Effects of Naloxone on Opioid-induced Hyperalgesia and Tolerance to Remifentanil under Sevoflurane Anesthesia in Rats

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ABSTRACT

Background: Opioid antagonists at ultra-low doses have been used with opioid agonists to prevent or limit opioid tolerance. The aim of this study was to evaluate whether an ultra-low dose of naloxone combined with remifentanil could block opioid-induced hyperalgesia and tolerance under sevoflurane anesthesia in rats.

Methods: Male adult Wistar rats were allocated into one of four treatment groups (n = 7), receiving remifentanil (4 $\mu g \cdot kg^{-1} \cdot min^{-1}$) combined with naloxone (0.17 $ng \cdot kg^{-1} \cdot min^{-1}$), remifentanil alone, naloxone alone, or saline. Animals were evaluated for mechanical nociceptive thresholds (von Frey) and subsequently anesthetized with sevoflurane to determine the baseline minimum alveolar concentration (MAC). Next, treatments were administered, and the MAC was redetermined twice during the infusion. The experiment was performed three times on nonconsecutive days (0, 2, and 4). Hyperalgesia was considered to be a decrease in mechanical thresholds, whereas opioid tolerance was considered to be a decrease in sevoflurane MAC reduction by remifentanil.

Results: Remifentanil produced a significant decrease in mechanical thresholds compared with baseline values at days 2 and 4 (mean ± SD, 30.7 ± 5.5, 22.1 ± 6.4, and 20.7 ± 3.7g at days 0, 2, and 4, respectively) and an increase in MAC baseline values (2.5 ± 0.3, 3.0 ± 0.3, and 3.1 ± 0.3 vol% at days 0, 2, and 4, respectively). Both effects were blocked by naloxone coadministration. However, both remifentanil-treated groups (with or without naloxone) developed opioid tolerance determined by their decrease in MAC reduction.

Conclusions: An ultra-low dose of naloxone blocked remifentanil-induced hyperalgesia but did not change opioid tolerance under inhalant anesthesia. Moreover, the MAC increase associated with hyperalgesia was also blocked by naloxone.

What We Already Know about This Topic

- Remifentanil may produce hyperalgesia and opioid tolerance
- Opioid-induced hyperalgesia and tolerance may decrease the sevoflurane sparing effect of opioids
- Ultra–low-dose naloxone can decrease or block the development of opioid tolerance in rodents

What This Article Tells Us That Is New

- Ultra–low-dose naloxone prevented remifentanil-induced hyperalgesia but not remifentanil-induced tolerance in rats anesthetized with sevoflurane
- Ultra–low-dose naloxone blocked the minimum alveolar concentration increase associated with opioid-induced hyperalgesia in rats
- These results provide further evidence that opioid-induced tolerance and opioid-induced hyperalgesia are separate phenomena

OPIOIDS produce excellent analgesia, but their effects are limited by the development of tolerance and hyperalgesia during treatment.[1] Opioids such as remifentanil are widely used in the intraoperative period to provide analgesia and for their inhalant anesthetic-sparing effects. Tolerance is defined as a decrease in the efficacy of a drug

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over time requiring an increase in opioid dose to maintain the same level of analgesia. This is produced by two main proposed mechanisms: an opposite reaction within the same opioid system in which the opioid produces its primary action (desensitization of the antinociceptive system), and the sensitization of pronociceptive systems that oppose the primary drug effect.[2] This latter effect, called opioid-induced hyperalgesia, results in increased pain sensitivity caused by opioid exposure.[3] The mechanism may involve alterations in opioid receptor signaling with disruption of G-protein coupling.[4]

Tolerance may develop over months of chronic opioid treatment,[5] but it has also been observed following acute administration over just days or hours.[6] Acute opioid tolerance to remifentanil leads to a decrease in the analgesic effect produced by this opioid and has been observed as little as 90 min following its administration to healthy volunteers.[6] This tolerance effect may also be associated with the decreased capacity of remifentanil to reduce the sevoflurane requirements of rats, including its ability to reduce the minimum alveolar concentration (MAC).[7] The clinical consequences may include an increase in the amount of inhalant anesthetic and opioid doses needed during surgery, potentially increasing their undesirable dose-dependent side effects, such as cardiorespiratory depression. Currently, it is not known whether this tolerance phenomenon is caused by sensitization of pronociceptive systems, alterations of opioid systems, or both.[2]

Opioid tolerance has been observed within a few days following treatment with opioids, such as morphine,[8] but it is unclear whether tolerance occurs in a similar way with remifentanil. Although most clinically used opioids share mu opioid receptor agonist activity, they may not share the same pronociceptive and antinociceptive mechanisms; for example, remifentanil, but not morphine or fentanyl, does not activate facilitation through serotoninergic descending pathways.[9] Remifentanil has been related to the development of hyperalgesia and opioid tolerance, which, in turn, may produce a decrease in the sevoflurane-sparing effect of opioids, which may be of clinical relevance.

Opioid antagonists, such as naloxone or naltrexone at ultra-low doses, are used in combination with opioids to enhance the opioid analgesic effect[10] and can decrease or block the development of opioid tolerance in rodents.[8,10–12] Ultra-low doses of naltrexone have been shown to enhance the antinociceptive effect of methadone,[13] whereas an ultra-low dose of naloxone produced an opioid-sparing effect in patients administered morphine for postoperative pain.[14] Furthermore, there is an oral opioid that combines a therapeutic amount of oxycodone with an ultra-low dose of the antagonist naltrexone for the treatment of moderate to severe chronic pain, aiming to prevent the development of tolerance in people[15] and animals.[16] However, there are no studies regarding the effect of opioid antagonists on remifentanil-induced tolerance or hyperalgesia.

Therefore, we hypothesized that ultra-low doses of naloxone may prevent the observed increase in sevoflurane requirements during remifentanil infusion, which are related to tolerance, as well as decrease mechanical nociceptive thresholds (MNTs), which are associated with hyperalgesia. The aim of this study was to determine whether ultra-low doses of naloxone may blunt or block the tolerance and hyperalgesia produced by remifentanil in rats.

Materials and Methods

Animals

Twenty-eight adult male Wistar rats (Charles River Laboratories, Barcelona, Spain) weighing 340 ± 47 g were housed in groups of four to six animals per cage (Macrolon Type IV) with a 12-h light–dark cycle at a relative humidity of 40–70% and 20° ± 2°C ambient temperature. Food (SAFE; Panlab, Barcelona, Spain) and water were provided *ad libitum*. The animals were allowed to acclimatize for at least 1 week. All of the studies were performed during the morning (starting at 8:30 AM). The protocol used in the study was endorsed by the Institutional Animal Care Committee of La Paz University Hospital, Madrid, Spain.

Behavior Test of Nociception

MNTs were evaluated by measuring the hind-paw withdrawal response to the application of electronically calibrated von Frey filaments (Electronic von Frey Aesthesiometer, Model 2393; IITC, Inc., Woodland Hills, CA). To minimize the influence of stress during the experimental procedure, the animals were acclimatized for 3 days to the testing procedure before baseline values were obtained. The animals were placed in a methacrylate cylinder (18 × 30 cm) with a wire grid bottom (1-cm^2 perforations).

The four filaments provided with the electronic von Frey device were used. These filaments were chosen to cover a pressure range between 10 and 50 g. The estimated pressure produced by every filament when applied was previously calculated as the mean of 25 tests. The thinnest filament (approximately 10 g) was used first, followed by an increase in pressure, after which pressure was increased or decreased according to the previous response. When a negative response was observed, the filament with the next greatest thickness was used; similarly, when a positive response was observed, the filament with the next lowest thickness was used. The maximum pressure applied, as determined by the device, was recorded. The threshold of each animal was calculated as the mean of the six applied pressures from the first crossover.

Anesthetic Induction and Instrumentation

Rats were placed in an induction chamber into which 8% sevoflurane was directed in a continuous oxygen flow of 3 l/min (Sevoflurane Vaporizer; Sevorane Dragër Vapor 2000; Lubeck, Germany). Endotracheal intubation was performed using a 14-gauge polyethylene catheter (Terumo Surflo

IV Catheter; Terumo Europe NV, Leuven, Belgium) with the animal positioned in sternal recumbency. A flexible, blunt-tipped guidewire was inserted into the trachea with an otoscope and used to direct the endotracheal catheter. After the catheter was positioned properly, it was connected to a small T-piece breathing system with minimum dead space. Fresh gas flow to the T-piece was adjusted to 1 l/min of oxygen (100%), and the sevoflurane concentration was adjusted to 1.5 × MAC (3.5–4 vol%). Rats were kept under spontaneous ventilation throughout the experiment. Remifentanil and naloxone was administered IV with an infusion pump (Syringe pump, Model Sep11S; Ascor S.A., Medical Equipment, Warsaw, Poland) using a 22-gauge polyethylene catheter inserted into a tail vein.

Heart rate and arterial oxygen hemoglobin saturation (*via* pulseoximetry) as well as respiratory rate were recorded continuously (RGB Medical Devices, Madrid, Spain). Rectal temperature was also monitored and maintained between 37.0° and 38.5°C using a water-circulating warming blanket (Heat Therapy Pump, Model TP-220; Gaymar, Orchard Park, NY) and a heating light.

Determination of the MAC

The MAC is a standard measure of volatile anesthetic potency and is defined as the concentration required to prevent gross purposeful movement in 50% of subjects in response to a noxious stimulus. The determination of reduction in the MAC produced by an opioid is an indirect, although clinically valuable, method of determining the analgesic potency of the opioid during the intraoperative period.

Intratracheal gas sampling was used to measure the anesthetic gas concentration and to determine the MAC. A fine 23-gauge needle was inserted through the endotracheal catheter with the needle tip located at the entrance of the endotracheal catheter, and gas samples were assayed using a sidestream infrared analyzer (Capnomac Ultima; Datex-Ohmeda, Hatfield, Hertfordshire, United Kingdom).

MAC determinations were evaluated by the same investigator (D.A.), who was not blinded to the drugs administered. A supramaximal noxious stimulus was applied with a long hemostat (8-inch Rochester Pean Hemostatic Forceps; RICA Surgical Products, Inc, Schiller Park, IL) clamped to the first ratchet lock on the tail for 60 s, or until a positive response was observed, immediately before the gas sample was obtained from the trachea. The tail was always stimulated proximally to a previous test site when the previous response was negative, or it was stimulated more distally if the response was positive, starting 6 cm from the tail base. A positive response was considered to be a gross purposeful movement of the head, extremities, or body. A negative response was considered to be the lack of movement or grimacing, swallowing, chewing, or tail flicking. When a negative response was seen, the sevoflurane concentration was reduced in decrements of 0.2 vol% until the negative response became positive. Similarly, when a positive response was seen, the sevoflurane concentration was increased by 0.2 vol% until the positive response became negative. The MAC was considered to be the concentration midway between the highest concentration that permitted movement in response to the stimulus and the lowest concentration that prevented such movement. Determination of the MAC was performed in a laboratory 650 m above sea level, which lowers the barometric pressure and results in MAC values that are higher than those obtained at sea level. Therefore, MAC values were corrected to the barometric pressure at sea level using the following formula: MAC (%) at sea level barometric pressure (760 mmHg) (altitude adjusted MAC) = measured MAC (%) × measured ambient barometric pressure (700 mmHg in Madrid)/sea level barometric pressure (760 mmHg).

Experimental Design and Drug Groups

The MAC was determined three times (MAC-1, MAC-2, and MAC-3) on nonconsecutive days (days 0, 2, and 4) in each animal. Thus, results were obtained from animals that completed all 3 days of infusion. First, all animals (n = 6–7 per group) were evaluated for MNTs using the von Frey filaments. Then, the rats were anesthetized and instrumented. A baseline MAC (MAC-1) was determined, and each animal acted as its own control. Then, drugs (remifentanil, naloxone, remifentanil plus naloxone, or saline) were infused continuously into the tail vein starting thirty minutes later. The MAC was redetermined (MAC-2), and the third MAC was determined approximately 90 min later (MAC-3). Periods of 30 min were allotted between MAC determinations, and periods of 40–60 min were usually necessary to determine the MAC value. Overall, each experiment lasted over 4 h (fig. 1).

Hyperalgesia was defined as a decrease in MNTs as determined by the von Frey test. Acute tolerance was defined as a decrease in the degree of remifentanil MAC reduction during opioid infusion (MAC-3 compared with MAC-2) on each day of the study, and delayed, or subacute, tolerance was considered a decrease in remifentanil MAC reduction (MAC-2) on different days (days 2 and 4 compared with day 0).

Four groups of rats were assessed according to the drugs administered: remifentanil, naloxone, naloxone plus remifentanil, and saline (control group). Drug doses used in rats are commonly higher compared with human doses, and extrapolation between species should be based on allometric escalation.[17] The naloxone loading dose was 10 ng/kg IV, followed by a constant rate of infusion at 0.17 $ng \cdot kg^{-1} \cdot min^{-1}$ (2 ng/ml). This dose was selected based on its ability to block opioid hyperalgesia and tolerance in rats.[18] The remifentanil dose was 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (50 μg/ml) with no loading dose, a dose reported to achieve a significant MAC reduction.[7] Sevoflurane (Sevorane) was obtained from Abbott (Madrid, Spain), naloxone (Naloxona) was obtained from Kern Pharma (Tarrasa, Spain), and remifentanil (Ultiva) was obtained from Glaxo-Wellcome (Madrid, Spain).

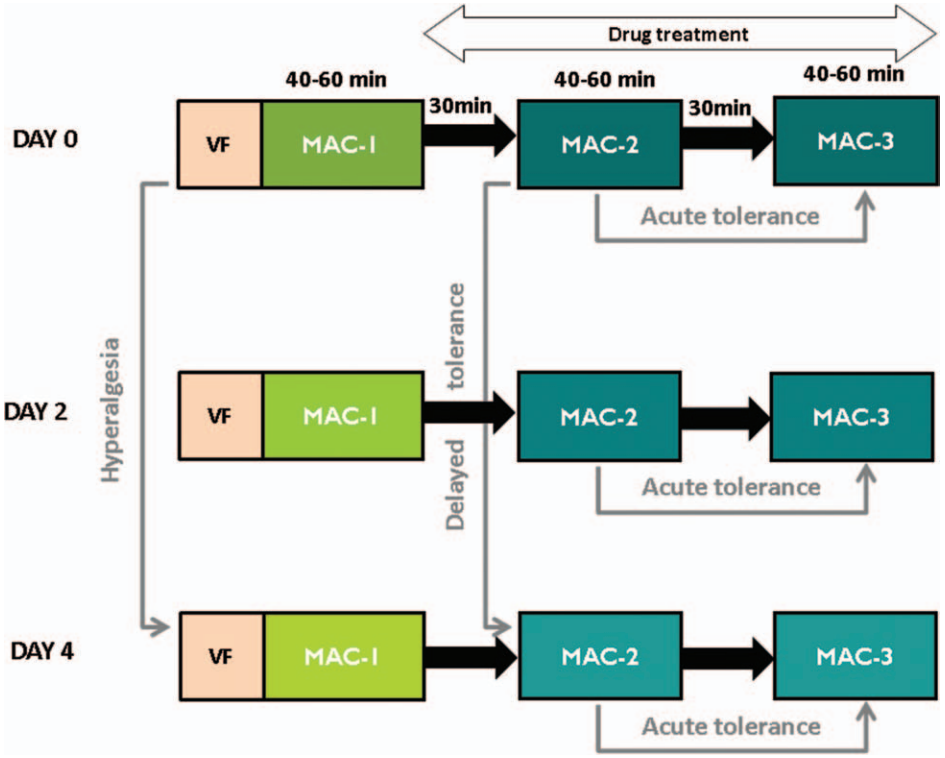

Fig. 1. Experimental design. Mechanical nociceptive thresholds determined with the von Frey test (VF) and minimum alveolar concentration (MAC) at baseline (MAC-1) and following remifentanil administration (MAC-2 and MAC-3) on days 0, 2, and 4. Hyperalgesia was defined as a decrease in mechanical nociceptive thresholds. Acute tolerance was defined as a decrease in the degree of remifentanil MAC reduction during opioid infusion (MAC-3 over MAC-2) on each day, and delayed tolerance was considered to be the decrease in remifentanil MAC reduction (MAC-2) between different days (days 2 and 4 over day 0).

Statistical Analysis

Previous work from our laboratory and sample size calculations indicated that an n value of 6 was necessary to determine differences in MAC reduction produced by remifentanil as well as remifentanil tolerance or hyperalgesia with a power of 80% and a P value of 0.05 to determine at least a 10% change in MAC.[7] The mean and SD required were obtained from a previous study,[7] and the statistical package used was the nQuery Advisor (version 2.0; Statistical Solutions, Saugus, MA).

The results are presented as the mean ± SD. Rats in each experiment were allocated randomly using a random number generator (Excel 2007, Microsoft Office; Microsoft Corp., Redmond, WA). The data were tested for normality with the Kolmogorov-Smirnov test. To assess the effects of remifentanil, naloxone, and their combination on the MAC and MNT values (*i.e.*, the absolute and relative percentages of variation), the two-way repeated measures ANOVA test was used, and the treatment (group) was the between-subjects factor. When an interaction effect between treatments and time was found, the following tests were used. The one-way ANOVA test was used to compare differences between the four groups on each day, but also to assess the appearance of hyperalgesia within the same treatment group by comparing the MNTs and MAC-1 on days 2 and 4 with that on day 0. The test was further used to assess the appearance of subacute or delayed opioid tolerance by comparing the MAC-2 on days 2 and 4 with that on day 0. The Bonferroni *post hoc* test was used to compare groups. To assess the appearance of acute opioid tolerance, the paired Student's t test was used to compare the MAC-2 and MAC-3 of each treatment group on each day of the study (days 0, 2, and 4). The analyses were two-tailed and a value of $P < 0.05$ was set to indicate statistical significance. The effect size between two groups was determined with the Cohen's d and the effect size of a factor (treatment group or time) with the partial η^2. All analyses were performed using the SPSS statistical package (version 19 for Windows; IBM Corp., Armonk, NY).

Results

Naloxone Combined with Remifentanil Reduced Opioid-induced Hyperalgesia

The baseline MNT at day 0 determined in all rats was 29.9 ± 5.6 g (n = 26) and was similar between the groups (P = 1.000, η^2 = 0.058). Two animals, one in the control group and another in the remifentanil plus naloxone group, showed a nervous behavior during MNT testing at day 0, and these missing data could not be included in the analysis.

Remifentanil produced a significant decrease in MNT values at days 2 and 4 compared with baseline (P = 0.022, d = 1.57, n = 7, both groups; and P = 0.007, d = 1.84, n = 7, both groups, respectively). Conversely, naloxone produced

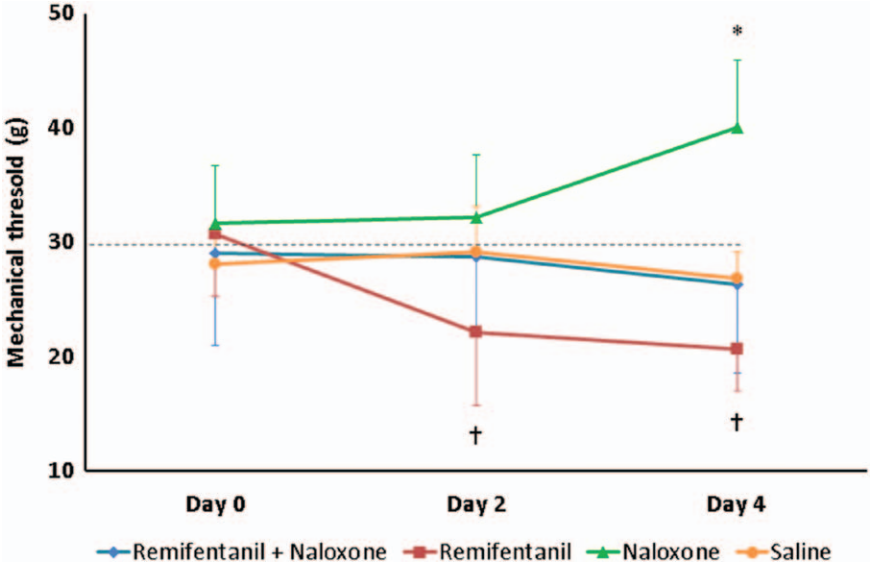

Fig. 2. The mechanical nociceptive thresholds determined with the von Frey test before minimum alveolar concentration (MAC) determination and the administration of 4 μg·kg^{-1}·min^{-1} remifentanil with 0.17 ng·kg^{-1}·min^{-1} naloxone, 4 μg·kg^{-1}·min^{-1} remifentanil alone, 0.17 ng·kg^{-1}·min^{-1} naloxone alone, or saline on days 0, 2, and 4. Data are expressed as the mean ± SD; n = 6–7 animals per group. * Significantly different from the control group (saline), $P < 0.05$. † Significantly different from baseline (day 0), $P < 0.05$.

a significant increase in MNTs at day 4 in comparison with the control group (saline) ($P = 0.002$, $d = 1.68$, n = 6, both groups). Adding naloxone to remifentanil did not produce any changes in MNTs ($P = 1.000$ on days 2 and 4, $d = 0.04$ and 0.34, respectively, n = 7, both groups) and did not differ from the control group ($P = 1.000$, $d = 0.36$, n = 7, both groups, at day 2; and $d = 0.23$, n = 6 both groups, at day 4) (fig. 2).

Remifentanil Produced an Increase in the Baseline MAC (Sevoflurane Requirement) That Was Blocked by Naloxone

The baseline MAC (MAC-1) at day 0 determined in all rats was 2.5 ± 0.2 vol% (n = 28) and was similar between groups ($P = 1.000$, $\eta^2 = 0.054$). A gradual though slight increase in MAC baseline (MAC-1) over time was determined in the control group and reached statistical significance by day 4

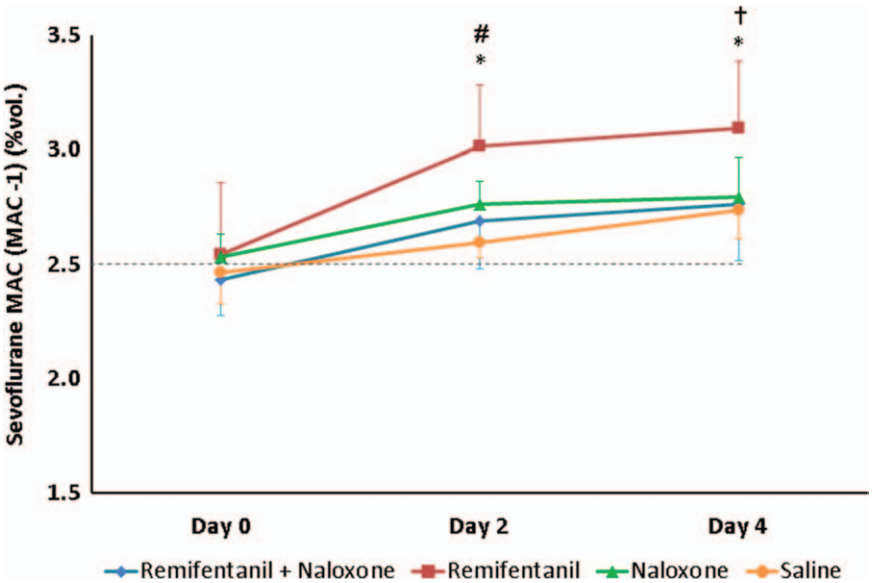

Fig. 3. Minimum alveolar concentration (MAC) of sevoflurane (vol%) at baseline and following the administration of the treatment drugs (4 μg·kg^{-1}·min^{-1} remifentanil with 0.17 ng·kg^{-1}·min^{-1} naloxone, 4 μg·kg^{-1}·min^{-1} remifentanil alone, 0.17 ng·kg^{-1}·min^{-1} naloxone alone, or saline) on days 0, 2, and 4. Data are expressed as the mean ± SD; n = 6–7 animals per group. * Significantly different from the control group, $P < 0.05$. † All groups were significantly different from baseline (MAC-1 at day 0) on day 4, $P < 0.05$. # All groups except the control group were significantly different from baseline (MAC-1 on day 0) on day 2 (hyperalgesia MAC), $P < 0.05$.

(10% increase; $P = 0.001$, $d = 1.99$, n = 7, both groups). A similar 10% increase in baseline MAC was observed on days 2 and 4 in rats receiving naloxone or remifentanil plus naloxone (day 2: $P = 0.023$, $d = 2.38$, n = 6, both groups; and $P = 0.098$, $d = 2.70$, n = 7, both groups; day 4: $P = 0.010$, $d = 2.70$, n = 6, both groups; and $P = 0.026$, $d = 2.08$, n = 7 at day 0 and n = 6 at day 2, respectively), whereas remifentanil alone produced a significantly higher increase in MAC of approximately 20% ($P = 0.023$, $d = 1.49$, n = 7, both groups; and $P = 0.008$, $d = 1.74$, n = 7, both groups, days 2 and 4, respectively) (fig. 3).

Naloxone Did Not Block the Acute Opioid Tolerance Produced by Remifentanil

When naloxone was administered alone, the MAC was not modified at any time point on any studied day ($P = 1.000$, $\eta^2 = 0.007$, n = 6, all groups). When administered alone, remifentanil produced an immediate reduction of the MAC (MAC-2) by 36 ± 2%, 28 ± 4%, and 26 ± 5% at days 0, 2, and 4, respectively. When naloxone was coadministered with the remifentanil infusion, the MAC reduction was similar at 35 ± 3%, 30 ± 4%, and 20 ± 12% for days 0, 2, and 4, respectively.

An acute opioid tolerance was observed approximately 1.5 h later with a lower MAC reduction in MAC-3 compared with MAC-2 ($P < 0.001$) when remifentanil was administered with (day 0, $d = 5.52$, n = 6, both groups; day 2, $d = 2.10$, n = 7, both groups) or without (day 0, $d = 6.68$, n = 7, both groups; day 2, $d = 3.06$, n = 6, both groups; day 4, $d = 2.31$, n = 7, both groups) naloxone except on day 4, when rats received remifentanil and naloxone; the differences between MAC-3 and MAC-2 were not significant ($P = 0.582$, $d = 0.24$, n = 7, both groups) (fig. 4).

Adding naloxone to remifentanil produced a slight nonsignificant decrease (5–10%) in the acute opioid tolerance effect, considered to represent a MAC decrease during opioid administration (MAC-3 over MAC-2), over remifentanil alone on all days (days 0, 2, and 4).

Naloxone Did Not Produce Any Effect on Delayed Tolerance to Remifentanil

Remifentanil alone or combined with naloxone reduced MAC (MAC-2) close to 35% ($P = 0.001$) at day 0 similarly ($d = 0.02$, n = 7, both groups). On day 2, the MAC reduction decreased to 30%; thus, the MAC decreased, that is, 17–20% less than on day 0 (remifentanil alone or combined with naloxone, $P = 0.001$, $d = 3.03$, n = 7 at day 0 and n = 6 at day 2; and $P = 0.027$, $d = 2.06$, n = 6 at day 0 and n = 7 at day 2, respectively). On day 4, a further decrease in MAC reduction was observed in both groups up to 25%; this finding represented 27–33% decreases from day 0 (remifentanil alone or combined with naloxone, $P < 0.001$ in both groups, $d = 4.00$, n = 7, both groups; and $d = 6.03$, n = 7 at day 0 and n = 6 at day 2, respectively) (fig. 5).

Discussion

The opioid antagonist naloxone administered at ultra-low doses inhibited remifentanil-induced hyperalgesia and the

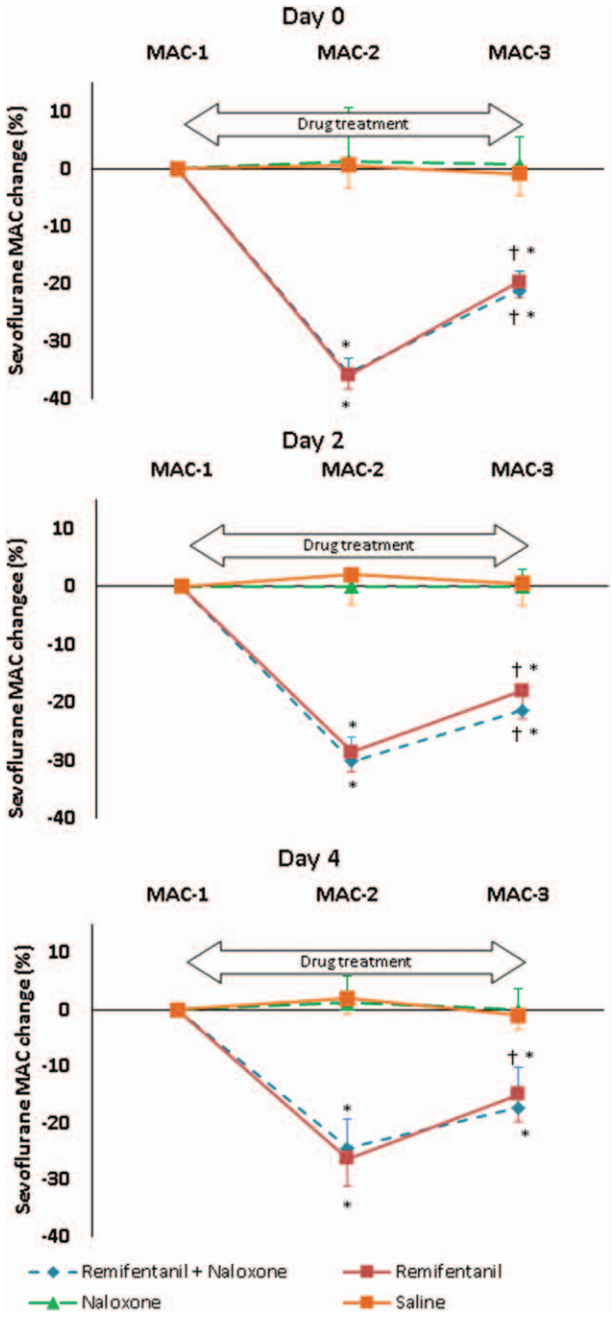

Fig. 4. Reduction of the minimum alveolar concentration (MAC) of sevoflurane (%) produced by the treatment drugs (4 μg·kg^{-1}·min^{-1} remifentanil with 0.17 ng·kg^{-1}·min^{-1} naloxone, 4 μg·kg^{-1}·min^{-1} remifentanil alone, 0.17 ng·kg^{-1}·min^{-1} naloxone alone, or saline). Data are expressed as the mean ± SD; n = 6–7 animals per group. * Significantly different from the control group, $P < 0.01$. † MAC-3 significantly different from MAC-2, $P < 0.05$.

associated MAC increase but failed to inhibit opioid tolerance to remifentanil determined in terms of MAC reduction. These results further suggest that both opioid-induced tolerance and hyperalgesia are two separate phenomena, although they may occur simultaneously. A dichotomy between opioid-induced hyperalgesia and tolerance has

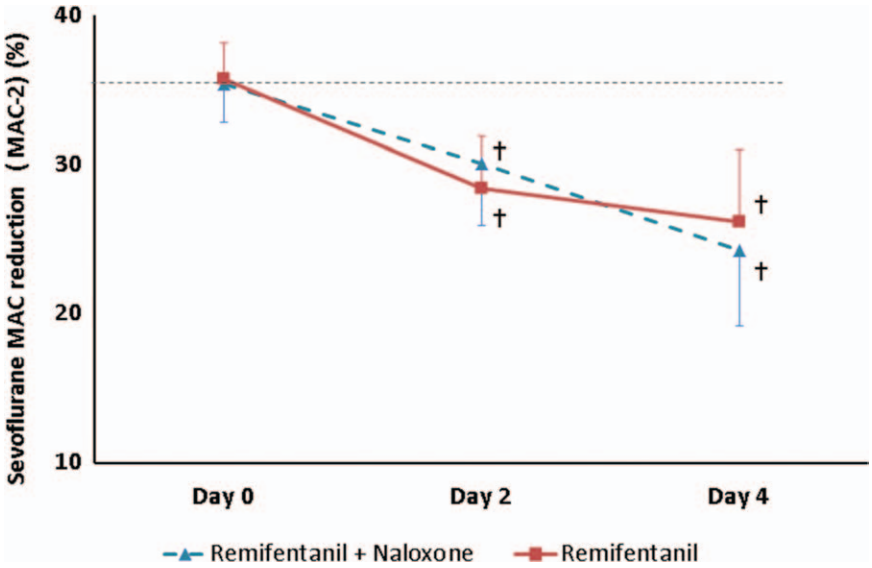

Fig. 5. Reduction of the minimum alveolar concentration (MAC) of sevoflurane (%) compared with MAC-2 produced by the treatment drugs (4 μg·kg^{-1}·min^{-1} remifentanil with 0.17 ng·kg^{-1}·min^{-1} naloxone, or 4 μg·kg^{-1}·min^{-1} remifentanil alone). Data are expressed as the mean ± SD; n = 6–7 animals per group. † All groups are significantly different from baseline (MAC-2 reduction at day 0), $P < 0.05$.

also been observed in low-back-pain patients treated with morphine that developed tolerance to remifentanil in the absence of opioid-induced hyperalgesia.[19]

In rodents, the use of antagonists to opioid receptors at low doses during several days combined with opioid administration, such as morphine, may produce an increased efficacy of the opioid analgesic effect[10,18] but also a decrease or inhibition of tolerance developed over days,[8,10–12,18] weeks, or months.[20,21] This effect has also been observed in people in whom a low dose of opioid antagonists potentiated opioid analgesia and minimized the development of tolerance.[22,23]

A drug combination of an ultra-low dose of naltrexone with oxycodone has been proposed for the treatment of moderate to severe chronic pain patients with similar analgesic action, minimal withdrawal symptoms, and better safety compared with oxycodone alone.[15] Similarly, buprenorphine has been combined with naloxone, enhancing the antinociceptive effect of buprenorphine in patients.[24,25]

Acute opioid tolerance to remifentanil, determined within 90 min, has been documented previously in rats[7,26–28] and was determined during the 2-h remifentanil infusion. In addition, remifentanil also produced a delayed, or subacute, tolerance effect with a 30% lower efficacy to reduce MAC on the third day of infusion compared with the first day of the study. The ultra-low dose of naloxone administered together with remifentanil failed to block opioid tolerance developed over hours (acute) or days (subacute) following the opioid infusion. However, opioid antagonists used in combination with morphine have been shown to decrease or block the development of opioid tolerance in rodents.[8,10–12] This differential effect between opioid agonists may be explained by a different mechanism of action of tolerance between different opioids,[9] but also by sevoflurane anesthesia, which may interfere with naloxone's effects. Also, ketamine, which prevents opioid tolerance,[29] did not have any effect on acute remifentanil tolerance under inhalational anesthesia.[26] Nevertheless, the effects of sevoflurane on the interaction between remifentanil and naloxone cannot be ruled out, and a different experimental design in the conscious animal would be required to make these determinations.

Remifentanil produced a significant decrease (30%) in MNTs[30] that was blocked by an ultra-low dose of naloxone. Ultra-low doses of naltrexone also inhibited acute thermal hyperalgesia elicited by low-dose morphine in mice.[31] However, no previous studies have assessed the role of opioid antagonists in remifentanil-induced hyperalgesia. Hyperalgesia has been widely reported in the postoperative period[32,33] and may be elicited when anesthesia is based on intraoperative high doses of opioids such as remifentanil.[34] Thus, an ultra-low dose of naloxone might improve postoperative pain relief in those patients undergoing remifentanil-based anesthesia or any other clinical situation where opioid-induced hyperalgesia may be expected. The decrease in MNTs produced by remifentanil (hyperalgesia) was associated with an increase in MAC of approximately 20%. This effect was previously reported in rats where remifentanil administration was associated with a similar increase in sevoflurane MAC 24 h after its administration, and this increase lasted several weeks.[35] Extrapolation of these data to the clinical setting suggests an increase in inhalant anesthetic requirements when opioids have been administered previously. However, a small and gradual increase in baseline MAC in control rats has also been observed. This effect may be attributable to local hyperalgesia produced by repeated tail clamping stimulation. However, such stimuli did not produce systemic hyperalgesia

because MNTs remained unchanged over time. In contrast, a decrease in MAC has been observed after repetitive MAC determinations.[36]

Although several studies failed to demonstrate antinociception produced by naloxone or naltrexone at low doses in rats,[8,10] increased pain and mechanical hyperalgesia have been observed in people.[37] In our study, naloxone did not produce an antinociceptive effect by day 2, although an increase in MNTs was observed by day 4, suggesting an analgesic action. This dual pronociceptive–antinociceptive action has been reported previously in rodents and may be dose-dependent[38,39]: lower doses produced an analgesic effect, whereas higher doses produced hyperalgesic effects in rats. This differential effect has also been observed in postoperative patients.[40] A mechanism of action has been proposed in which opioid antagonists may bind with high affinity to filamin A that interacts with the mu opioid receptor, disrupting chronic opioid-induced G-protein coupling.[41] However, two affinity states, nanomolar and picomolar, have been observed on filamin A, and this 200-fold difference in affinity may be responsible for the enhancement of opioid analgesia and the prevention of tolerance.[41]

Although other reports have suggested enhanced opioid analgesia with morphine,[14,20] buprenorphine,[24,25] and methadone,[13] in our study, no additional antinociceptive effects were observed in terms of MAC reduction, when naloxone was associated with remifentanil. However, other studies also failed to demonstrate any analgesic advantage of ultra-low doses of naloxone for opioid requirements in postoperative patients treated with patient-controlled morphine administration,[42] or for fentanyl requirements in critically ill children.[43] Reasons for this may include the 24-fold higher dosage of naloxone in patients or the 14-fold lower morphine/naloxone ratio.[42] Opioids were administered as infusions between 1 and more than 4 days in patients, and a tolerance effect was observed in rats during only 2 h of remifentanil infusion. Thus, a tolerance effect cannot be ruled out and may account for the lack of efficacy of naloxone in patients.

Naloxone binds a pentapeptide segment of the scaffolding protein, filamin A, preventing a G-protein coupling switch (Gi/o to Gs) by the mu opioid receptor.[44,45] This mechanism of action may explain the inhibition of opioid tolerance perhaps as a result of the desensitization of the antinociceptive opioid system.[18] Furthermore, naloxone actions involve other mechanisms of action that may explain these antihyperalgesic effects when coadministered with remifentanil. These may include attenuated *N*-methyl-D-aspartate receptor neurotransmission in the spinal cord,[8] which is related to the development of opioid-induced hyperalgesia.[46,47] In addition, naloxone acts on toll-like receptor 4, inhibiting neuroinflammation,[11] which is implicated in opioid-induced hyperalgesia.[48]

Another limitation of this study is the methods used to determine the interaction of naloxone and remifentanil (MAC reduction).[49] MAC determinations were performed by a person not blinded to the drugs administered. However, the movement response to a supramaximal noxious stimulus is a highly objective and reproducible measure. Furthermore, all MAC determinations were assessed by the same person, thus reducing the bias of the results. A direct link between analgesic potency and MAC reduction cannot be established intraoperatively during inhalation anesthesia, and effects other than changes in analgesia may account for changes in sevoflurane MAC.[36] Potent analgesic drugs, such as opioids[50] and sedatives,[51,52] decrease the MAC to a clinically relevant extent. Therefore, anesthetic immobility and analgesia are not necessarily linked,[53] and a variable effect on the MAC has been determined when different types of analgesic drugs are considered. Nevertheless, the MAC method mimics the intraoperative period and is of clinical relevance.

Extrapolation of the results from the rat to human patients has obvious limitations, and extrapolation should not be applied directly to the clinical setting. The analgesic or antihyperalgesic doses used in rodents are higher, as may be expected from allometric scaling between species, and must be adjusted to obtain clinically relevant effects.[54] Although the dose of remifentanil used in rats produces a clinically relevant MAC reduction, it is actually one to two orders of magnitude higher than that typically used in humans. This difference may produce variations in the effect of the opioid, which may explain the differences in the modulation of tolerance and hyperalgesia observed between people and rodents. In a pilot study, higher doses of naloxone (10-fold) were assessed but did not induce any improvement in remifentanil's ability to decrease MAC or affect the opioid tolerance blockade.

In conclusion, an ultra-low dose of naloxone prevented remifentanil-induced hyperalgesia but not opioid tolerance, as determined under inhalant anesthesia in rats. Moreover, naloxone blocked the MAC increase associated with opioid-induced hyperalgesia. Although this finding might improve pain relief in the postoperative period, the clinical relevance of the results should be determined in the clinical setting.

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Paul Wood's Ambulance Training near Allentown

In 1917 a Columbia University student interrupted his medical studies for training in Allentown, Pennsylvania, to prepare him for future deployment around Piave, Italy, in the United States Army Ambulance Service. Besides the usual marching around in basic training, Paul Meyer Wood received specialized training in rescue procedures using the very types of ambulance vehicles (*above*) that would be employed at the Italian Front. (Copyright © the American Society of Anesthesiologists, Inc.)

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