Regional Anesthesia and Pain

Protective effect of prior administration of magnesium on delayed hyperalgesia induced by fentanyl in rats

[L'effet protecteur de l'administration préalable de magnésium sur l'hyperalgésie secondaire produite par le fentanyl chez le rat]

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Purpose: Magnesium exerts a physiological block of the ion channel on the N-methyl-D-aspartate receptor, and may therefore prevent the induction of central sensitization. The purpose of this study was to assess whether systemic magnesium can prevent long-lasting hyperalgesia induced by sc fentanyl administration in uninjured rats.

Methods: Long-lasting hyperalgesia was induced in male Sprague Dawley rats with sc fentanyl (four injections, 60 $\mu\text{g}\cdot\text{kg}^{-1}$ per injection at 15-min intervals). Magnesium sulphate (100 $\text{mg}\cdot\text{kg}^{-1}$) was injected *ip* 30 min prior to the first sc fentanyl injection. Sensitivity to nociceptive stimuli (paw-pressure test) was assessed for several days after injections.

Results: Subcutaneous fentanyl led to delayed hyperalgesia associated with a decrease in the nociceptive threshold lasting two days (35% decrease for the maximum effect). Intraperitoneal magnesium sulphate partially but significantly ($P < 0.05$) prevented the delayed decrease in the nociceptive threshold following sc administration of fentanyl.

Conclusions: This study shows that magnesium may prevent the delayed and prolonged hyperalgesia following fentanyl administration in rats.

Objectif: Le magnésium exerce un blocage physiologique du récepteur N-méthyl-D-aspartate, et peut donc prévenir l'induction d'une sensibilisation centrale. Le but de cette étude était de déterminer si l'administration systémique de magnésium pouvait prévenir l'hyperalgésie prolongée induite par le fentanyl sc chez le rat naïf.

Méthode: Une hyperalgésie prolongée était induite chez le rat mâle Sprague Dawley avec du fentanyl sc (quatre injections, 60 $\mu\text{g}\cdot\text{kg}^{-1}$ par injection à 15 min d'intervalle). Du sulfate de magnésium (100 $\text{mg}\cdot\text{kg}^{-1}$) était injecté par voie *ip* 30 min avant la première injection de fentanyl. La sensibilité aux stimuli nociceptifs (paw-pressure test) était évaluée durant plusieurs jours après les injections.

Résultats: Le fentanyl a entraîné une hyperalgésie prolongée associée à une diminution du seuil nociceptif durant deux jours (diminution de 35 % de l'effet maximum). L'administration *ip* de sulfate de magnésium a prévenu partiellement mais significativement ($P < 0.05$) la diminution du seuil nociceptif secondaire à l'administration sc de fentanyl.

Conclusion: Cette étude démontre que le magnésium peut prévenir l'hyperalgésie secondaire et prolongée après administration de fentanyl chez le rat.

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Accepted for publication May 29, 2006.

Revision accepted September 18, 2006.

Competing interests: None declared.

A growing body of evidence suggests that an active pronociceptive process is initiated by opioid administration.¹ Recent preclinical studies have shown that systemic opioid administration in rats can lead to a long-lasting reduction of nociceptive threshold, a sign indicating central sensitization.^{2–5} Decreased analgesia and abnormal pain (thermal hyperalgesia and tactile allodynia) after administration of opioids suggest that neuropathic pain and opioid induced abnormal pain sensitivity share common pathophysiologic mechanisms.^{6,7} Among these neural mechanisms the N-methyl-D-aspartate (NMDA) receptor has been shown to play a critical role.^{8–10}

Magnesium exerts a physiological block of the ion channel on the NMDA receptor and is considered as a natural NMDA receptor antagonist.¹¹ Such NMDA antagonism may therefore prevent the induction of central sensitization,¹² and, thus, is likely to prevent opioid-induced pain sensitivity. The magnesium block is removed as part of the molecular sensitization process and we hypothesized that systemically administered magnesium could reduce sensitization following opioid-induced pain sensitivity by maintaining channel block at the NMDA receptor.

The purpose of this study was to investigate whether systemic magnesium might prevent long-lasting hyperalgesia induced by *sc* fentanyl administration in uninjured rats.

Methods

Animals

Experiments were performed on adult male Sprague-Dawley rats (CD1 Charles River; IFFA-CREDO, L'Arbresle, France; weight, 250–275 g at the beginning of the experiment) housed in individual standard cages under a 12-hr light 12-hr dark cycle (lights on at 8.00 AM) at a constant room temperature of $22 \pm 2^\circ\text{C}$ one week before experiments. Animals had access to food (Harland Teklod, Blackthorn, Bicester, Oxon, England) and water *ad libitum*. Experiments were approved by the Institution's Animal Care and Use Committee and were carried out in accordance with guidelines from the International Association for the Study of Pain Committee for Research and Ethical Issues.¹³

Drugs

The following drugs were used: magnesium (magnesium sulphate; Sigma-Aldrich Co., Saint Quentin Fallavier, France) and fentanyl (fentanyl; Sigma-Aldrich Co., Saint Quentin Fallavier, France). All drugs were diluted in physiologic saline (0.9%). Fentanyl

was injected subcutaneously ($100 \mu\text{L} \cdot 100 \text{ g}^{-1}$ body weight). Subcutaneous injections were performed on the back of unanesthetized rats with a 25-G needle. Magnesium was injected *ip* ($2 \text{ mL} \cdot \text{kg}^{-1}$). Control animals received an equal volume of saline.

Measurement of nociceptive threshold

The threshold of response to increasing pressure was measured by a modification of the Randall–Selitto method,¹⁴ the paw-pressure vocalization test, with the use of an Analgesy Meter (Ugo Basile, Biosed, Camerio, Milan, Italy). The right hindpaw was positioned under a pressure pad, the probe tip (diameter 1 mm) being applied at the metacarpal level between the third and the fourth finger. A constantly increasing pressure was applied until the rat squeaked. A 600-g cutoff value was used to prevent tissue damage.^{4,15} The experiments were performed in a quiet room by the same investigator blinded to the treatment used.

General procedure

After arrival in the laboratory, animals were allowed five days to become accustomed to the colony room, gently handled daily for five minutes, and left in the test room for two hours (from 11:00 AM to 1:00 PM). All experiments began at 11:00 AM and were performed during the light part of the cycle. As previously described,⁴ to ensure nociceptive threshold stability, the basal nociceptive threshold was measured twice (with 30 min between the measurements) on the two days preceding the planned experimental day (D_{-2} and D_{-1}). On the experimental day (D_0), the basal nociceptive threshold was also determined twice before drug injections (30 min between measurements). Experiments with fentanyl were initiated only if no statistical changes were observed in basal nociceptive thresholds when estimated on days D_{-2} , D_{-1} , and D_0 . The rats were assigned randomly to the different experimental groups, and the investigator was unaware of the treatment used.

Experimental protocol

The study was performed in two phases. First, a study was performed to assess the action of systemic magnesium on fentanyl-induced hyperalgesia. Second, serum magnesium concentration, red cell magnesium concentration, and cerebrospinal fluid (CSF) magnesium concentration were measured in the fentanyl-induced hyperalgesic phase in naïve and magnesium-treated rats.

In phase I, there were four groups of animals. There were eight animals per group. According to the study of Célèrier *et al.*,⁴ and in order to induce a

long-lasting hyperalgesia, fentanyl was injected four times ($60 \mu\text{g}\cdot\text{kg}^{-1}$ per injection) at 15-min intervals resulting in a total dose of $240 \mu\text{g}\cdot\text{kg}^{-1}$ (groups A and B). Supplemental O_2 $3\text{--}4 \text{ L}\cdot\text{min}^{-1}$ was administered via a facemask throughout the procedure. Magnesium sulphate ($100 \text{ mg}\cdot\text{kg}^{-1}$) was injected *ip* 30 min prior to the first *sc* fentanyl injection in group A whereas *ip* saline was injected in group B. As described previously *ip* magnesium sulphate $100 \text{ mg}\cdot\text{kg}^{-1}$ was unlikely to induce signs of toxicity.¹⁶ Control groups received *sc* saline with *ip* magnesium sulphate (group C) or *sc* saline with *ip* saline (group D). On D_0 , the nociceptive threshold was estimated every 30 min for a period of 240–360 min after the last fentanyl injection. Subsequent to D_0 , the nociceptive threshold was measured twice daily (30 min between both measurements) for five days ($\text{D}_{+1}\text{--}\text{D}_{+5}$).⁴

In phase II, there were two groups of animals. There were eight animals per group. According to the same protocol as in phase I, delayed hyperalgesia was induced in both groups with fentanyl. One group received *ip* magnesium, whereas the control group received *ip* saline. Twenty-four hours after *ip* injections, a lethal dose of *ip* pentothal was administered, and blood samples as well as CSF samples were collected from the rat's heart and at the level of the cisterna magna, respectively.

Data and statistical analysis

The mean of the two measurements performed daily on days D_{-2} , D_{-1} , and D_0 were compared [one-way analysis of variance (ANOVA) for repeated measures]. The basal reference value of the nociceptive threshold was chosen as the first measurement of the nociceptive threshold performed on day D_0 . Normal distribution was verified with the Kolmogorov-Smirnov test. The nociceptive threshold was compared between groups and between days (D_0 , D_{+1} to D_{+5}) using ANOVA (two-way, for repeated measures) followed by a Neuman-Keuls test as appropriate and unpaired Student's *t* tests were used to assess comparisons between serum, red cells, and CSF magnesium concentrations. Data are expressed as the mean $\pm$ SD, and $P < 0.05$ was considered statistically significant.

Results

No statistically significant differences were found among the basal nociceptive thresholds of each experimental group: $340 \pm 40 \text{ g}$ ($n = 8$), $350 \pm 40 \text{ g}$ ($n = 8$), $340 \pm 30 \text{ g}$ ($n = 8$), $340 \pm 10 \text{ g}$ ($n = 8$) for groups A, B, C, and D respectively (one-way ANOVA, $P > 0.05$). The mean baseline nociceptive threshold value was $340 \pm 30 \text{ g}$ ($n = 32$).

FIGURE 1 Short-lasting effect (A) and long-lasting effect (B) induced by *sc* fentanyl $60 \mu\text{g}\cdot\text{kg}^{-1} \times 4$ compared with that of *sc* saline as measured by the paw-pressure vocalization test ($n = 8$ for each group). Mean nociceptive thresholds ($\pm$ SD) are expressed in grams. * $P < 0.05$ (one-way ANOVA) compared with the basal nociceptive threshold value. Fentanyl or saline were injected four times at 15-min intervals. Intraperitoneal saline was injected 30 min prior to the first *sc* fentanyl or saline injection.

Effects of *sc* fentanyl on the nociceptive threshold

Subcutaneous fentanyl ($4 \times 60 \mu\text{g}\cdot\text{kg}^{-1}$) first caused a statistically significant short-lasting increase in nociceptive thresholds ($P < 0.05$; Figure 1A), and induced a statistically significant decrease in nociceptive thresholds on D_{+1} and D_{+2} ($P < 0.05$; Figure 1B). In contrast, *sc* saline did not alter the nociceptive threshold throughout the experiment ($P > 0.05$; Figures 1A and 1B).

Effects of *ip* magnesium pretreatment on the *sc* fentanyl induced changes in nociceptive thresholds

Intraperitoneal magnesium $100 \text{ mg}\cdot\text{kg}^{-1}$ did not modify the short-lasting effect ($P > 0.05$; Figure 2A), but significantly reduced the long-lasting decrease in nociceptive thresholds of *sc* fentanyl on D_{+1} and D_{+2} ($P < 0.05$; Figure 2B).

Effects of *ip* magnesium alone or *ip* saline alone on nociceptive thresholds in naïve rats

Neither *ip* magnesium $100 \text{ mg}\cdot\text{kg}^{-1}$ nor *ip* saline altered the nociceptive threshold from D_0 to D_{+5} ($P > 0.05$; Figure 3).

FIGURE 2 Effects of *ip* magnesium pretreatment *vs ip* saline pretreatment on the short-lasting effect (A) and on the long-lasting effect (B) induced by *sc* fentanyl 60 $\mu\text{g}\cdot\text{kg}^{-1} \times 4$ as measured by the paw-pressure vocalization test ($n = 8$ for each group). Mean nociceptive thresholds ($\pm$ SD) are expressed in grams. * $P < 0.05$ (one-way ANOVA) compared with the basal nociceptive threshold value for the magnesium group. # $P < 0.05$ (one-way ANOVA): long-lasting hyperalgesia induced by *sc* fentanyl 60 $\mu\text{g}\cdot\text{kg}^{-1} \times 4$ after *ip* saline pretreatment *vs* after *ip* magnesium pretreatment. Fentanyl was injected four times at 15-min intervals. Intraperitoneal saline or magnesium were injected 30 min prior to the first *sc* fentanyl injection.

Magnesium concentrations

Serum magnesium concentration, red cell magnesium concentration, and CSF magnesium concentration were similar in both groups (Table).

Discussion

The main finding of this study is that systemic magnesium sulphate partially prevented the delayed hyperalgesia initiated by systemic fentanyl in uninjured rats.

Clerier *et al.* have demonstrated that systemic administration of fentanyl in uninjured rats induced delayed hyperalgesia, a sign indicating central sensitization, by activating NMDA pain facilitatory processes.⁴ Likewise, a growing body of evidence indicates that pronociceptive processes associated with central sensitization are associated primarily with amino acid activity at the NMDA receptor level,^{8–10} and studies have shown that opioid induced hyperalgesia is prevented by the NMDA receptor antagonists ketamine

FIGURE 3 Effects of *ip* magnesium *vs ip* saline pretreatment on the nociceptive threshold for days in opioid-nave rats as measured by the paw-pressure vocalization test ($n = 8$ for each group). Mean nociceptive thresholds ($\pm$ SD) were expressed in grams. There were no statistical differences between groups. Saline was injected four times at 15-min intervals. Intraperitoneal saline or magnesium were injected 30 min prior to the first *sc* saline injection.

TABLE

Magnesium concentrations (mmol·L ⁻¹) in serum, red cell, and CSF		
	Control group	Magnesium group
Serum magnesium concentration	1.12 $\pm$ 0.52	0.97 $\pm$ 0.14
Red cell magnesium concentration	2.65 $\pm$ 0.29	2.58 $\pm$ 0.51
CSF magnesium concentration	0.92 $\pm$ 0.5	0.91 $\pm$ 0.04

CSF = cerebrospinal fluid. Values are mean $\pm$ SD. There were no significant differences between groups.

and MK-801.^{4,17,18} This is in accordance with previous studies which demonstrate that opioid-induced central sensitization and neuropathic pain share common pathophysiologic mechanisms.^{6,7} On the other hand, Begon *et al.* have shown in a preclinical study in rats that systemic magnesium and systemic MK-801 similarly reverse mechanical hyperalgesia in diabetic and mono-neuropathic rats.¹⁹ In agreement with these data, the present study therefore suggests that magnesium may prevent opioid-induced central sensitization through physiological block of the NMDA receptor.

Our study is also in accordance with preclinical studies, which report that intrathecal magnesium suppresses neuropathic pain responses in different rat models,^{20–22} and delays the development of tolerance when co-

administered with opioids.²³ However, concern may arise as to the actual effect of systemically administered magnesium at the spinal level. Magnesium crosses the blood-brain barrier by active transport.²⁴ Even when *iv* magnesium is given in large doses no significant amount crosses the blood-brain barrier.²⁵

The phase II of our study failed to demonstrate any significant difference in serum, red cell, and CSF concentrations of magnesium when measured 24 hr after *ip* injections of magnesium or saline after fentanyl-induced hyperalgesia in uninjured rats. We chose to measure magnesium concentrations at that time because the maximum decrease in nociceptive threshold has been shown to occur on days D₊₁ in this model of opioid-induced delayed hyperalgesia.⁴ This is in accordance with the results of Ko *et al.* who recently measured CSF magnesium concentration and serum magnesium concentration after perioperative *iv* magnesium sulphate infusion or placebo in patients undergoing abdominal hysterectomy.²⁶ Although the serum magnesium concentration was significantly reduced in the control group, and whereas the serum magnesium concentration of the magnesium group exceeded twice the serum concentration observed in the control group, the CSF magnesium concentrations were similar in the two groups. However, a limitation of the study of Ko *et al.* and of our results is that magnesium concentrations in the CSF were measured only once. Therefore, CSF magnesium concentration was not compared in dependent samples in each group, and further studies evaluating the long-term dynamic relationship between CSF and serum magnesium concentrations are warranted. Furthermore, previous clinical studies have shown that systematically administered magnesium is effective in the management of neuropathic pain, and is therefore likely to act at the spinal level.^{27,28}

Despite these observations, even if a spinal action of systemic magnesium on opioid-induced hyperalgesia exists, other mechanisms cannot be excluded. Magnesium is involved in several processes including gating of calcium channels.¹¹ Conversely, impairment of motor function might have affected the results of the Randall-Selitto test. However, when systemic magnesium was administered alone there was no effect on the nociceptive threshold, and no motor impairment modified the Randall-Selitto test compared with saline (Figure 3). Cognitive impairment and impairment of vigilance have not been assessed in the study. A possible role of cognitive or vigilance impairment cannot therefore be excluded. On the other hand, hypercapnia subsequent to fentanyl administration was not assessed during the phase of increase in nociceptive

threshold on D₀. Potential prolonged neurological side-effects of hypercapnia interfering with nociceptive threshold assessment on days D₊₁ to D₊₅ cannot be ruled out.

In conclusion, our study shows that systemic magnesium administration confers a partial but significant prevention of opioid induced secondary hyperalgesia following systemic fentanyl in uninjured rats. Although these findings are encouraging, mechanistic clarifications are needed, and further evaluation of side effects and dose range studies are warranted before clinical applications can be considered.

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