Gabapentin Prevents Delayed and Long-lasting Hyperalgesia Induced by Fentanyl in Rats

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Background: Opioid-induced hyperalgesia can develop rapidly after opioid exposure. Neuropathic pain and opioid-induced hyperalgesia share common pathophysiologic mechanisms. Gabapentin is effective for the management of neuropathic pain and may therefore prevent opioid-induced hyperalgesia. This study tested the effectiveness of gabapentin for prevention of long-lasting hyperalgesia induced by acute systemic fentanyl in uninjured rats. Involvement of the $\alpha_2\delta$ auxiliary subunits of voltage-gated calcium channels in the prevention of opioid-induced hyperalgesia by gabapentin also was assessed.

Methods: Hyperalgesia was induced in male Sprague-Dawley rats with subcutaneous fentanyl (four injections, 20, 60, or 100 μg/kg per injection at 15-min intervals). Intraperitoneal (30, 75, 150, or 300 mg/kg) or intrathecal (300 μg) gabapentin was administered 30 min before or 300 min after (intraperitoneal 150 mg/kg) the first fentanyl injection. Sensitivity to nociceptive stimuli (paw-pressure test) was assessed on the day of the experiment and for several days after injections. The effects combining gabapentin with intrathecal ruthenium red (20 ng) also were assessed.

Results: Fentanyl administration was followed by an early increase (analgesia) and by a later and sustained decrease (hyperalgesia) in nociceptive thresholds. Gabapentin did not significantly modify the early analgesic component but dose-dependently prevented the delayed decrease in nociceptive threshold. Ruthenium red partially, but significantly, opposed the prevention of opioid-induced hyperalgesia by gabapentin.

Conclusions: Intraperitoneal and intrathecal gabapentin prevents the development of hyperalgesia induced by acute systemic exposure to opioids. This prevention may result, at least in part, from binding of gabapentin to the $\alpha_2\delta$ auxiliary subunits of voltage-gated calcium channels.

ACCUMULATING evidence suggests that administration of opioids leads not only to analgesia, but also to an enhancement in pain sensitivity induced by central sensitization.[1] This phenomenon is referred to as *opioid-induced hyperalgesia* (OIH). Preclinical studies have demonstrated that long-lasting hyperalgesia may occur even after acute administration of opioids in uninjured

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rats,[2,3] and recent clinical studies have shown that opioid-induced pain sensitivity can develop rapidly after short-term exposure in uninjured humans[4] and therefore may, paradoxically, facilitate postoperative pain.[5,6] Decreased analgesia and abnormal pain (thermal hyperalgesia and tactile allodynia) occurring after administration of opioids suggest that neuropathic pain and opioid-induced abnormal pain sensitivity share common pathophysiologic mechanisms.[7] Drugs effective for the management of neuropathic pain are therefore likely to be effective for the prevention of OIH.

Gabapentin, a 3-alkylated analogue of γ-aminobutyric acid, was originally developed as an adjunct for the management of epileptic seizures.[8] Subsequent trials established that gabapentin is also effective for the management of neuropathic pain,[9] and reduces pain, hyperalgesia, and allodynia after tissue or nerve injury through several possible mechanisms.[10] This suggests that gabapentin might be effective for the prevention of OIH. However, a preemptive effect of gabapentin on OIH after acute administration has never been studied.

Fentanyl has been shown to induce hyperalgesia which may last for days after acute systemic administration in uninjured rats.[3] Consequently, the aim of this study was to test the hypothesis that gabapentin could prevent OIH after acute systemic administration of fentanyl in uninjured rats. Prevention of long-lasting hyperalgesia after systemic fentanyl was assessed after either systemic or intrathecal administration of gabapentin. Gabapentin acts by binding to the $\alpha_2\delta$ subunit of spinal voltage-gated calcium channel (VGCC).[11–13] Ruthenium red has been shown to modulate gabapentin binding to the $\alpha_2\delta$ subunit in mouse cerebral cortex and to inhibit the antiallodynic effect of gabapentin.[14] To explore whether the $\alpha_2\delta$ subunit of spinal voltage-gated calcium channel is involved in the preventive effect of gabapentin, a second purpose of the current study was created to further examine whether ruthenium red affects the prevention of OIH by gabapentin.

Materials and Methods

Animals

Experiments were performed in male Sprague-Dawley rats (250–275 g; CD1 Charles River, IFFA-CREDO, L'Arbresle, France) housed in groups of three per cage, under a 12-h light, 12-h dark cycle (lights on at 8:00 AM), at a constant room temperature of 22° ± 2°C, 1 week before experiments. Animals had access to food and water *ad libitum*. Experiments were approved by the

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Institution's Animal Care and Use Committee and were performed in accordance with guidelines from the International Association for the Study of Pain Committee for Research and Ethical Issues.[15] This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. At the end of the experiment, the rats were killed with isoflurane. Accordingly, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (J.-X.M.).

Drugs

The following drugs were used for the experiment: fentanyl citrate, ruthenium red, and carrageenan (Sigma-Aldrich Co., Saint Quentin Fallavier, France), gabapentin (Sigma-Aldrich Co., Steinheim, Germany), and lidocaine (Astra-Zeneca, Rueil-Malmaison, France). Gabapentin was stored in an opaque container. Gabapentin, carrageenan, and lidocaine were diluted with physiologic saline (0.9%). To induce OIH, fentanyl was injected subcutaneously (100 μl/100 g body weight) on the back of nonanesthetized rats with a 25-gauge needle. To induce localized inflammatory pain, carrageenan (0.2 ml of a 1% solution of carrageenan in saline) was subcutaneously injected in the right hind paw. To facilitate injection, the animals were placed in a plastic cage, and the injected paw was pulled through a hole at the base of the cage; all the injections were performed with a 25-gauge needle. For those animals given intrathecal drugs, a single intrathecal injection was accomplished by lumbar puncture between L4 and L5 as previously described.[16] The skin of the back was shaved in the lumbar region and prepared with 10% povidone-iodine. Rats were briefly anesthetized with isoflurane. To flex the lower thoracic and lumbar vertebral column, foam was placed under the animal's abdomen. The dead space of the 25-gauge hypodermic needle hub used for puncture was 25 μl. Before intrathecal injection, the needle hub was flushed with 25 μl of the mixture to be injected. A reflexive flick of the tail was considered to be a sign of the accuracy of each injection. To ascertain correct intrathecal injection, 20 μl lidocaine (2%, 400 μg) was injected with gabapentin, ruthenium red diluted in 10 μl saline, or 10 μl plain saline, resulting in a total volume of 30 μl. Only animals that developed transient (10- to 20-min) bilateral hind limb motor weakness or paralysis within 30 s of the injection were included in the study. Animals with persistent motor deficiency lasting more than 30 min after injection were rejected from further study. For those animals given intraperitoneal drugs, a single intraperitoneal injection was accomplished by abdominal puncture using a 25-gauge hypodermic needle. In preparation of injection, the skin of the abdomen was shaved and prepared with 10% povidone-iodine. Control animals received an equal volume of subcutaneous, intraperitoneal, or intrathecal saline injections.

Measurement of Nociceptive Threshold

Mechanical hyperalgesia was measured in handheld rats as the threshold of response to increasing pressure by a modified Randall-Selitto method,[17] the paw-pressure test, using an analgesimeter (Ugo Basile, Biosed, Camerio, Milan, Italy). The right hind paw was positioned under a pressure pad, and the probe tip (diameter 1 mm) was applied at the metacarpal level between the third and fourth fingers. A constantly increasing pressure was applied to the right hind paw of the rat to determine the minimum stimulus necessary to evoke an obvious nociceptive response (sharp paw withdrawal). A 600-g cutoff value was used to prevent tissue damage.[3] The experiments were performed in a quiet room by the same investigator (A.V.E.) who was blinded to the treatment that was given.

General Procedure

After arrival in the laboratory, animals were allowed 5 days to become accustomed to the colony room, were gently handled daily for 5 min, and left in the test room for 2 h (from 11:00 AM to 1:00 PM). All experiments began at 11.00 AM and were performed in groups of 6–12 animals during the light part of the cycle. As previously described,[3] to ensure nociceptive threshold stability, the basal nociceptive threshold was measured twice (with 30 min between the measurements) on the 2 days preceding the planned experimental day (i.e., D_{-2} and D_{-1}) and was repeated on the experimental day (D_0). Two comparisons were performed: between the first and second measurements of the nociceptive threshold performed daily on days D_{-2}, D_{-1}, and D_0 before treatment (Student t test, $P > 0.05$) and among the first daily measurements on days D_{-2}, D_{-1}, and D_0 (one-way analysis of variance, $P > 0.05$). Experiments with fentanyl and gabapentin were initiated only when no statistical changes in basal nociceptive thresholds were observed when estimated on days D_{-2}, D_{-1}, and D_0 (one-way analysis of variance, $P > 0.05$).[3] The reference value of each pain parameter was chosen as the basal value on D_0.

On D_0, the nociceptive threshold was estimated every 30 min for a period of 240–300 min after the last fentanyl injection. The nociceptive threshold was then measured twice daily (30 min between measurements) for 5 days (D_{+1}–D_{+5}).[3]

Experimental Protocol

In a first set of experiments, according to the study of Célèrier et al.,[3] fentanyl was injected four times (60 μg/kg per injection) at 15-min intervals, resulting in a total dose of 240 μg/kg administered over 1 h to induce long-lasting hyperalgesia. Supplemental oxygen was ad-

Table 1. Summary of Treatment Groups with Intraperitoneal Gabapentin

Group	Subcutaneous Fentanyl	Subcutaneous Saline	Intraperitoneal Gabapentin	Intraperitoneal Saline	Carrageenan
F60-IPG30	+	−	+	−	−
F60-IPG75	+	−	+	−	−
F60-IPG150	+	−	+	−	−
F60-IPG300	+	−	+	−	−
F60-PRE-IPG	+	−	+	−	−
F60-POST-IPG	+	−	+	−	−
F60-IPS	+	−	−	+	−
S-IPG	−	+	+	−	−
S-IPS	−	+	−	+	−
CAR-F100-IPG	+	−	+	−	+
CAR-F100-IPS	+	−	−	+	+
CAR-S-IPG	−	+	+	−	+
CAR-S-IPS	−	+	−	+	+

Treatment given in the 13 groups. Fentanyl or saline was injected subcutaneously. Gabapentin or saline was injected intraperitoneally. Gabapentin was injected either before or after fentanyl injections. Intraplantar injection of carrageenan was performed in the right hind paw.

CAR = carrageenan; F = fentanyl; IPG = intraperitoneal gabapentin; IPS = intraperitoneal saline; S = saline.

ministered *via* facemask throughout the procedure. To evaluate the possible site of action (central or peripheral) of gabapentin, separate groups of animals received either an intraperitoneal injection or an intrathecal injection of gabapentin. To assess potential early and long-term effects on nociceptive thresholds of intraperitoneal gabapentin or intraperitoneal saline, experiments were conducted with intraperitoneal gabapentin (group F60-IPG) and intraperitoneal saline (group F60-IPS) (table 1). To assess the potential early and long-term effects on the nociceptive thresholds of intrathecal gabapentin + lidocaine or intrathecal saline + lidocaine, experiments were conducted with intrathecal gabapentin + lidocaine (group F60-ITG) and intrathecal saline + lidocaine (group F60-ITS) (table 2). Control groups consisted of subcutaneous saline and intraperitoneal gabapentin (group S-IPG) (table 1), subcutaneous saline and intraperitoneal saline (group S-IPS) (table 1), subcutaneous saline and intrathecal gabapentin + lidocaine (group S-ITG) (table 2), and subcutaneous saline and intrathecal saline + lidocaine (group S-ITS) (table 2). Intraperitoneal or intrathecal gabapentin was administered 30 min before the first subcutaneous injection of fentanyl. Given the interrelations between the neural mechanisms underlying hyperalgesia and morphine tolerance,[7] the

doses of intraperitoneal and intrathecal gabapentin were chosen according to previous studies in which 150 mg/kg intraperitoneal gabapentin[18] and 300 μg intrathecal gabapentin[19] had been shown to prevent morphine tolerance in rats. Intraperitoneal gabapentin was diluted in 1 ml saline, whereas intrathecal gabapentin was diluted in 10 μl saline. Control animals received an equal volume of intraperitoneal or intrathecal saline.

To study the effects of various doses of gabapentin on fentanyl-induced hyperalgesia, an additional experiment was conducted with intraperitoneal gabapentin at 30 mg/kg (group F60-IPG30), 75 mg/kg (group F60-IPG75), or 300 mg/kg (group F60-IPG300) (table 1).

To investigate the possible long-term effects on nociceptive thresholds of the timing of gabapentin administration, intraperitoneal gabapentin at 150 mg/kg was administered either 30 min before the first subcutaneous fentanyl injection (group F60-PRE-IPG) or 300 min after the last subcutaneous fentanyl injection (group F60-POST-IPG) (table 1).

To assess whether gabapentin enhances the analgesic effect of a lower dose of fentanyl, subcutaneous fentanyl was injected four times (20 μg/kg per injection) at 15-min intervals, resulting in a total dose of 80 μg/kg administered over 1 h. The experiment was conducted

Table 2. Summary of Treatment Groups with Intrathecal Gabapentin

Group	Subcutaneous Fentanyl	Subcutaneous Saline	Intrathecal Gabapentin	Intrathecal Saline	Intrathecal Ruthenium
F60-ITG	+	−	+	−	−
F60-ITS	+	−	−	+	−
S-ITG	−	+	+	−	−
S-ITS	−	+	−	+	−
F60-ITG-ITRuth	+	−	+	−	+
F60-ITS-ITRuth	+	−	−	+	+
F60-ITG-ITS	+	−	+	+	−
F60-ITS	+	−	−	+	−

Treatment given in the eight groups. Fentanyl or saline was injected subcutaneously. Gabapentin or saline was injected intrathecally. Ruthenium red or saline was injected intrathecally with gabapentin.

F = fentanyl; ITG = intrathecal gabapentin; ITRuth = intrathecal ruthenium red; ITS = intrathecal saline; S = saline.

with either intraperitoneal gabapentin (group F20-IPG) or intraperitoneal saline (F20-IPS) (table 1).

To evaluate the potential effects of intraperitoneal gabapentin on hyperalgesia induced by a higher dose of subcutaneous fentanyl (four injections at 15-min intervals, 100 μg/kg per injection) in a rat model of inflammatory pain, intraplantar carrageenan injection was performed after the first subcutaneous fentanyl injection. Experiments were conducted with 150 mg/kg intraperitoneal gabapentin (group CAR-F100-IPG) and intraperitoneal saline (group CAR-F100-IPS) (table 1). Control groups consisted of intraplantar carrageenan, subcutaneous saline, and intraperitoneal gabapentin (group CAR-S-IPG), and intraplantar carrageenan, subcutaneous saline, and intraperitoneal saline (group CAR-S-IPS) (table 1).

In a second set of experiments, we studied the potential effect of ruthenium red on OIH prevention by gabapentin to test the hypothesis that gabapentin could prevent OIH by interfering with the $\alpha_2\delta$ subunit of spinal VGCC. To induce long-lasting hyperalgesia, subcutaneous fentanyl was injected four times (60 μg/kg per injection) at 15-min intervals, resulting in a total dose of 240 μg/kg administered over 1 h. Intrathecal gabapentin, 300 μg, and 20 ng intrathecal ruthenium red were administered 30 min before the first fentanyl injection (group F60-ITG-ITRuth) (table 2). The dose of ruthenium red was chosen according to a previous study where 20 ng intrathecal ruthenium red attenuated the antiallodynic effect of 300 μg intrathecal gabapentin in a rat model of postoperative pain.[14] Control groups consisted of intrathecal saline and intrathecal ruthenium red (group F60-ITS-ITRuth), intrathecal gabapentin and intrathecal saline (group F60-ITG-ITS), and intrathecal saline (group F60-ITS) (table 2).

Data and Statistical Analysis

The means of the two measurements performed daily on days D_{-2}, D_{-1}, and D_0 were compared (one-way analysis of variance for repeated measures). The basal reference value of the nociceptive threshold was chosen as the first measurement of the nociceptive threshold performed on D_0. Normal distribution was verified with the Kolmogorov-Smirnov test. The stability of the control group over time was checked using one-way analysis of variance for repeated measures. The nociceptive threshold was compared between groups and between days (D_0, D_{+1}–D_{+5}) using analysis of variance (two way, for repeated measures) followed by a Dunnett test as appropriate. The presence of an interaction (time × groups) was considered for assessing the difference between groups.

To evaluate the time-course effects of treatments on the nociceptive threshold, a one-way analysis of variance followed by post hoc analysis using the Newman-Keuls test was performed on D_0; another analysis was performed on each of the groups on the days after treatment.

For further analysis of the effects of various doses of gabapentin on hyperalgesia induced by fentanyl, an algesic index (i.e., amplitude of hyperalgesia) was determined for each experimental group. As already described,[3] this index was represented by the mean of the surface areas of trapezia evaluated for each rat. Surface areas were calculated by summing the nociceptive threshold values measured every day after D_0. Paired saline group–gabapentin group comparisons of algesic indices on days D_{+1} to D_{+5} after various intraperitoneal doses of gabapentin were performed using the Dunnett test.

To evaluate the amplitude of the effects in experiments using different pharmacologic treatments in carrageenan-treated rats and in ruthenium red–treated rats, algesic indexes were expressed as a mean percentage ± SD of the reference index (100%: algesic index associated with hyperalgesia observed in the saline-treated rats); the Mann–Whitney test was used to compare the algesic indexes.

Data are expressed as mean ± SD, and $p < 0.05$ was considered statistically significant.

All analyses were performed with the statistical package StatView (Stat-View for Windows and Macintosh Version 5.0; Abacus Concepts, Inc., Berkeley, CA).

Results

No statistically significant differences were found among the basal nociceptive thresholds of each experimental group (one-way analysis of variance, $P > 0.05$). The mean baseline nociceptive threshold value was 340 ± 4 g (n = 179). No rat was excluded because of inadequate motor weakness due to the intrathecal lidocaine injection. Experiments were not initiated in seven rats because of an instable threshold baseline before fentanyl treatments. A significant interaction (group × time) was found between carrageenan-treated rats and ruthenium red–treated rats.

Preventive Effect of Gabapentin on Long-lasting Hyperalgesia Induced by Fentanyl

As expected, subcutaneous administration of fentanyl on D_0 (four boluses of 60 μg/kg) (figs. 1A and B) but not subcutaneous saline (figs. 1C and D) caused a statistically significant short-lasting increase in nociceptive thresholds (analgesia) for 2 h followed by a statistically significant long-lasting decrease in nociceptive thresholds on D_{+1} and D_{+2} (one-way analysis of variance, $P < 0.05$).

Intraperitoneal (fig. 1A) and intrathecal (fig. 1B) gabapentin did not modify the short-lasting analgesic effect of subcutaneous fentanyl (one-way analysis of variance, $P > 0.05$) but prevented the delayed decrease in nociceptive threshold (one-way analysis of variance, $P <$
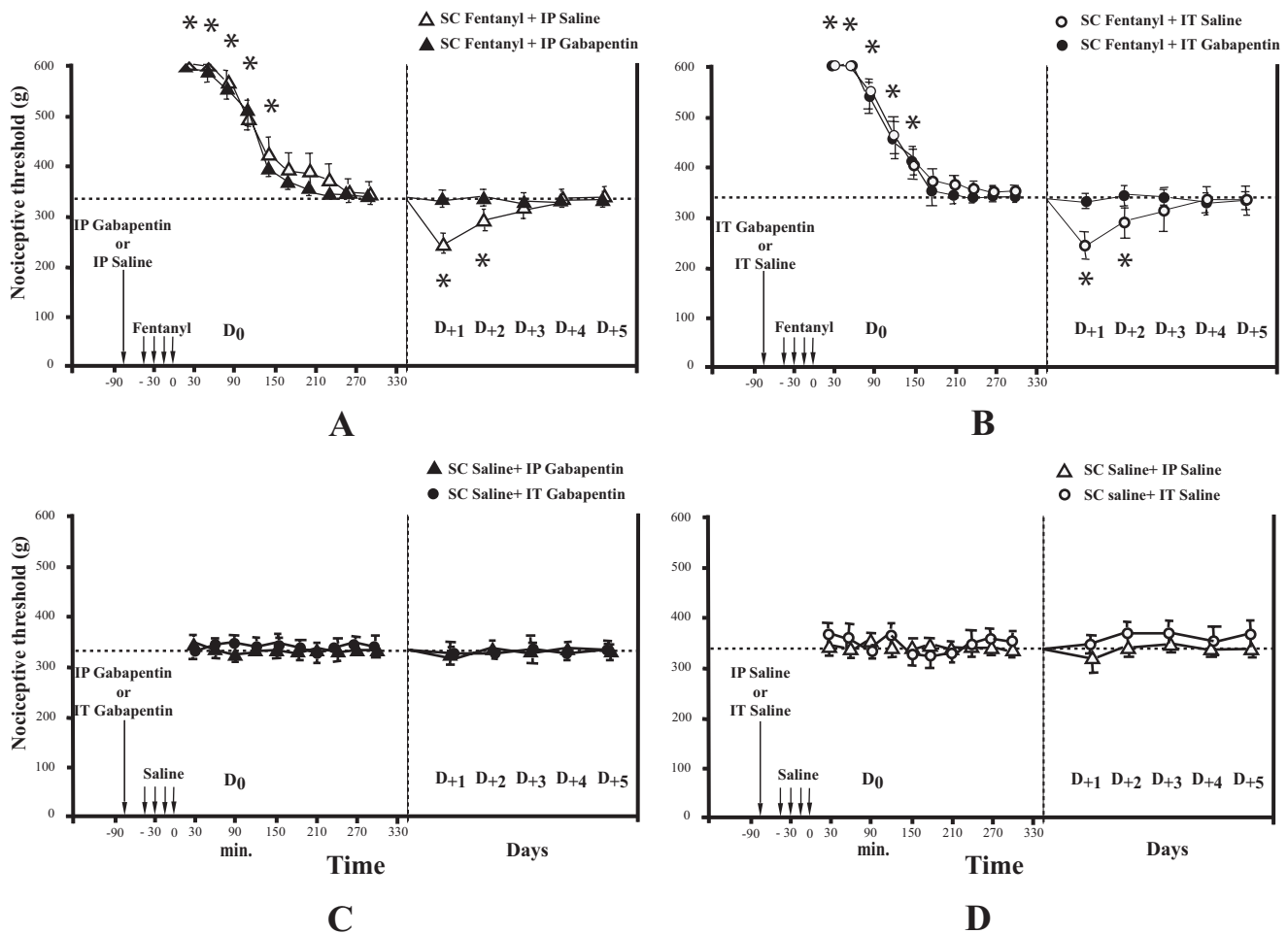
Fig. 1. Effect of gabapentin on delayed hyperalgesia induced by subcutaneous fentanyl in uninjured rats. Four fentanyl (60 μg/kg; *A* and *B*) or saline (1 ml/kg; *C* and *D*) injections were performed every 15 min on the day of the test (D_0). Intraperitoneal gabapentin (150 mg/kg; *A–C*), intrathecal gabapentin (300 μg; *B* and *C*), intraperitoneal saline (1 ml; *A–D*), or intrathecal saline (10 μl; *B–D*) was administered 30 min before the first subcutaneous injection. The basal nociceptive threshold was evaluated after treatment for 5 h on D_0 and then daily for 5 days (D_{+1}–D_{+5}). (*A*) Subcutaneous fentanyl and intraperitoneal gabapentin (n = 10) or saline (n = 8). (*B*) Subcutaneous fentanyl and intraperitoneal gabapentin (n = 10) or saline (n = 8). (*C*) Subcutaneous saline and intraperitoneal (n = 8) or intrathecal (n = 8) gabapentin. (*D*) Subcutaneous saline and intraperitoneal (n = 6) or intrathecal (n = 6) saline. Mean nociceptive thresholds (±SD) are expressed in grams. * $P < 0.05$ with the Newman-Keuls test when compared with the basal nociceptive threshold value. IP = intraperitoneal; IT = intrathecal; SC = subcutaneous.

0.05; figs. 1A and B). Neither intraperitoneal nor intrathecal gabapentin alone altered the nociceptive threshold from D_0 to D_{+5} (one-way analysis of variance, $P > 0.05$; fig. 1C).

Neither subcutaneous nor intraperitoneal nor intrathecal saline altered the nociceptive threshold from D_0 to D_{+5} (one-way analysis of variance, $P > 0.05$; fig. 1D).

As shown by evaluation of the algesic indices, the higher the gabapentin dose used, the less pronounced the delayed effect of fentanyl; therefore, gabapentin prevented hyperalgesia in a dose-dependent manner (fig. 2). Further analysis indicated that the algesic indices obtained with 75, 150, and 300 mg/kg gabapentin were statistically different from that observed with saline.

Variations of the nociceptive threshold after administration of gabapentin either before or after fentanyl injections are shown in figure 3. No statistically significant changes in nociceptive threshold were observed for sev-

eral days (D_{+1} to D_{+5}) in both groups (one-way analysis of variance, $P > 0.05$).

The effects of gabapentin pretreatment on the early analgesic effect of a low dose of fentanyl are shown in figure 4. The analgesic effect of the combined bolus injections of 4 × 20 μg/kg subcutaneous fentanyl and 150 mg/kg intraperitoneal gabapentin was not enhanced compared with that observed with fentanyl alone (two-way analysis of variance, $P > 0.05$).

Preventive Effect of Gabapentin on Fentanyl Enhancement of Carrageenan-induced Long-lasting Hyperalgesia

As expected, carrageenan induced a decrease in the nociceptive threshold in the injected hind paw 4 h after administration (one-way analysis of variance, $P < 0.05$; fig. 5A). A long-lasting hyperalgesia was induced, as revealed by a sustained decrease in the nociceptive threshold
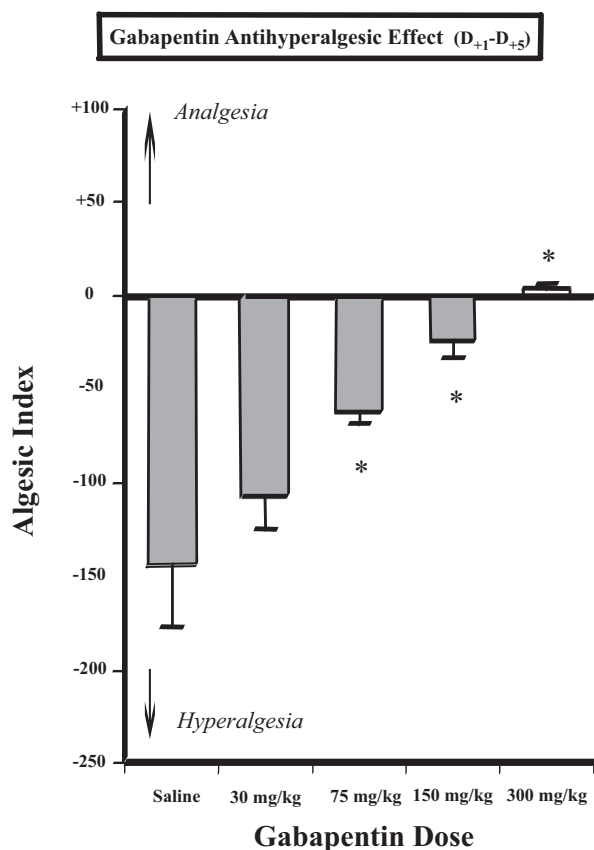
Gabapentin Antihyperalgesic Effect (D_{+1}-D_{+5})

Fig. 2. Dose-response of the effects of gabapentin on fentanyl-induced hyperalgesia as measured from D_{+1} to D_{+5}. Four fentanyl (60 μg/kg) injections performed every 15 min on the day of the test (D_0) resulted in a decrease in the nociceptive threshold below the basal value as measured by the paw-pressure test. Intraperitoneal gabapentin or saline were administered 30 min before the first subcutaneous fentanyl injection. The algesic index was determined by the mean of the surfaces (±SD) calculated by summing the nociceptive threshold values measured every day from D_{+1} to D_{+5}. The histogram represents the algesic index after various intraperitoneal doses (30, 75, 150, or 300 mg/kg) of gabapentin (n = 8, 7, 10, and 7, respectively). * P < 0.05 with the Dunnett test when compared with the saline group (n = 6).

for 3 days (D_{+1} to D_{+3}) (one-way analysis of variance, $P < 0.05$; fig. 5A). However, preventive administration of gabapentin significantly reduced this decrease in nociceptive threshold. The decrease in nociceptive threshold was significantly less on D_{+2} and D_{+3} (respectively, 305 ± 24 vs. 248 ± 17 g on D_{+2} and 318 ± 5 vs. 302 ± 8 g on D_{+3}; two-way analysis of variance, $P < 0.05$; fig. 5A). As expected, fentanyl administration (four boluses of 100 μg/kg) initially opposed the hyperalgesic effect of carrageenan. This analgesic effect disappeared 4 h after the last fentanyl injection (one-way analysis of variance, $P < 0.05$; fig. 5B). Subsequently, long-lasting hyperalgesia was induced for 4 days (D_{+1}-D_{+4}) (one-way analysis of variance, $P < 0.05$; fig. 5B). Preventive administration of gabapentin significantly reduced the long-lasting nociceptive threshold decrease in fentanyl-treated rats for 3 days, from D_{+2} to D_{+4} (two-way analysis of variance,

$P < 0.05$; fig. 5B). The long-lasting hyperalgesia from D_{+1} to D_{+8} after the combined carrageenan, fentanyl, and gabapentin injections was not different from that observed after the combined carrageenan, saline, and gabapentin injections (two-way analysis of variance, $P > 0.05$; fig. 5C). In carrageenan-treated rats, the algesic index observed with fentanyl injections significantly exceeded that which was observed with saline injections (reference algesic index) (Mann–Whitney test, $P < 0.05$; fig. 5D), whereas the algesic index observed with fentanyl and gabapentin injections was not statistically different from the reference algesic index (Mann–Whitney test, $P > 0.05$; fig. 5D).

Inhibitory Effect of Ruthenium Red on Prevention of Fentanyl-induced Hyperalgesia by Gabapentin

The effects of intrathecal ruthenium red on the prevention of fentanyl-induced long-lasting hyperalgesia by intrathecal gabapentin are shown in figure 6. As expected, 300 μg intrathecal gabapentin totally prevented fentanyl-induced long-lasting hyperalgesia (one-way analysis of variance, $P < 0.05$). Intrathecal ruthenium red partially, but significantly, opposed the prevention of fentanyl-induced long-lasting hyperalgesia by intrathecal gabapentin (two-way analysis of variance, $P < 0.05$). Intrathecal ruthenium red had no effect on its own on the nociceptive threshold changes when compared with intrathecal saline (two-way analysis of variance, $P > 0.05$).

Discussion

The main finding of our study was that either systemic or intrathecal delivery of gabapentin prevented the delayed hyperalgesia induced by short-term use of systemic fentanyl in uninjured rats. Our results are in accord with those of previous studies[3,20,21] and have confirmed that short-term use of systemic fentanyl in rats induces a delayed and sustained decrease in the nociceptive threshold below the basal value, i.e., the enhancement of pain sensitivity indicative of hyperalgesia. However, gabapentin had no analgesic effect per se and did not enhance the analgesic effect of various doses of fentanyl. This is in accord with the results of previous studies and confirms that gabapentin reduces hyperalgesia after central sensitization but has no effect on pain transmission in normal skin.[22] Given the interrelations between neural mechanisms underlying hyperalgesia and morphine tolerance,[7] the doses of intrathecal and intraperitoneal gabapentin were chosen according to previous studies in which gabapentin was shown to prevent morphine tolerance in rats.[18,19] It should be emphasized that the dose of gabapentin required after spinal delivery is considerably lower than that required after systemic delivery. Although a supraspinal effect cannot be excluded, peripheral redistribution after spinal delivery unlikely or
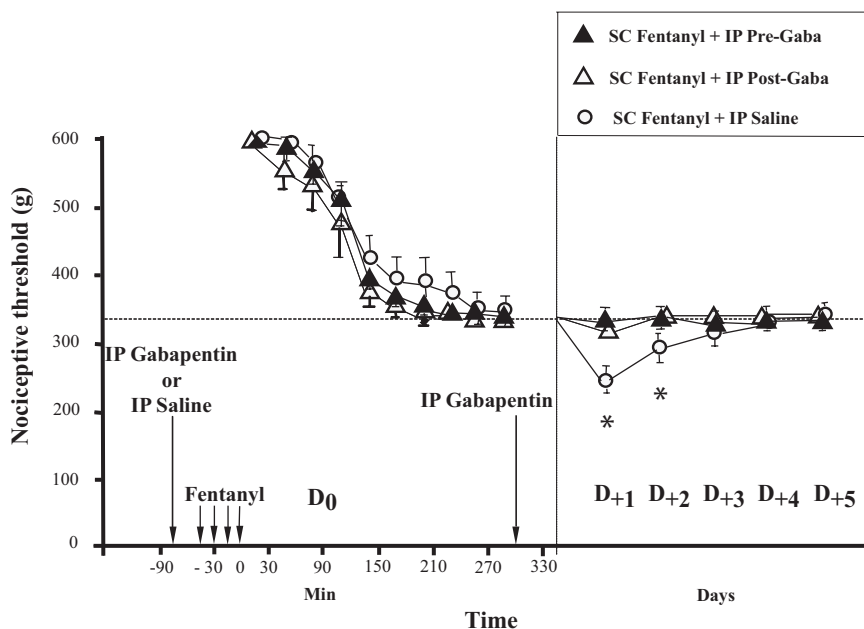
Fig. 3. Effect of gabapentin on delayed hyperalgesia induced by subcutaneous administered fentanyl in uninjured rats when administered either before or after fentanyl injections. Four fentanyl (60 μg/kg) injections were performed every 15 min on the day of the test (D_0). Intraperitoneal gabapentin (150 mg/kg) was administered either 30 min before the first subcutaneous fentanyl injection (n = 7) or 300 min after the last subcutaneous fentanyl injection (n = 7). The basal nociceptive threshold was evaluated after treatment for 5 h on D_0 and then daily for 5 days (D_{+1}–D_{+5}). Mean nociceptive thresholds ($\pm$SD) are expressed in grams. * $P < 0.05$ with the Newman-Keuls test when compared with the basal nociceptive threshold value. IP = intraperitoneal; SC = subcutaneous.

minimally accounts for the antihyperalgesic effect conferred by this route of administration. The single doses of gabapentin given in the study were able to counteract

hyperalgesia that occurred more than 24 h after gabapentin administration, far beyond the pharmacologic effect of the drug, suggesting a preemptive effect. Inter-
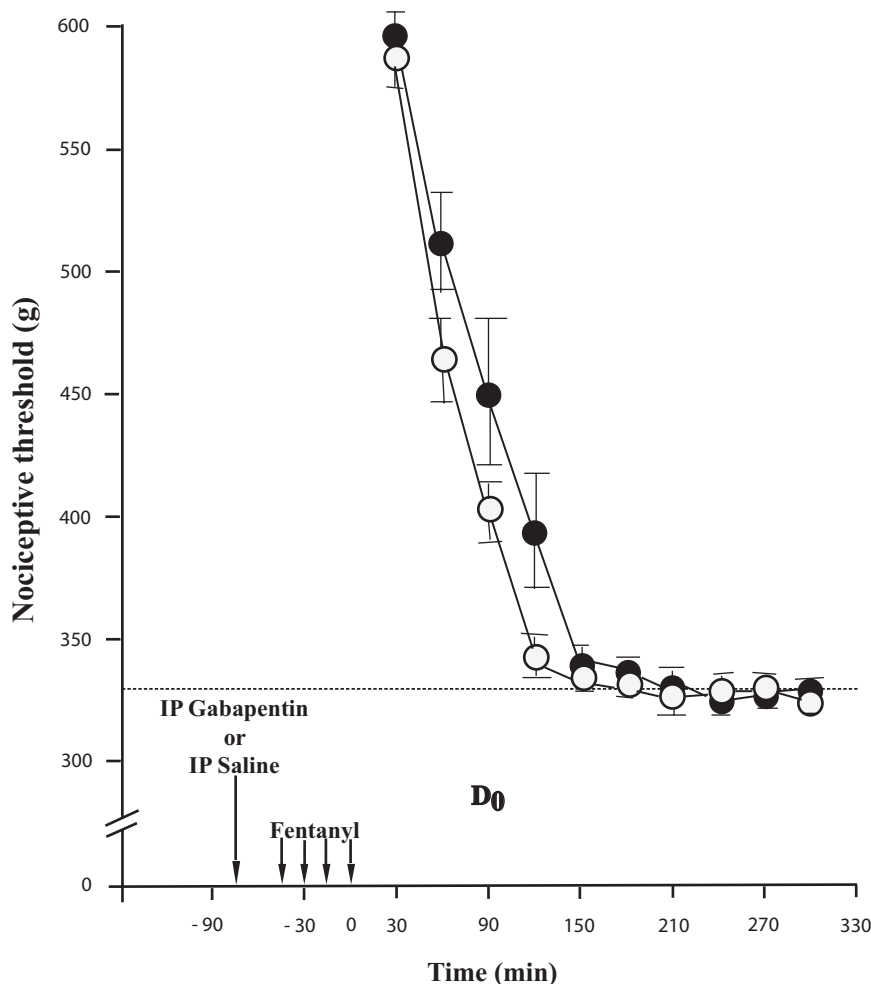

Fig. 4. Enhancement of the analgesic effect of a low fentanyl dose by gabapentin. Subcutaneous fentanyl was injected four times (20 μg/kg per injection) at 15-min intervals, resulting in a total dose of 80 μg/kg administered over 1 h. Experiments were conducted with either intraperitoneal gabapentin 150 mg/kg (*closed circles*, n = 6) or intraperitoneal saline (*open circles*, n = 6). The basal nociceptive threshold was evaluated after treatment for 5 h on D_0. Mean nociceptive thresholds ($\pm$SD) are expressed in grams. * Dunnett test, $P < 0.05$ for comparison between groups. IP = intraperitoneal.
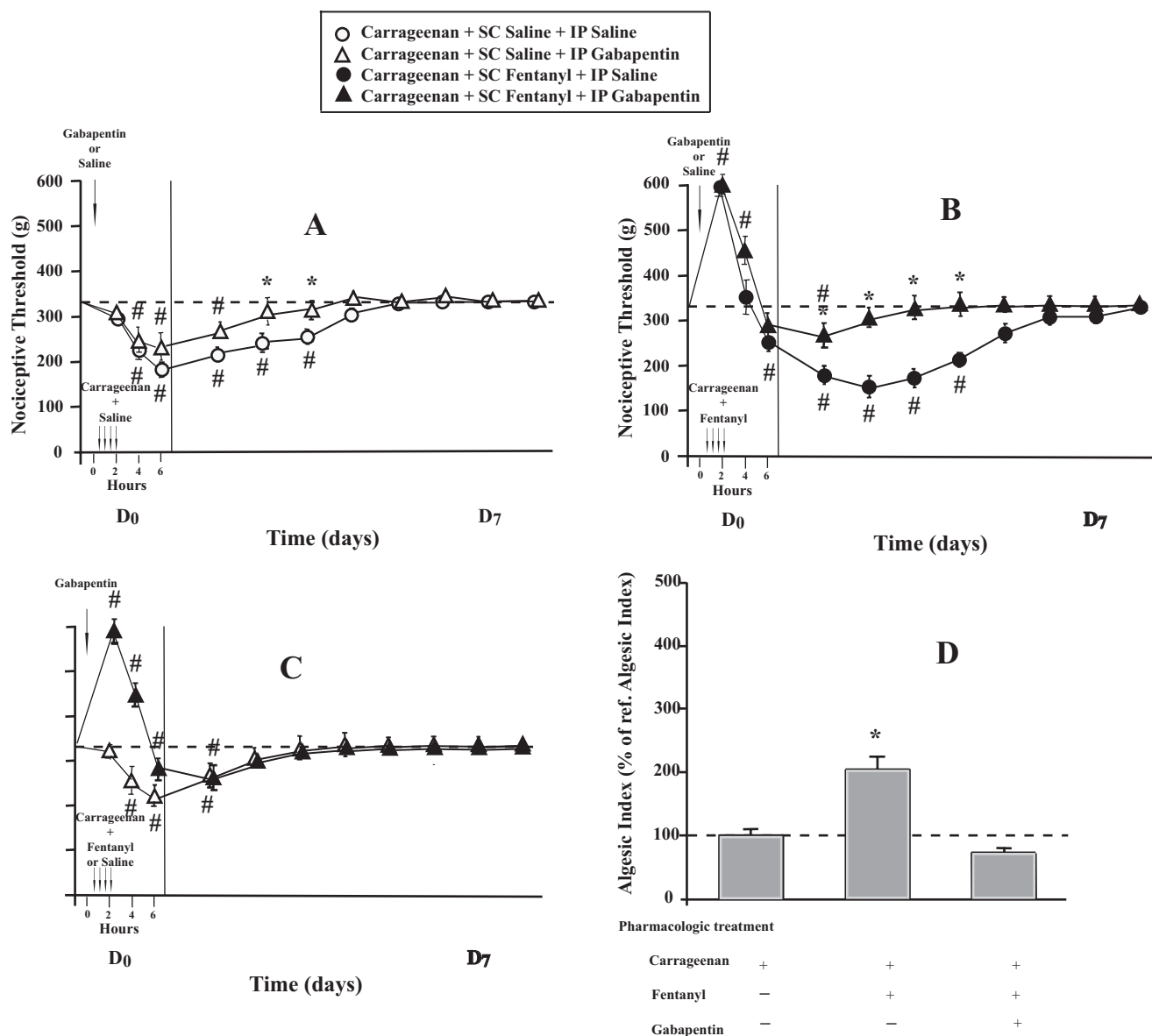
Fig. 5. Effects of gabapentin on hyperalgesia induced by subcutaneous fentanyl in a rat model of inflammatory pain. Four fentanyl (100 μg/kg) or saline injections were performed at 15-min intervals. Intraplantar injection of carrageenan (0.2 ml of a 1% solution in saline) was performed after the first fentanyl injection. Intraperitoneal injections of gabapentin 150 mg/kg or saline were performed 30 min before the first fentanyl injection. Control groups consisted of intraplantar carrageenan, subcutaneous saline and intraperitoneal gabapentin, or saline. The basal nociceptive threshold was evaluated after treatment for 5 h on D₀ and then daily for 5 days (D+1–D+8). (A) Subcutaneous saline, and intraperitoneal gabapentin (n = 8) or saline (n = 8). (B) Subcutaneous fentanyl, and intraperitoneal gabapentin (n = 8) or saline (n = 8). (C) Intraperitoneal gabapentin, and subcutaneous fentanyl (n = 8) or saline (n = 8). Mean nociceptive thresholds (± SD) are expressed in grams. # Newman-Keuls test, $P < 0.05$ compared with the basal nociceptive threshold value. * Dunnett test, $P < 0.05$ for comparison between groups. (D) Algesic indices of the effects of gabapentin on carrageenan and fentanyl–induced hyperalgesia. Plus symbol (+): pharmacologic treatments (carrageenan, fentanyl, gabapentin); minus symbol (−): saline injections. * Mann–Whitney test, $P < 0.05$ compared with the reference algesic index (carrageenan injection in saline-saline–treated rats). IP = intraperitoneal; SC = subcutaneous.

estingly, one-time exposure to gabapentin was able to prevent delayed hyperalgesia even after the acute effects of fentanyl had abated, *i.e.*, when OIH was potentially initiated. This suggests that gabapentin might also oppose or reverse OIH. Of note, our observation that the higher the gabapentin dose used, the better the prevention of fentanyl-induced hyperalgesia, suggests that the prevention of OIH by gabapentin is dose dependent.

In a recent preclinical study, a single injection of fentanyl in rats was shown to elicit a dose-reduction of the analgesic effect of a subsequent opioid administration.[23] The concept of short-term tolerance emerged simultaneously and was demonstrated in a recent preclinical study where an original hypothesis suggested that tolerance, especially short-term tolerance, is not mainly due to a decrease in opioid effectiveness, but might result from an enhancement in pain sensitivity leading to an
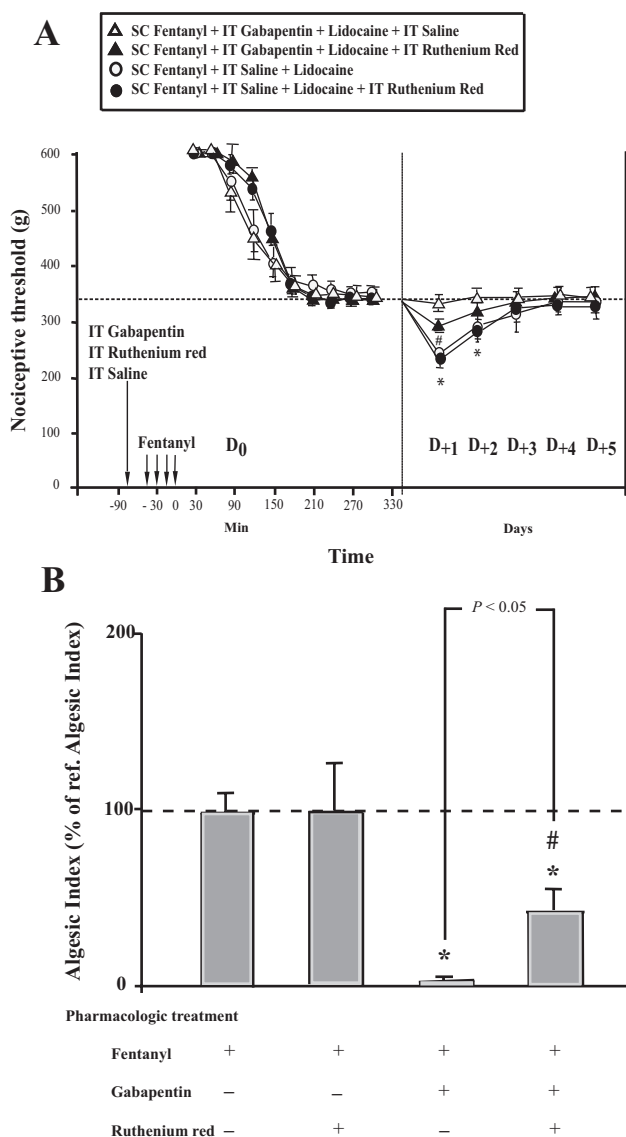
A

Legend:
- △ SC Fentanyl + IT Gabapentin + Lidocaine + IT Saline
- ▲ SC Fentanyl + IT Gabapentin + Lidocaine + IT Ruthenium Red
- ○ SC Fentanyl + IT Saline + Lidocaine
- ● SC Fentanyl + IT Saline + Lidocaine + IT Ruthenium Red

B

Fig. 6. (A) Effects of ruthenium red on the prevention of fentanyl-induced hyperalgesia by gabapentin. Four fentanyl injections (60 μg/kg) were performed subcutaneously at 15-min intervals. Intrathecal gabapentin, 300 μg, with intrathecal ruthenium red (20 ng) (n = 8) or saline (n = 8) was administered 30 min before the first fentanyl injection. The basal nociceptive threshold was evaluated after treatment for 5 h on D_0 and then daily for 5 days (D_{+1}–D_{+5}). Mean nociceptive thresholds (±SD) are expressed in grams. * Dunnett test, $P < 0.05$ for comparison between saline-injected *versus* gabapentin-injected groups. # Dunnett test, $P < 0.05$ for comparison between ruthenium red-injected *versus* saline-injected groups in gabapentin-administered rats. (B) Algesic indices of the effects of ruthenium red on the prevention of fentanyl-induced hyperalgesia by gabapentin. Plus symbol (+): pharmacologic treatments (fentanyl, gabapentin, ruthenium red); minus symbol (−): saline injections. * Mann–Whitney test, $P < 0.05$ compared with the reference algesic index: long-lasting hyperalgesia induced by fentanyl injections in saline-treated rats. # Mann–Whitney test, $P < 0.05$ for comparison between ruthenium red-injected *versus* saline-injected groups in gabapentin-administered rats. IT = intrathecal; SC = subcutaneous.

apparent decrease in the effectiveness of morphine.[20] From a clinical viewpoint, these data and our results suggest that a single opioid treatment for surgery may induce pain vulnerability for a long time, and therefore

support a role for gabapentin–opioid combination in the setting of opioid-induced hypersensitivity.

The precise mechanisms that may account for opioid tolerance remain to be elucidated. Opioid-induced hypersensitivity has been shown to be associated with spinal changes through modulation of glutamate receptors *N*-methyl-D-aspartate[7] and 2-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid/kainate[24] resulting from activation and translocation of protein kinase C[7,25,26] or increased spinal dynorphin expression involving activation of descending pain facilitatory systems.[27] To date, such a pronociceptive effect of spinal dynorphin expression has not been demonstrated after short-term opioid exposure. Spinal changes involve plasticity initiated by opiate receptor activation[28]; it is noteworthy that a fentanyl-induced up-regulation of μ-opioid receptor messenger RNA *via* activation of cyclic adenosine monophosphate signaling has been demonstrated in a recent study.[29] This could be relevant to understanding the molecular mechanisms of opioid tolerance.

Recent preclinical studies may explain, at least in part, the mechanisms by which gabapentin prevents OIH. It has been demonstrated that gabapentin may inhibit excitatory amino acid release from presynaptic terminals.[30] It has also been reported that the antinociceptive effect of gabapentin correlates with the suppression of noxious-evoked release of excitatory amino acid in the spinal cord[31]; conversely, gabapentin has been shown to induce a decrease in the neurotransmitter glutamate.[32] Postsynaptic opioid receptor occupation by an exogenous ligand initiates production of nitric oxide.[7] Nitric oxide may diffuse out of the postsynaptic neuron enhancing presynaptic release of endogenous glutamate, resulting in positive feedback.[7] In this way, exogenous opiates may increase the basal level of presynaptic glutamate release. The underlying hypothesis is that the inhibition of glutamate release by gabapentin might oppose this positive feedback and might therefore underlie at least in part its ability to reduce OIH. On the other hand, it has been recently proposed that reduction of presynaptic neurotransmitter release requires high affinity binding to the $\alpha_2\delta$ auxiliary subunit of presynaptic VGCC.[33] Gabapentin acts by binding to the $\alpha_2\delta$ subunit of spinal VGCC.[11-13] Ruthenium red has been shown to modulate gabapentin binding to the $\alpha_2\delta$ subunit of spinal VGCC, and to noncompetitively inhibit the antiallodynic effect of gabapentin in a rat model of postoperative pain.[14] Of note is our finding that ruthenium red significantly attenuated the prevention of fentanyl-induced long-lasting hyperalgesia by gabapentin but had no effect on fentanyl-induced hyperalgesia *per se*. In the current study, the effective molar ratio of ruthenium red (20 ng) to gabapentin (300 μg) was approximately 3×10^{-5}, much less than 1. Therefore, the attenuating effects of ruthenium red are unlikely to be attributable to chemical interactions or chelation with gabapentin.

Taken as a whole, this information suggests that gabapentin may decrease glutamate release *via* VGCC inhibition by binding to the $\alpha_2\delta$ subunit of spinal VGCC. However, given that the complete reversal of gabapentin's effect was not obtained at this effective dose of ruthenium red in the current study, these preliminary results suggest that further studies including dose-range and specific experiments aimed at demonstrating that prevention of OIH by gabapentin actually results, at least in part, from a specific interaction with the $\alpha_2\delta$ subunit of spinal VGCC, would be valuable.

The results of a recent preclinical study by Richebé *et al.*[20] suggest that exaggerated postoperative pain is not only associated with an excess of surgical nociceptive input but is also due to pain hypersensitivity induced by perioperative opioid use itself. A single opioid treatment for surgery may induce sustained pain vulnerability and initiate a latent pain sensitization that could facilitate the development of chronic pain. A widely used rat model for inflammatory pain is the carrageenan model, which shows hyperalgesia peaking after 1–4 h (acute pain) and lasting 24–96 h (long-lasting hyperalgesia).[34] It has been suggested that this evolution may be compared with the postoperative time course.[35] In accord with a previous study,[36] our results show that fentanyl strongly enhanced long-lasting inflammation-induced hyperalgesia by carrageenan. Interestingly, gabapentin partially prevented the sensitization of inflammation-induced hyperalgesia at the tested dose and totally opposed its enhancement by fentanyl. Although this experimental carrageenan model may be different from inflammatory responses involved in postoperative pain development, our results are in accord with clinical data showing that patients receiving a large dose of intraoperative opioids are associated with severe postoperative pain.[5,6] Taken as a whole, this information suggests that the management of postoperative pain could not be limited to analgesic agents such as opioids but also requires antihyperalgesic agents able to prevent the development of pain sensitization processes when large doses of intraoperative opioids are used. Promising preclinical studies suggest that cyclooxygenase inhibitors[37] and nefopam[38] might be used as antihyperalgesic agents in this field. On the other hand, ketamine has already been shown to oppose postoperative pain hypersensitivity and subsequent short-term tolerance induced by perioperative opioid use.[20] In addition, a growing body of preclinical evidence suggests that several anticonvulsant medications, whose specific mechanisms include sodium and calcium channel antagonism and decreased glutamate transmission, suppress opioid analgesic tolerance.[39] Fentanyl and related compounds are widely used in human surgery, and from a clinical viewpoint, although speculative, the results of the current study suggest that gabapentin administered before opiates used during and after surgical procedures may prevent long-lasting enhancement in pain sensitivity. The results of our study support the use of a combination of opioids and antihyperalgesic agents in the perioperative period and suggest that gabapentin may be an effective antihyperalgesic drug for the preemptive treatment of transient hyperalgesia after short-acting opioid-based anesthesia. Accordingly, recent clinical studies that have evaluated the use of a single dose of preemptive gabapentin have convincingly demonstrated reductions in postoperative pain scores and analgesic consumption with inconsequential adverse effects.[32]

On a methodologic note, the tail-flick test has been used for years to detect differences in nociceptive thresholds. However, this test often uses a steep stimulation curve with a fast-increasing stimulation intensity that could mask changes in baseline nociceptive thresholds.[40] In contrast, tests that use a slow-increasing stimulation curve, such as the paw-pressure withdrawal test, enable the detection of even subtle changes in nociceptive thresholds.[40] As a result, we used the Randall-Selitto test, in which a constantly increasing pressure is applied to the rat hind paw.[17] However, given that the motor effects of high-dose fentanyl may have affected the motor reflex component of the paw-pressure withdrawal test, using the paw-withdrawal vocalization test would have provided a more integrated measure of pain involving supraspinal functions. A single intrathecal injection was used in this study, as successfully used previously in our laboratory,[16] and we avoided the use of catheter implantation[41] with its well-known deleterious effects.[42–44] In the current study, correct placement of the needle into the spinal canal for single intrathecal injection was evoked by a reflexive flick of the tail, and was confirmed by transient bilateral motor hind limb weakness or paralysis after 400 μg intrathecal lidocaine.[45] However, concerns may arise as to lidocaine-induced hyperalgesia. We have already shown in a previous study that animals receiving 400 μg intrathecal lidocaine alone did not develop delayed hyperalgesia.[16] It is therefore unlikely that lidocaine interfered with the development of delayed hyperalgesia after systemic administration of fentanyl. Given that the intrathecal saline + lidocaine group results were significantly different from those of the intrathecal gabapentin + lidocaine group, it is tempting to conclude that gabapentin alone suppresses fentanyl-induced hyperalgesia. However, one cannot exclude the influence of a gabapentin–lidocaine interaction on the observed results.

In summary, our study demonstrates for the first time the preventive effect of gabapentin on hyperalgesia induced by short-term administration of opioids. Our results support a role for addition of gabapentin to opioids in the setting of hyperalgesia after acute administration of opioids. Further investigations are needed, however, to confirm these results in specific clinical settings and patient populations. Finally, the data reported in the

current work suggest that gabapentin exerts its preventive effect on OIH, at least in part, by its specific interaction with the $\alpha_2\delta$ subunit of spinal VGCC. Further studies are required, however, to explore the exact sites and mechanisms through which gabapentin exerts its preventive effect on OIH.

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