of magnesium treatment. The experiments were performed in a quiet room by a unique experimenter.

Formalin Test

The animals were placed in the glass box (40 × 30 × 20 cm) with mirrors placed all around the cage to allow an unobstructed view of the paws. After 15 min for habituation, the rats were injected (50 μl, subcutaneous) with formalin (5%) into the plantar surface of the right hind paw. Scoring of nociceptive behavior began immediately after formalin injection and then was followed up for 60 min according to the method described by Dubuisson *et al.*[25]

Pharmacologic Experiments

Three series of experiments were performed with each of the experimental models. In these protocols, low doses of morphine were chosen to avoid a ceiling antinociceptive effect, which would blunt the effect of magnesium–morphine combination.

In Mononeuropathic Rats. Vocalization thresholds were determined before and 14 days after the CCI as control predrug values, and a pretreatment was administered to the eight groups (n = 7 per group). Pretreatments consisted of the following: (1) Magnesium 90: a cumulative dose up to 90 mg/kg of magnesium (magnesium sulfate; Sigma-Aldrich Co., Saint Quentin Fallavier, France), divided into three intraperitoneal injections (30 mg/kg magnesium or 2 ml/kg saline). Each injection was administered with an interval of 1 h. (2) Magnesium 30: a single dose of magnesium (30 mg/kg intraperitoneal). In each group, the pretreatment (magnesium or saline, 2 ml/kg intraperitoneal) was administered blindly using the method of equal blocks. Thirty minutes after the last injection of pretreatment 1 or 2, 0.3 mg/kg morphine (morphine hydrochloride; Cooper, Rhone Poulenc Rorer, Melun, France) were injected by the intravenous route. In the CCI model, the low dose of morphine was chosen according to Christensen *et al.*[13] The vocalization threshold was assessed at 15, 30, 45, 60, 120, and 180 min after the intravenous injection treatment. The measurement was performed on the ipsilateral side to the ligature and on the contralateral paws. The randomized treatments were performed blindly to avoid uncontrollable environmental influence that could induce a modification in behavioral response.

In Diabetic Rats. Vocalization thresholds were determined before and 21 days after induction of diabetes as control predrug values. Pretreatments were administered to the eight groups (n = 7 per group) as described in the CCI model. Thirty minutes after the last injection of pretreatment 1 or 2, an intravenous injection of 1 mg/kg morphine or saline (1 ml/kg) was performed. The dose of morphine was chosen according to Courteix *et al.*[26] The assessment of the vocalization threshold was performed as previously described.

In Rats Injected with Formalin. The pretreatments were administered to eight groups (n = 8 per group) of rats as previously described. Thirty minutes after the last injection of pretreatment 1 or 2, morphine (1.5 mg/kg) or saline (2 ml/kg) were injected subcutaneously in these animals blindly. The dose of morphine was chosen according to Coderre *et al.*[27] and Jourdan *et al.*[28] Immediately after morphine injection, the animals were placed for habituation in the glass box, and 15 min later, rats were given a subcutaneous injection (50 μl) of formalin (5%) into the plantar surface of the right hind paw. Scoring of nociceptive behaviors began immediately afterward and lasted for 60 min.

Expression of Results and Statistical Analysis

Data analysis of the vocalization thresholds or scores of nociceptive behaviors in the formalin test, both expressed as mean ± standard error of the mean, was performed for each time of measurement by a two-way analysis of variance, followed when the F value was significant by a Dunnett test to compare the corresponding values of the drug-treated group with the saline group. The significance levels were as follows: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

Results

Effect of Magnesium and Morphine on the Vocalization Threshold in Mononeuropathic Rats

As expected, mechanical hyperalgesia occurred in the operated hind paw 12 days after the sciatic nerve ligature (figs. 1A and B). No change in the vocalization threshold was observed after a single injection of 30 mg/kg magnesium alone (fig. 1A). However, a dose of magnesium (30 mg/kg × 3, intraperitoneal) reduced the mechanical hyperalgesia (fig. 1A). In comparison with the control saline-treated group, the lowering of the vocalization threshold became significant ($P < 0.01$) 45 min after the last injection of magnesium (160.5 ± 7.8 *vs.* 228.8 ± 13.5 g, respectively). Morphine alone (0.3 mg/kg, intravenous) induced a fairly modest but significant antihyperalgesic effect (fig. 1B). When injected in combination with magnesium, it produced,

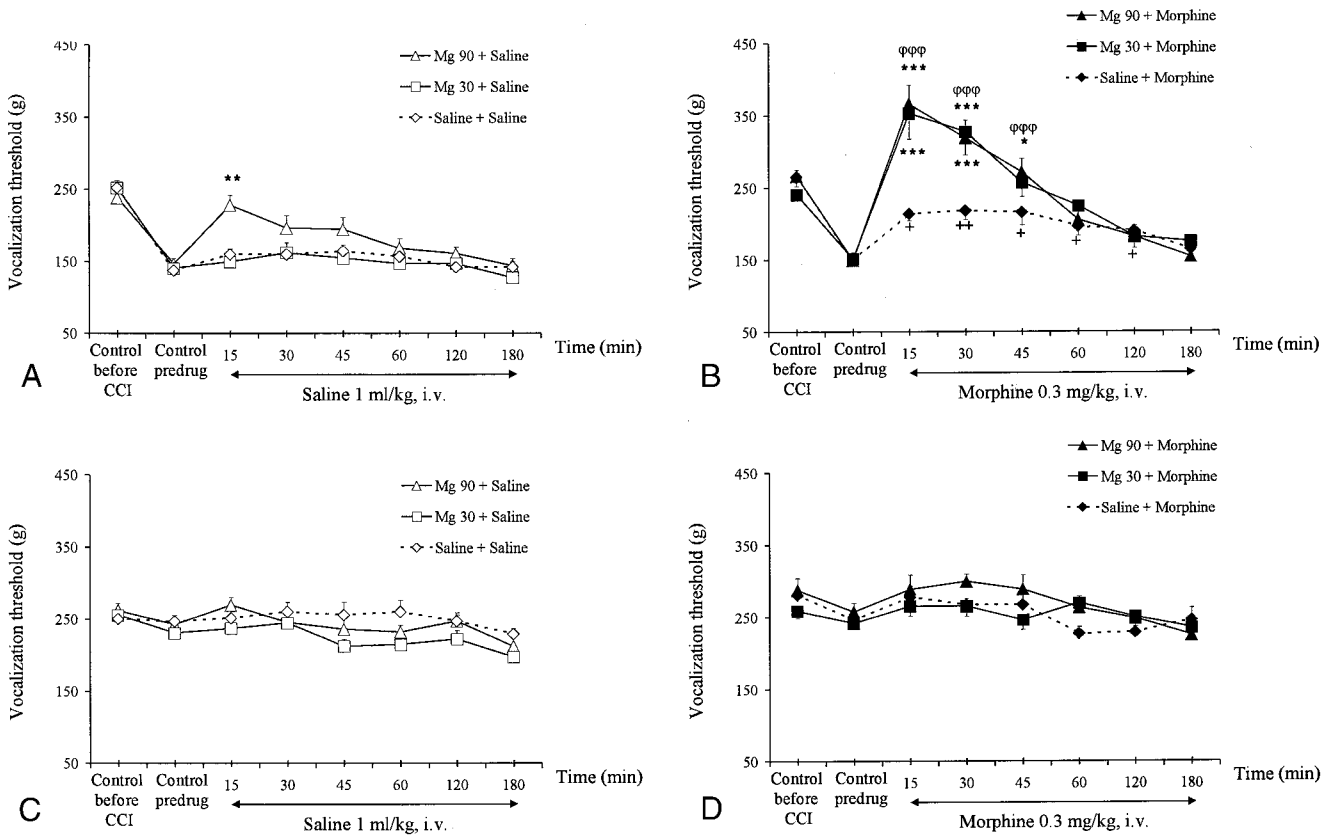

Fig. 1. Time course of the effect of a unique (30 mg/kg, intraperitoneal) or a cumulative (30 mg/kg × 3, intraperitoneal) dose of magnesium (Mg), administered alone (*A, C*) or in combination with morphine (0.3 mg/kg, intravenous [i.v.]) or saline (1 ml/kg, intravenous) (*B, D*) on mechanical pain threshold in mononeuropathic rats. The measurements were assessed both on the ipsilateral (chronic constrictive injury [CCI]) (*A, B*) and the contralateral (*C, D*) paws. Vocalization threshold, expressed in grams, was determined before CCI and then before and six times after morphine or saline injection over a period of 180 min. Data are represented as mean ± standard error of the mean. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with the control saline plus saline group (*A*) or saline plus morphine–treated group (*B*). ψψψ$P < 0.001$, values of magnesium 90 plus morphine–treated group compared with the control magnesium 90 plus saline group (*B*). +$P < 0.05$; ++$P < 0.01$, values of saline plus morphine–treated group compared with the control saline plus saline group (*B*).

whatever the pretreatment with magnesium, a significant ($P < 0.001$) antinociceptive effect in comparison with the saline plus morphine group. The magnitude of the maximal effect occurring 15 min after morphine injection was respectively +216.4 ± 21.3 and +201.4 ± 15.9 g for magnesium 90 plus morphine and magnesium 30 plus morphine. The magnitude of the analgesic response of morphine is significantly ($P < 0.001$) amplified by pretreatment with magnesium; however, in our experimental conditions, the effect of morphine was not dependent on the dose of magnesium. None of the tested drugs, alone or in combination, induced changes in the vocalization threshold for the contralateral paw in the CCI model (figs. 1C and D).

Effect of Magnesium and Morphine on the Vocalization Threshold in Diabetic Rats

In the model of diabetic neuropathy, mechanical hyperalgesia developed in the animals 3 weeks after the induction of diabetes, according to the time schedule described by Courteix *et al.*[23] The decrease in vocalization threshold ranged from 40.2 ± 2.6 to 46.4 ± 2.5% in the different groups of rats. The pretreatment with the cumulative dose of magnesium (30 mg/kg × 3, intraperitoneal) induced a significant antihyperalgesic effect (fig. 2A). Forty-five minutes after the last injection of magnesium, the vocalization threshold increased significantly ($P < 0.01$) in comparison with the saline plus saline–treated group. The pretreatment with a single injection of magnesium (30 mg/kg, intraperitoneal) did not change the vocalization threshold in diabetic rats. Morphine (1 mg/kg, intravenous) injected alone (fig. 2B) induced no change in the nociceptive threshold. The combination of morphine plus magnesium induced a significant and dose-dependent antinociceptive effect (fig. 2B). The peak effect, which occurred 30 min after morphine injection (353.6 ± 33.0 g) for the magnesium 90 plus morphine–treated group and 15 min after morphine injection (311.3 ± 25.0 g) ($P < 0.001$) for the magnesium 30 plus morphine–treated group, was significantly higher ($P < 0.001$) than this observed in the saline plus morphine–treated group (180.0 ± 16.0 and

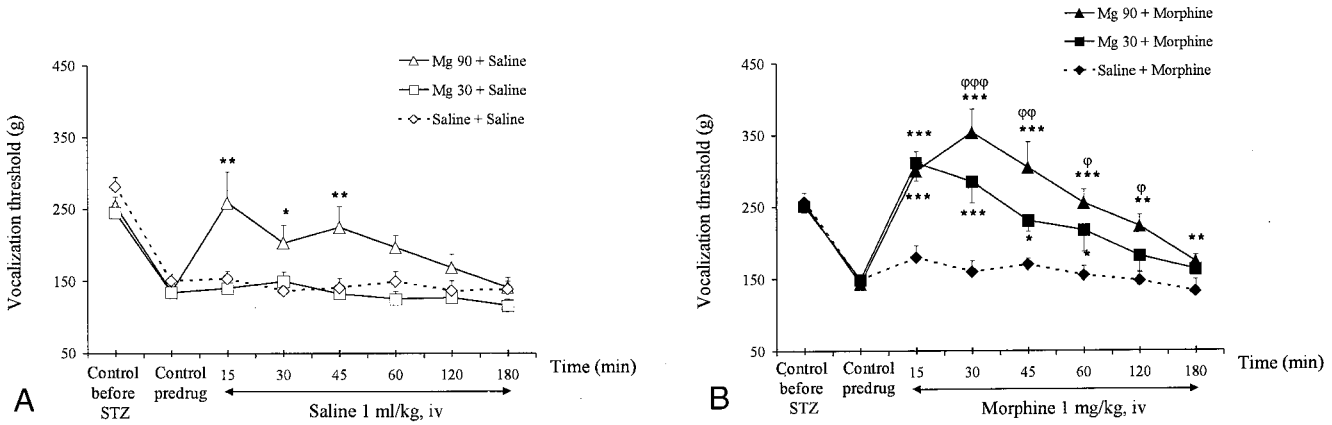

Fig. 2. Time course of the effect of a unique (30 mg/kg, intraperitoneal) or cumulative (30 mg/kg × 3, intraperitoneal) dose of magnesium (Mg), administered alone, with saline (1 ml/kg, intravenous [iv]) (*A*), or in combination with morphine (1 mg/kg, intravenous) (*B*) on mechanical pain threshold in diabetic rats. Vocalization threshold, expressed in grams, was determined before induction of diabetes and then before and six times after morphine or saline injection over a period of 180 min. Data are represented as mean ± standard error of the mean. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with the control saline plus saline group (*A*) or saline plus morphine–treated group (*B*). φ$P < 0.05$; φφ$P < 0.01$; φφφ$P < 0.001$, values of magnesium 90 plus morphine–treated group compared with the control magnesium 90 plus saline group (*B*). STZ = streptozocin.

160.0 ± 15.8 g, at 15 and 30 min after the morphine injection, respectively). In these experimental conditions, the analgesic effect of morphine seemed to be dependent ($P < 0.01$) on the dose of magnesium, as shown by comparison with the area under the curve (0–180 min).

Effect of Magnesium and Morphine on the Vocalization Threshold in Formalin Test

Regardless of dose, the pretreatments with magnesium did not modify the behavioral scores in phase 1 or 2 of the formalin test (fig. 3A). Morphine (1.5 mg/kg, subcutaneous) alone did not have any effect in comparison with the control group (saline plus saline) (fig. 3B). Nevertheless, the pretreatment with 30 or 90 mg/kg magnesium combined with morphine blocked phase 2 of the formalin test (fig. 3B), thus revealing a strong increased effect of morphine.

Discussion

In each of the experimental models of neuropathic pain, the single dose of magnesium (30 mg/kg) had no antinociceptive effect by itself. However, the cumulative dose of magnesium (30 mg/kg × 3) administered alone induced an antihyperalgesic effect. The repeated pattern of magnesium administration *via* the systemic route seems to be essential. Because magnesium crosses the blood–brain barrier by active transport,[29] this may indicate that cumulative doses are required to reach a sufficiently high concentration in the central nervous system to obtain an analgesic effect.

In the formalin test, no effect on the phase 1 was observed with magnesium alone or in combination with morphine. This is in accordance with other studies, which reported that $MgSO_4$,[30] like other NMDA antagonists,[5] has no antinociceptive effect on acute pain.[17,18] In regard to the phase 2 response, which is NMDA-receptor dependent, an antinociceptive effect with the

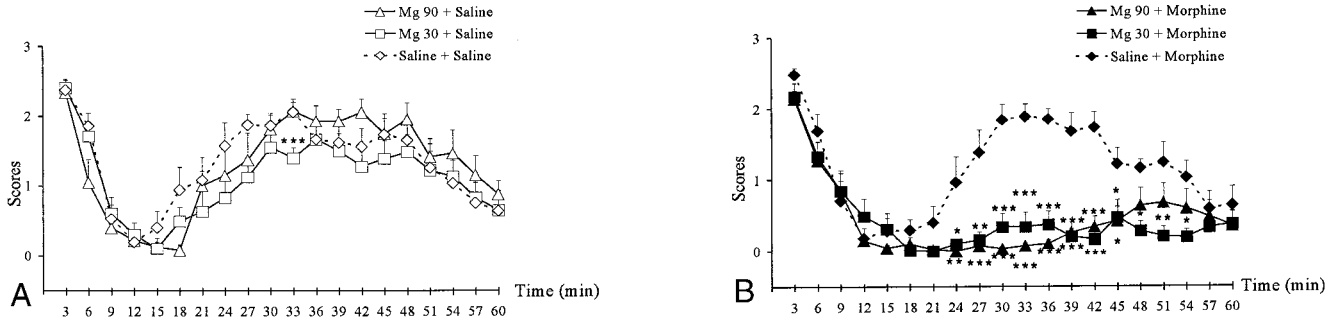

Fig. 3. Time course of the effect of a unique (30 mg/kg, intraperitoneal) or cumulative (30 mg/kg × 3, intraperitoneal) dose of magnesium (Mg), administered alone (*A*) or in combination with morphine (1.5 mg/kg, subcutaneous) or saline (2 ml/kg, subcutaneous) (*B*) on the behavioral scores after the paw injection of formalin (5%; 50 μl). Data are represented as mean ± standard error of the mean. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with the control saline plus saline group (*A*) or saline plus morphine–treated group (*B*).

combination of magnesium and morphine is strongly marked, even though magnesium alone has no effect. Our results contrast with those of Takano *et al.*,[18] who reported that intrathecal injection of magnesium was able to decrease the phase 2 response. This discrepancy may be due to the difference in the routes of administration and, as a consequence, in the magnesium concentration at spinal level.

Our results underline the interest of the combination of magnesium plus morphine for neuropathic pain relief or for pain associated with a subacute inflammation that is considered as a model of postsurgical inflammatory pain. As far as we can ascertain, no experimental study has shown antinociceptive efficacy of the magnesium–morphine association in experimental models of neuropathic pain. However, Kroin *et al.*[31] showed an antinociceptive effect of the magnesium–morphine combination in mechanical allodynia induced by an incision in the plantar surface of one hind paw, modifying the withdrawal threshold by using Von Frey filaments. The same authors demonstrated a synergism of action between magnesium and morphine in naive rats. McCarthy *et al.*[30] also observed an increased analgesic effect of morphine on the tail-flick test in normal rats after an intrathecal infusion of magnesium. They also demonstrated that magnesium delays the development of morphine tolerance.

Clinically, loading doses of magnesium have been shown to lead to partial or total relief[32] in neuropathic patients. Tanaka *et al.*[33] observed in patients with postherpetic neuralgia or causalgia that repetition of magnesium administration once a week decreased the pain visual analog scale score after a few weeks of treatment, with no side effects. The combination of magnesium and morphine has been tested with success. Preoperative or peroperative administration of magnesium has been shown to reduce postoperative morphine consumption.[34,35] These clinical data are in agreement with our findings about increased opioid analgesia by magnesium. However, no such combination of magnesium plus morphine has been assessed in patients with neuropathic pain.

Having previously demonstrated a similar antinociceptive effect of magnesium and MK-801, an NMDA receptor antagonist, in two models of neuropathic pain,[19] these data reinforce the hypothesis of a similar mechanism of action between these two drugs. Similar findings were published by Yamamoto and Yaksh,[36] who observed that NMDA receptor antagonist MK-801 potentiated the action of inoperative morphine in a chronic pain model. Christensen *et al.*[13] showed also in a mechanical test that a systemic pretreatment with a competitive NMDA receptor antagonist (HA-966) dose-dependently enhanced the effect of morphine in the CCI model. In accordance with these findings, some clinical studies[34,35] strengthen the hypothesis that magnesium could behave like a noncompetitive NMDA antagonist.

Our experimental results may have a direct application to pain management in patients. These results obtained with the formalin test suggest that magnesium, when used at pharmacologic doses in inflammatory pain, amplifies the analgesic properties of morphine. In this way, the coadministration of magnesium and morphine should allow a significant reduction in morphine administration for postoperative pain alleviation. Some clinical studies confirm[34,35] this hypothesis, but it must be said that others are less conclusive.[37,38] These discrepancies can be explained by the quantity of magnesium administered or by the route of administration. Above all, they can result from an inadequate anticipation of magnesium administration in relation to the surgical procedure, a factor of considerable importance if one refers to studies showing that magnesium does not cross the blood–brain barrier rapidly.[29]

The second possible area of application of the magnesium–morphine combination could be neuropathic pain management. Despite the fact that, here as well, certain results seem contradictory, the majority of clinical trials point to the unsatisfactory level of opiate efficiency in neuropathic pain.[39] We know that in this kind of pain, excitatory amino acids in general and NMDA receptor channels in particular have a key role. Some clinical trials have shown that noncompetitive NMDA receptor antagonists can have an effect when used alone but also can reveal the analgesic properties of morphine.[40] The use of currently available NMDA antagonists is unfortunately limited because of the nature and severity of clinical side effects, which are apparent when efficient doses are reached.[14] Given that magnesium possesses pharmacologic properties that are comparable to those of NMDA antagonists, it seems justified, on the basis of our experimental results, to test this association in patients with neuropathic pain.

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