Buprenorphine Prevents Remifentanil-Enhanced Mechanical Allodynia in a Rat Inflammatory Pain Model

Liqin DENG[1, 2], Tetsuro NIKAI[1$], Ryosuke ISHIDA[1] and Yoji SAITO[1]

[1]*Department of Anesthesiology, Shimane University Faculty of Medicine, Izumo 693-8501, Japan.*
[2]*Department of Anesthesiology, General Hospital of Ningxia Medical University, Yinchuan 750004, China.*

Purpose Remifentanil is a μ-receptor agonist known to cause pain hypersensitivity. Buprenorphine targets multiple opioid receptors and exhibits good anti-hyperalgesic characteristics. We investigated whether pre-treatment with buprenorphine could prevent remifentanil-enhanced mechanical allodynia in an inflammatory pain model.

Methods Inflammatory pain was induced by subcutaneous carrageenan injection into the hind paw of rats. In experiment 1, rats were randomized to receive intravenous remifentanil （10 μg·kg^{-1}·min^{-1} or 30 μg·kg^{-1}·min^{-1}） or saline for 30 min. In experiment 2, buprenorphine （25 μg/kg） or saline was injected intravenously 10 min before remifentanil administration. Mechanical thresholds of hind paws were evaluated using von Frey filaments at 1 and 3h after carrageenan injection and daily thereafter for 7 days.

Results Intravenous infusion of remifentanil significantly enhanced bilateral mechanical allodynia induced by carrageenan. Pre-treatment with buprenorphine prevented remifentanil-enhanced bilateral mechanical allodynia significantly.

Conclusion Pre-treatment with buprenorphine effectively prevents remifentanil-enhanced mechanical allodynia in an inflammatory pain model.

INTRODUCTION

Opioid-induced pain hypersensitivity （OIH）, including hyperalgesia and allodynia, has been identified as one of the most frequent adverse effects of opioid therapy ［1］. Some clinical reports have actually suggested that remifentanil－a short-acting opioid given to patients during surgery－may aggravate postoperative pain ［2, 3］. In line with these observations, we have previously reported that intravenous infusion of remifentanil per se induces transient withdrawal hyperalgesia, which lasts up to 60 min in treatment-naïve rats ［4］. Other animal studies have also shown that remifentanil causes hypersensitivity for several days in an incision pain model ［5, 6］. It is well recognized that surgery produces tissue injury with consequent release of histamine and inflammatory mediators, which induce central and/or peripheral sensitization leading to postoperative pain hypersensitivity ［7］. Although inflammation is the major factor of postoperative pain hypersensitivity, there is little knowledge regarding the relationship between remifentanil and inflammation-induced hyperalgesia. We sought to investigate whether intravenous infusion of remifentanil enhances mechanical allodynia induced by carrageenan, the most commonly employed inflammatory pain model in rats.

Central sensitization in the spinal cord is one of the mechanisms of opioid-induced hypersensitivity. However, an effective method to prevent remifentanil-induced pain hypersensitivity has not been established. Buprenorphine, as a partial μ- and ORL1-receptor agonist and κ- and δ-receptor antagonist, acts distinctly from pure μ-receptor agonists. Buprenorphine has a high affinity but low efficacy and slow dissociation from the μ-receptor, thus producing longer analgesia with better anti-hyperalgesic characteristics related to its κ-opioid receptor antagonistic properties ［8］. In fact, buprenorphine has already been found to prevent or markedly decrease

[$]Correspondence and reprint requests to: Tetsuro Nikai
Department of Anesthesiology, Shimane University Faculty of Medicine. 89-1 Enya-cho, Izumo City, Shimane 693-8501, Japan
Tel:+81-853-20-2295
Fax:+81-853-20-2292
E-mail:tetsunatsu2000@yahoo.co.jp

the development of central hypersensitivity activated by neuropathic pain [8, 9]. Currently, no animal or human data are available regarding buprenorphine pre-treatment for the prevention of opioid-induced hyperalgesia. In the present study, we tested the following hypothesis: (1) intravenous infusion of remifentanil may enhance mechanical allodynia induced by unilateral carrageenan administration in rats and (2) pre-treatment with buprenorphine may prevent remifentanil-enhanced mechanical allodynia in carrageenan pain model in rats.

MATERIALS AND METHODS

Animals

All experiments were performed on male Sprague-Dawley rats weighing 200-300 g (CLEA Japan, Tokyo, Japan) with the institutional approval of the Animal Care and Use Committee of Shimane University (approval no. IZ23-89) and conformed to the *Guide for the Care and Use of Laboratory Animals*. Rats were housed at 22 ± 1°C with a 12-h light-dark cycle and were fed food and water freely. Rats were habituated to the cage and experimental environment in the daytime for 3 days before a Hydrocoat™ catheter (3Fr; Norfolk Medical Inc., USA) was implanted into the right jugular vein. The catheter was implanted under pentobarbital (50-60 mg/kg, intraperitoneally) anesthesia 3 days before carrageenan injection. Animals were allowed to recover for 3 days after catheterization and were weighed daily. If a rat lost weight during the experiments, it was excluded from the study. Experiments were performed by the same experimenter in a blinded manner in the same daytime environment.

Carrageenan pain model and drugs administration

To evaluate the effects of remifentanil on mechanical allodynia induced by inflammatory stimulation, we selected a carrageenan-induced pain model in rats. This model is a widely used and well-established inflammatory pain model that shows hyperalgesia peaking at 1-4 h (acute pain) after administration and lasting for 24-96 h (long-lasting hyperalgesia) [10]. λ-Carrageenan (0.1 ml of a 1% solution of carrageenan in saline; Sigma Chemical, St. Louis, MO, USA) was injected subcutaneously into the intraplantar region of the left hind paw with a 27-gauge needle.

Remifentanil (Ultiva®; Janssen Pharmaceuticals, Tokyo, Japan) dissolved in normal saline at a pre-determined concentration and infusion velocity (10-30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ at 25 $\mu L/min$) from our prior pilot experiments was infused intravenously through the indwelled intravenous catheter using an infusion syringe pump (KD Scientific, Holliston, MA). Buprenorphine (Lepetan®; Otsuka Pharmaceuticals, Tokyo, Japan) was diluted with normal saline (25 $\mu g/mL$) and injected intravenously through the same catheter. The dose of buprenorphine (25 $\mu g/kg$, intravenously) was the optimal and minimal dose to prevent the acute pain induced by carrageenan and not to induce bilateral mechanical allodynia when the time course was pursued for 7 days after carrageenan injection in our preliminary study.

Assessment of bilateral mechanical allodynia

Mechanical allodynia was measured based on the hind paw withdrawal response to calibrated von Frey filaments (North Coast Medical, Morgan Hill, CA). Rats were placed on a wire mesh platform through which the von Frey filaments were applied. Beginning with the 2.0-g filament probe, the upper-limit value was 15.0 g. The 50% withdrawal threshold (in grams) was calculated using a modification of the "up and down" method [11]. Both ipsilateral and contralateral hind paws were alternately tested. The baseline of the paw withdrawal threshold was obtained before treatment. Rats with a baseline mechanical threshold of less than 10.0 g were excluded from the study. Paw withdrawal thresholds in both hind paws were assessed at 1 h, 3 h, and 1-7 days after intraplantar carrageenan injection.

Procedures

In experiment 1, rats were randomly divided into the following 3 groups (n = 7 per group): control, Remi 10, and Remi 30. Rats in the Remi 10 and Remi 30 groups were intravenously infused with remifentanil 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively, from 5 min before until 25 min after carrageenan injection. Rats in the control group received an intravenous infusion of saline. The experimental setup of this first experiment is shown in Fig. 1.

In experiment 2, rats were randomly assigned to one of the following 5 groups (n = 6 per group): Saline+Saline, Saline+Remi 10, BPN+Remi 10, Saline+Remi 30, and BPN+Remi 30. Rats in the BPN+Remi 10 and BPN+Remi 30 groups received a single intravenous injection of buprenorphine (25 µg/kg) at 10 min before remifentanil (10 and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively) administration for 30 min. Rats in the Saline+Remi 10 and Saline+Remi 30 groups were injected an equal volume of saline before remifentanil (10 and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively) infusion. Rats in the Saline+Saline group were given saline instead of buprenorphine and remifentanil. Carrageenan (1%, 100 µL) was injected subcutaneously into the intraplantar region of the left hind paw at 5 min after remifentanil infusion.

Statistical analysis

All data are presented as the mean ± standard er-

ror of the mean (SEM). All statistical analyses were performed with SPSS software (version 16.0; SPSS Inc., Chicago, IL). Treatment effects over time were compared using 2-way repeated-measures analysis of variance (ANOVA), and comparisons among groups were analyzed by one-way ANOVA followed by Scheffe test for multiple comparison analysis. Statistical significance was set at $P < 0.05$.

RESULTS

Remifentanil enhances carrageenan-induced bilateral mechanical allodynia

Mechanical allodynia, manifested as a decrease in paw withdrawal threshold as compared with the baseline in the control group, was observed bilaterally in the hind paws after carrageenan injection. In the ipsilateral side, remifentanil (10 and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) decreased the paw withdrawal thresholds
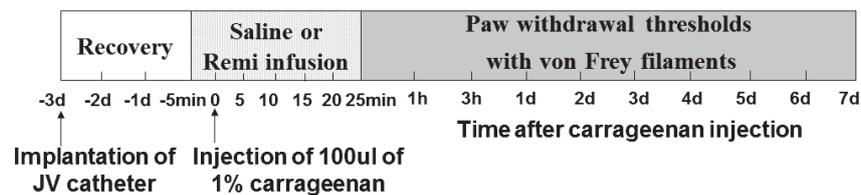
Fig. 1. Schematic representation of the setup of experiment 1. After a 3-day recovery period from the implantation of the catheters into the right jugular vein (JV), rats were infused with remifentanil (Remi; 10 or 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or saline for 30 min. Carrageenan (1%, 100 µL) was injected subcutaneously into the left hind paw 5 min after infusion. Paw withdrawal thresholds in both hind paws were assessed at different time points from 1 h to 7 days.
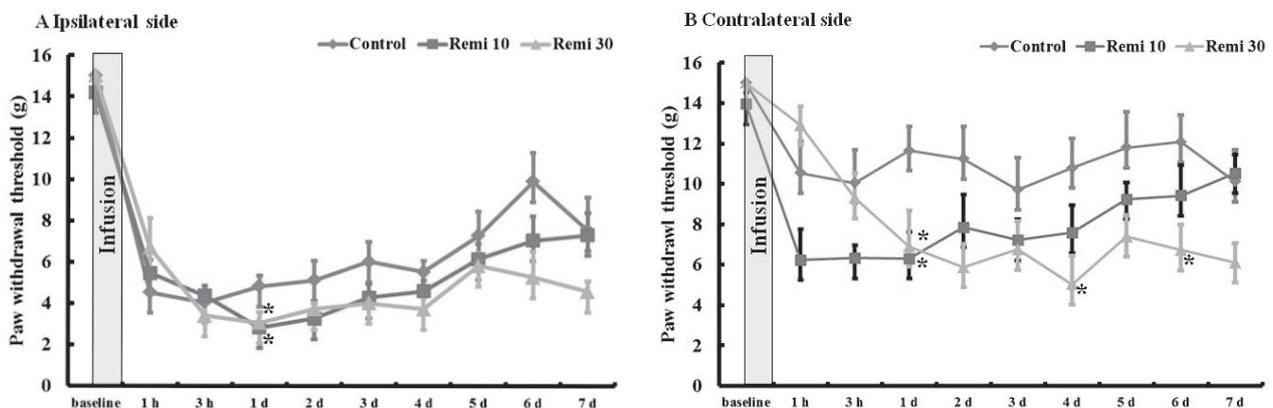

Fig. 2. Effects of intravenous remifentanil (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ [Remi 10] and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ [Remi 30]) on mechanical allodynia induced by unilateral carrageenan injection in both hind paws of rats. The gray-shaded surfaces indicate remifentanil or saline infusion. (A) Ipsilateral side; (B) contralateral side (n =7 per group). Results are expressed as mean values; vertical bars indicate the SEM. *$P < 0.05$ compared to control by one-way ANOVA with Scheffe test.

at 1 day after carrageenan injection (Fig. 2A). In the contralateral side, remifentanil at 10 µg·kg^{-1}·min^{-1} decreased the paw withdrawal threshold at 1 day after carrageenan injection as compared with the control group. At the concentration of 30 µg·kg^{-1}·min^{-1}, remifentanil significantly decreased the paw withdrawal threshold at 1, 4, and 6 days after carrageenan injection as compared with the control group (Fig. 2B).

Buprenorphine prevents remifentanil-enhanced bilateral mechanical allodynia

The effect of buprenorphine was investigated in another experiment, in which we confirmed that remifentanil enhanced mechanical allodynia in both hind paws after carrageenan injection (Fig. 3 and 4). The paw withdrawal thresholds in the BPN+Remi 10 and BPN+Remi 30 groups were increased in comparison with the Saline+Remi 10 and Saline+Remi 30 groups in both hind paws. In the ipsilateral hind paw, pre-treatment with buprenorphine prevented remifentanil-induced decrease in paw withdrawal threshold; this tendency lasted for 3 days (Fig. 3A and 4A). In the contralateral hind paw, pre-treatment with buprenorphine prevented not only remifentanil (10 and 30 µg·kg^{-1}·min^{-1})-enhanced allodynia but also carrageenan-induced allodynia, an effect that lasted for 7 days (Fig. 3B and 4B). As a consequence, the paw withdrawal threshold was similar between
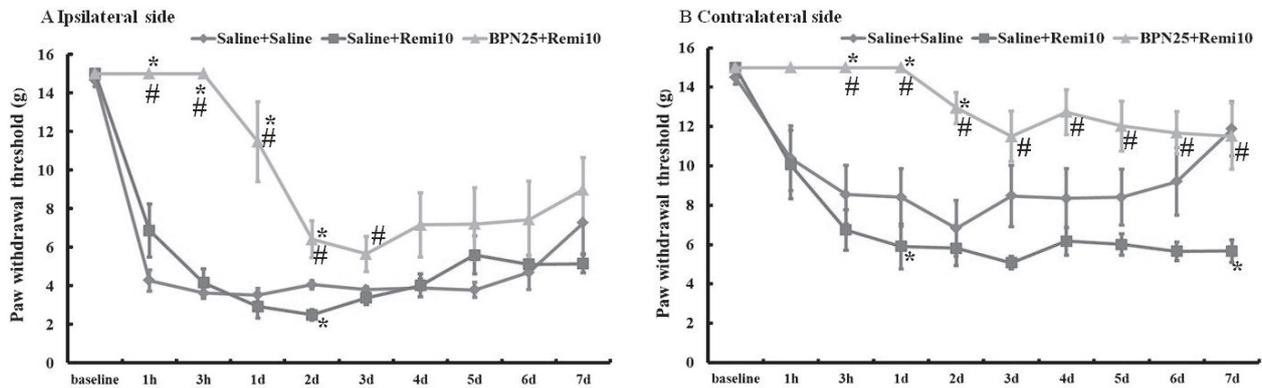

Fig. 3. Effects of buprenorphine (25 µg/kg) pre-treatment on mechanical allodynia enhanced by intravenous remifentanil (10 µg·kg^{-1}·min^{-1}) in both hind paws in carrageenan-induced inflammatory pain in rats. (A) Ipsilateral side; (B) contralateral side (n = 6 per group). Results are expressed as mean values; vertical bars indicate the SEM. *$P < 0.05$ compared to the Saline+Saline group; #$P < 0.05$ compared to the Saline+Remi 10 group by one-way ANOVA with Scheffe test.
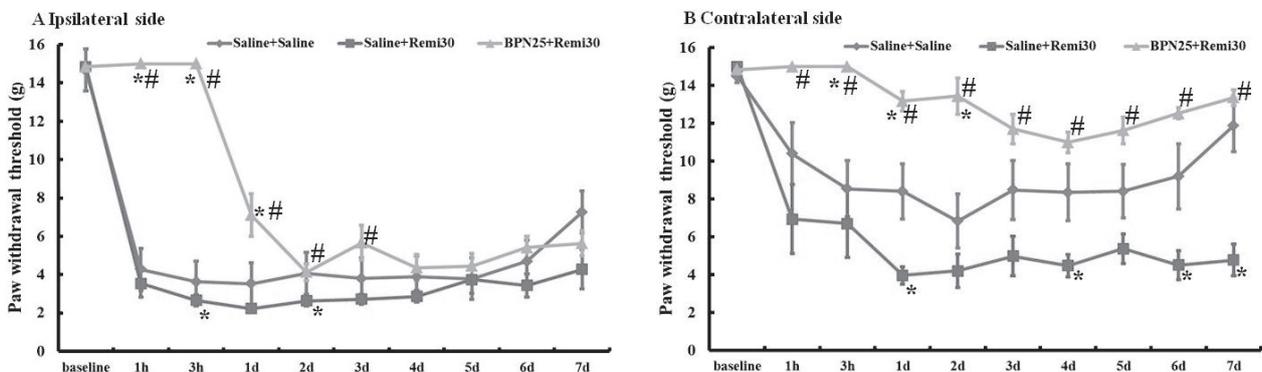
Fig. 4. Effects of buprenorphine (25 µg/kg) pre-treatment on mechanical allodynia enhanced by intravenous remifentanil (30 µg·kg^{-1}·min^{-1}) in both hind paws in carrageenan-induced inflammatory pain in rats. (A) Ipsilateral side; (B) contralateral side (n = 6 per group). Results were expressed as mean values; vertical bars indicate the SEM. *$P < 0.05$ compared to the Saline+Saline group. #$P < 0.05$ compared to the Saline+Remi 30 group by one-way ANOVA with Scheffe test.

baseline and BPN+Remi 10 and BPN+Remi 30 groups.

DISCUSSION

This study has 2 main findings: first, intravenous infusion of remifentanil (10 and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) significantly enhanced carrageenan-induced bilateral mechanical allodynia; second, pre-treatment with a single dose of buprenorphine (25 μg/kg, intravenously) prevented remifentanil-enhanced bilateral mechanical allodynia effectively.

Unilateral hind paw injection of carrageenan induces long-lasting hyperalgesia [12]. It is well known that mechanical allodynia at the site of tissue injury is produced by peripheral and central sensitization. On the other hand, mechanical allodynia at a site adjacent to, or remote from, the site of injury uniquely results from central sensitization [13]. Our data indicate that remifentanil might increase pain hypersensitivity induced by inflammation. This phenomenon might be related to central sensitization in the spinal cord. Rivat et al. reported that the mechanisms by which central sensitization is elicited by opioids share pathways with those underlying central sensitization elicited by nociceptive inputs, especially those associated with inflammatory pain [14]. In fact, there are several central sensitization mechanisms implicated in opioid-induced hypersensitivity, including enhanced descending facilitation from the rostral ventromedial medulla to the spinal cord dorsal horn [15, 16], activation of N-methyl-D-aspartate (NMDA) receptors in the central system [3, 12, 14, 17], role of nitric oxide [18], increased spinal dynorphin [6], and downregulation of δ-receptors [5]. We previously reported that remifentanil itself causes spinal sensitization via ERK1/2 [4]. Taken together, central sensitization evoked by remifentanil could play an important role in the development of pain hypersensitivity induced by inflammation. Our previous study also showed that induction of remifentanil withdrawal-induced hyperalgesia in treatment-naïve rats is dose-independent [4]. On the contrary, in painful animal models, hyperalgesia has been demonstrated to be dependent on the dose of remifentanil. Indeed, Cabanero et al. reported that high doses of remifen-

tanil induce longer-lasting mechanical hyperalgesia than lower doses in a mouse model of incisional pain [19]. Furthermore, it has been reported that a high dose of remifentanil induces long-term potentiation (LTP) in the spinal cord and leads to longer-lasting hyperalgeia [20]. Our present data, which tend to show prominent allodynia at some measuring points on the contralateral side in the remifentanil group (30 $\mu g \cdot kg^{-1} \cdot min^{-1}$), might support remifentanil's dose effect.

It is well recognized that μ-opioid receptor activation contributes not only to opioid analgesia but also to opioid-induced hyperalgesia [21]. Buprenorphine — a partial μ- and ORL1-receptor agonist and κ- and δ-receptor antagonist — was herein shown to effectively prevent remifentanil-enhanced allodynia in a carrageenan pain model. Opioid-induced hyperalgesia has been demonstrated to related to tonic activation of descending facilitation, and then increase expression of spinal dynorphin [22]. Spinal dynorphin acts as an endogenous mediator promoting opioid-induced abnormal pain via an increase in the release of excitatory neurotransmitters [23]. A positive feedback is hence generated that amplifies further abnormal pain. It seems reasonable to speculate that buprenorphine might counteract remifentanil-induced hypersensitivity via its κ-receptor antagonistic properties because dynorphin has been believed an endogenous κ-receptor agonist [8]. In addition, buprenorphine can suppress nociceptive processing by acting at supraspinal ORL1 receptors on the state of central sensitization [24]. Therefore, ORL1 receptors might be key in mediating the anti-hyperalgesic effect of buprenorphine. To date, there are no scientific data to suggest ORL1's role in preventing opioid-induced hyperalgesia. In contrast, a recent study[25] has reported that an ultra-low dose of buprenorphine (0.1 μg/kg, intraperitoneally) results in immediate hyperalgesia in the rat [25]. However, in that study, buprenorphine (20-200 μg/kg, intraperitoneally) was not followed by delayed hyperalgesia, not even at 72 h after injection. This is in agreement with our results, which demonstrated that pre-treatment with buprenorphine (25 μg/kg) can prevent effectively remifentanil-induced longer-lasting hypersensitivity in carrageenan pain model. These findings imply that buprenorphine has

a good anti-hyperalgesic effect on central sensitization in inflammatory pain.

Recently, opioid rotation, decrease of opioid dose, and use of specific NMDA antagonists have all been suggested as effective means to treat opioid-induced hyperalgesia [26]. Silverman [26] suggested that sublingual buprenorphine as one of the candidates for treatment of opioid-induced hyperalgesia. Our present study corroborates by demonstrating that pre-treatment with buprenorphine might prevent remifentanil-induced hypersensitivity.

Notwithstanding the promising results, one may not directly extrapolate them to the clinics because of the gap between animal models and patients, from the doses used in the experiments to the ones used in clinical anesthesia, passing by possible inter-species differences in the modes of action. Further investigation is needed to dissect the precise mechanisms by which pre-treatment with buprenorphine prevents remifentanil-induced hyperalgesia.

In conclusion, intravenous infusion of remifentanil enhanced mechanical allodynia induced by unilateral paw inflammation in rats. Pre-treatment with buprenorphine effectively prevented remifentanil-enhanced mechanical allodynia.

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