Can J Anesth/J Can Anesth (2009) 56:126–135
DOI 10.1007/s12630-008-9023-4

REPORTS OF ORIGINAL INVESTIGATIONS

Effects of sevoflurane on carrageenan- and fentanyl-induced pain hypersensitivity in Sprague-Dawley rats

Effets du sévoflurane sur l'hypersensibilité à la douleur induite par carragénine et le fentanyl chez des rats Sprague-Dawley

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Received: 18 February 2008 / Revised: 19 November 2008 / Accepted: 24 November 2008 / Published online: 31 December 2008

Abstract

Purpose *Opioids are widely used for anesthesia but paradoxically induce postoperative pain hypersensitivity via N-methyl-D-aspartate (NMDA) receptor modulation. Sevoflurane effects on opioid-induced hyperalgesia have not been yet evaluated in vivo. Nevertheless, some experimental in vitro studies reported anti-NMDA receptor properties for sevoflurane. The aim of this study was to evaluate sevoflurane effects on fentanyl-induced hyperalgesia in opioid-naive rats and in rats with inflammatory pain.*

Methods *Sevoflurane effects on hyperalgesia were evaluated in Sprague-Dawley rats: opioid-naive rats, rats treated with fentanyl ($4 \times 60\ \mu g\ kg^{-1}$) and rats with inflammatory pain (carrageenan) treated with fentanyl ($4 \times 60\ \mu g\ kg^{-1}$). On day zero, subcutaneous fentanyl injections were administered and inflammatory pain was induced with one carrageenan injection in one hind paw. Rats were exposed to low concentrations of sevoflurane (1.0 or 1.5%) on day zero prior to fentanyl injections and inflammatory pain induction, and for the duration of the fentanyl analgesic effect. The nociceptive threshold (Randall-Selitto test) was evaluated daily for 7 days. On day seven, naloxone was injected and the nociceptive threshold was assessed 5 min later.*

Results *In rats without inflammatory pain but treated with fentanyl on day zero, sevoflurane 1.0% reversed the early (day zero) and long-lasting (day zero to day three) hyperalgesia classically described after high-doses of fentanyl ($P < 0.05$). This sevoflurane concentration antagonized the hyperalgesia induced by naloxone on day seven ($P = 0.33$). In a second experiment in rats with inflammatory pain, exposure to low concentrations of sevoflurane (1.0 and 1.5%) did not reduce fentanyl-induced hyperalgesia ($P > 0.05$), but nevertheless antagonized the naloxone induced hyperalgesia on day seven ($P = 0.061$).*

Conclusion *Relatively low sevoflurane concentrations (1.0%) reverse fentanyl-induced hyperalgesia in rats without inflammatory pain. Nevertheless, the lack of effect of sevoflurane concentrations of 1.0% and 1.5% to oppose hyperalgesia following high-dose fentanyl and inflammatory pain suggests that sevoflurane anti-hyperalgesic properties are weak.*

Résumé

Objectif *Les opioïdes sont largement utilisés en anesthésie mais induisent paradoxalement une hypersensibilité postopératoire à la douleur via une modulation des récepteurs N-méthyl-D-aspartate (NMDA). Les effets du*

Accepted for presentation at the annual meetings of the Société Française d'Anesthésie et Réanimation, September 2005, Paris, France and the American Society of Anesthesiologists, October 2005, New Orleans, Louisiana, USA.

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sévoflurane sur cette hyperalgésie induite par les opioïdes n'ont pas été encore évalués in vivo. Néanmoins, certaines études expérimentales in vitro rapportent des propriétés anti-NMDA pour le sévoflurane. Le but de notre étude était d'évaluer, chez des rats naifs et des rats avec douleurs inflammatoires, les effets du sévoflurane sur l'hyperalgésie induite par le fentanyl.

Méthode *Le sévoflurane a été testé chez des rats Sprague Dawley: rats naïfs, rats traités avec fentanyl au jour zéro ($4 \times 60\ \mu g \cdot kg^{-1}$) et enfin rats avec douleur de type inflammatoire (carragénine) et traités avec du fentanyl ($4 \times 60\ \mu g \cdot kg^{-1}$). Les rats ont été exposés au sévoflurane (1 ou 1,5 %) juste avant les injections (fentanyl et carragénine) et durant toute la durée de l'effet analgésique du fentanyl. Le seuil nociceptif a été évalué (test de Randall-Selitto) plusieurs fois au jour zéro, puis une fois par jour pendant une semaine. À jour sept, une injection sous-cutanée de naloxone était réalisée et le seuil nociceptif évalué cinq minutes plus tard.*

Résultats *Chez les rats non douloureux mais ayant reçu du fentanyl au jour zéro, le sévoflurane à 1 % a permis de: 1) totalement abolir l'hyperalgésie à la fois précoce et de longue durée après administration de fortes doses de fentanyl ($P < 0,05$), 2) de prévenir totalement l'hyperalgésie induite par l'injection de naloxone à jour sept ($P = 0,33$). Chez les rats qui avaient une douleur de type inflammatoire (carragénine), le sévoflurane à 1 et 1.5 % n'était pas capable de réduire significativement l'hyperalgésie induite par les fortes doses de fentanyl ($P > 0,05$), mais réduisait l'hyperalgésie induite par l'administration de naloxone au jour sept ($P = 0,061$).*

Conclusion *Le sévoflurane permet la prévention totale de l'hyperalgésie induite par le fentanyl seul chez les rats. Néanmoins, l'absence d'effet significatif du sévoflurane à 1 ou 1,5 % sur l'hyperalgésie consécutive à l'association douleur inflammatoire et fortes doses de fentanyl suggère que ses propriétés anti-hyperalgésiques sont faibles.*

It has been shown that surgery can induce pain hypersensitivity leading to hyperalgesia, allodynia, and exaggerated spontaneous pain.[1] Both peripheral and central sensitization of sensory nerve fibres defines such clinical consequences as hyperalgesia.[2,3] Previous studies reported the role of glutamate acting via *N*-methyl-D-aspartate (NMDA) receptors in the development of central sensitization.[1,4] Paradoxically, opioids have also been reported to induce hyperalgesia and allodynia via an activation of the same NMDA receptors.[5,6] Enhanced hyperalgesia was observed for several days after injections of high doses of fentanyl were administered to animals that were subjected to inflammatory or surgical pain in response to nociceptive inputs.[7,8] In human volunteers, other opioids, such as remifentanil, induced not only analgesia but also hyperlagesia once the medication was discontinued.[9] Although opioids remain an essential component of acute postoperative pain management, anesthesiologists should take into account the potential for these drugs to exacerbate post-operative pain when high doses are administered to patients intraoperatively.[10–12] Moreover, it has been suggested that this enhancement of pain sensitization of opioids may be associated with long-term chronic pain.[13]

During the last decade, many intraoperative anti-hyperalgesic strategies were designed to oppose the development of central sensitization that leads to exaggerated acute postoperative pain and chronic pain.[14–17] Interestingly, the administration of low doses of pharmacological agents with NMDA receptor antagonistic properties, such as ketamine and nitrous oxide, reduced hyperalgesia, even though these drugs did not elicit analgesic effects per se.[8,18–20] These results were described on nociceptive-induced hyperalgesia (inflammation or surgery), high doses of fentanyl-induced hyperalgesia, and a combination of both.[8,18–20]

Halogenated anesthetics have in vitro anti-NMDA properties.[21–24] Other studies have shown that halogenated anesthetics induce hyperalgesia when administered at very low concentrations[25,26] and even decrease morphine analgesic effects.[25,27] However, in the context of current clinical practice, it is unresolved as to whether or not inhaled anesthetics elicit residual effects on postoperative hyperalgesia. To address this issue, we undertook a study to evaluate whether or not sevoflurane can antagonize fentanyl-induced hyperalgesia in rats, with or without inflammatory pain. The primary aim of this study was to evaluate the ability of sevoflurane to modify the hyperalgesia induced by high dose fentanyl in the absence of inflammatory pain and to compare the nociceptive threshold (NT) values to those obtained from rats without fentanyl injection. In the second part of this study, we tested potential clinical implications to ascertain whether sevoflurane, at concentrations of 1.0 or 1.5%, would decrease hyperalgesia associated with high dose fentanyl combined with inflammatory pain compared to a control group of fentanyl-treated rats without inflammatory pain.

Methods

Animals

The study protocol was approved by our local institutional review board and was in keeping with the official edict presented by the French Ministry of Agriculture (Paris, France) under the recommendations of the Declaration of Helsinki. Pharmacological tests and care of the animals were conducted in accordance with the Animals Care and

Use manual of the National Institute of Health (1999). The rats were euthanized with carbon dioxide when the experiments were completed. The experiments were performed on adult male (300–350 g) Sprague-Dawley rats (Charles River Laboratories, l'Abresle, France) that were housed in groups of five per cage in a 12 h light-dark cycle (lights on at 7:00 a.m.) at a constant room temperature of 23 ± 2°C. The animals had access to food and water ad libitum.

Drugs

Fentanyl citrate, naloxone, and carrageenan λ (Sigma–Aldrich, Saint-Quentin Fallavier, France) were dissolved in 0.9% physiologic saline. After pinching the skin, fentanyl 60 μg kg^{-1} *sc* and naloxone 1 mg kg^{-1} *sc* were administered (1 ml kg^{-1} body weight) in the lower posterior part of the neck between the two scapula. The control animals received an equal volume of saline injection. Carrageenan (0.2 ml of a 1% carrageenan solution in saline) was prepared 24 h before each experiment. Sevoflurane (Abbott, Paris, France) was delivered via bypass vaporizers with medical air.

Exposures to gas

All exposures to anesthetic gas were performed in a Plexiglas chamber (42 cm long × 26 cm wide × 26 cm high) with a sliding door on one side to insert the rats. Five rats per group were introduced into each chamber for each of three sessions, for a total of 15 rats in each group in this study. As fresh gases were fed into the chamber through an inlet port (4 l min^{-1}), the gases inside the chamber were purged by a vacuum at the same rate as the inflow. Oxygen and sevoflurane concentrations were continuously monitored to confirm their concentrations. All gas exposures were initiated 30 min before the beginning of each experiment and were maintained for an additional 4-h exposure; the total halogenated gas exposure time was 4.5 h. The gas concentrations were monitored with an infrared analyzer (Baxter, Colin BP-508, type S; Nippon Colin Co., Komaki, Japan) and were continuously recorded by gas chromatography during the exposure period. A circuit for delivery and scavenging of the volatile anesthetic was connected to the enclosure via a gas-tight fitting at each end of the Plexiglas box. Two concentrations of sevoflurane, 1.0 and 1.5%, in medical air (Air Liquide Santé France, Paris, France) were administered.

Measurement of NT

The nociceptive thresholds in hand-held rats were determined by the Randall-Selitto method[28]; i.e., they were based on a rat's vocalization following hind paw pressure. This test involves constantly increasing the pressure applied to the rat's hind paw until it squeaks. The Basile analgesimeter (Apelex, Massy, France; stylus tip diameter, 1 mm) was used. A 600-g cut-off value was determined to prevent tissue damage. Nociceptive threshold measurements were taken for the two days (D) preceding the surgery (i.e., on D_{-2} and D_{-1}) and were repeated before tissue injury on the day of the experiment (D_0). Experiments were initiated when no change in basal NT was observed on three consecutive days (D_2, D_1, and D_0 one-way analysis of variance (ANOVA); $P > 0.05$). The reference value of the NT was chosen as the basal value on D_0 evaluated at the left hind paw.

Carrageenan injection

On D_0, the basal value of the NT was evaluated and the rats were placed in a Plexiglas box. They received the first subcutaneous fentanyl injection in the lower dorsal part of the neck between the two scapulae 50 min after sevoflurane 1.0 or 1.5% exposure was initiated. Fifteen minutes after the first fentanyl injection, and immediately before the second one, carrageenan (0.2 ml of a 1% carrageenan solution in saline) was injected into the plantar aspect of the left hind paw subcutaneously. The injections were administered with a 25-G needle.

General procedures

After their arrival in the laboratory, the animals were acclimatized to the animal care unit for four days. To avoid stress resulting from experimental conditions that might affect measurement of the NT, the same experimenter performed the experiments in quiet conditions in a testing room close to the animal care unit. For two weeks before the experiments, the animals were weighed daily, handled gently for 5 min, and placed in the testing room for 2 h (from 9:00 a.m. to 11:00 a.m.) where they were left to become acclimatized. During the same 2-week period, the rats were also acclimatized to the Plexiglas chamber used for gas administration with an air inflow rate set at 4 l min^{-1}. All experiments began at 9:00 a.m. and were performed during the light part of the cycle. Nociceptive threshold assessments were taken for the two days preceding the experimental day (i.e., on D_{-2} and D_{-1}) and were repeated on the experimental day (D_0) before the exposure to gas, pharmacological agent (fentanyl or saline), and the carrageenan injection. Next, the NT was determined several times on D_0, according to the various experimental protocols, and once daily until the rats recovered the basal values. Experiments were initiated when no change of the basal NT was observed for three

successive days (D_{-2}, D_{-1}, and D_0, one-way ANOVA; $P > 0.05$). The reference value of the NT was chosen as the basal value on D_0.

Experimental protocols (Fig. 1)

Experiment 1

Administration of sevoflurane 1.0% in medical air to fentanyl- or saline-treated rats: The rats were allocated on D_0 to one of the following groups: (1) saline group: medical air exposure for 4.5 h and subcutaneous saline injections (total of four injections: first injection 50 min after gas exposure began and then every 15 min); (2) fentanyl group: medical air exposure for 4.5 h and subcutaneous fentanyl injections (total of four injections: first injection 60 μg kg^{-1} 50 min after gas exposure began and then 60 μg kg^{-1} every 15 min); (3) sevoflurane-saline group: sevoflurane 1% in medical air exposure for 4.5 h and subcutaneous saline injections (total of four injections: first injection 50 min after gas exposure began and then every 15 min); (4) sevoflurane-fentanyl group: sevoflurane 1% in medical air exposure for 4.5 h and fentanyl subcutaneous injections (total of four injections: first injection 60 μg kg^{-1} 50 min after gas exposure began and then 60 μg kg^{-1} every 15 min). A total of nine NT measurements were performed on D_0. Nociceptive threshold measurements were taken before gas exposure, 20 min after gas exposure began, 1 h after the first fentanyl injection, and then every 30 min for 8 h. After D_0, the rats were tested once daily during the seven subsequent days (D_1–D_7). When the rats returned to the basal NT value (D_7), one naloxone injection 1 mg kg^{-1} *sc* was administered, and the NT was measured 5 min later.

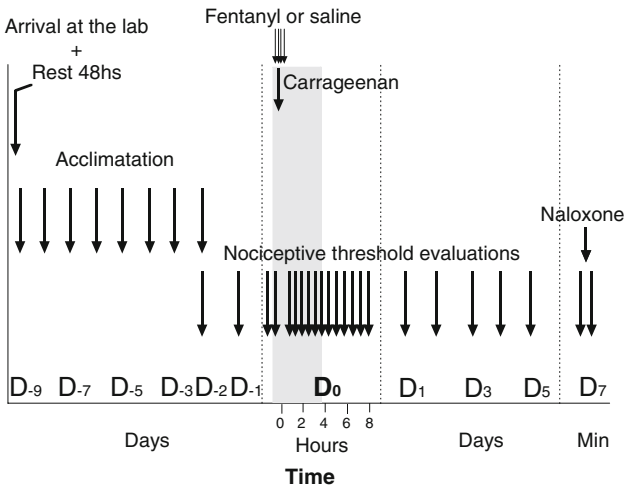

Fig. 1 Design of the experimental study in rats. Rats were allowed to rest for 2 days after arrival at the lab. Rats were then acclimatized to the testing room, the tests, the Plexiglas chamber for gas exposure, and the experimenter. The nociceptive threshold was assessed from day D_2 to D_7. On D_0, the nociceptive threshold was evaluated every 30 min for 8 h (for experiment 1) or every 2 h for 8 h (for experiments 2 and 3). In experiment 1, on D_0, rats were treated with sevoflurane (inhaled concentration: 1.0 or 1.5%) or air, fentanyl (4×60 μg kg^{-1}) or saline. In experiments 2 and 3, carrageenan was injected in the plantar aspect of the left hind paw; subcutaneous fentanyl was administered in the back part of the neck, between the two scapulae, and these rats were subjected to air, sevoflurane 1.0% and sevoflurane 1.5%. On D_7, subcutaneous naloxone was administered, and the nociceptive threshold was evaluated 5 min later

Experiment 2

Administration of sevoflurane 1.0% in medical air to fentanyl- and carrageenan-treated rats: On D_0, the rats were allocated to one of the following groups: (1) control group: medical air exposure for 4.5 h and subcutaneous fentanyl 60 μg kg^{-1} injections, 50 min after gas exposure began and then 60 μg kg^{-1} every 15 min (total of four injections), in rats also treated with carrageenan on D_0; and (2) sevoflurane group: sevoflurane 1% in medical air exposure for 4.5 h and subcutaneous fentanyl 60 μg kg^{-1} injections, 50 min after gas exposure began and then 60 μg kg^{-1}every 15 min (total of four injections), in rats also treated with carrageenan on D_0.

The carrageenan plantar injection was performed in both groups 15 min after the first fentanyl injection. Nociceptive threshold measurements were taken on D_0 before gas exposure and 2, 4, 6, and 8 h after the first fentanyl injection. After D_0, the rats were evaluated daily during the seven subsequent days (D_1–D_7). When the rats returned to the basal NT value (D_7), one naloxone injection 1 mg kg^{-1} *sc* was administered, and the NT was measured 5 min later.

Experiment 3

Administration of sevoflurane 1.5% in medical air to fentanyl- and carrageenan-treated rats: Other than the fact that the sevoflurane concentration was 1.5% in experiment 3, the experimental design was identical to that of experiment 2.

Statistical analysis

All data are expressed as mean ± SD. The reference value of the NT was considered as the basal value on D_0. One-way repeated measures ANOVA was used to assess time effects of treatments on NT within each group. Two-way ANOVA was used to test for differences between two groups. Two different statistical analyses with one-way ANOVA were performed for two different periods of time: (1) Considering NT values from the basal value on D_0 until the last NT evaluation on D_0 (since the period of time between the two NT evaluations on D_0 differed from the period of time separating the two evaluations on D_1 to D_7);

(2) Basal values taken on D_0, before any injection, compared to NT values on D_1 to D_7. After ANOVA one-way was performed for each group, a post-hoc analysis was done using the Dunnett's test to investigate for time effects. After two-way ANOVAs were completed for comparison between groups, post-hoc Dunnett's tests were administered to test for comparison between groups. Paired Student's *t* tests were used to compare the hyperalgesic effect induced by naloxone on D_7. The accepted value for significance was $P < 0.05$. Statistical analyses were performed by using Statistica® computer software (StatSoft, Maisons-Alfort, France) (Table 1).

Results

Effects of sevoflurane 1.0% on NT in saline- or fentanyl-treated rats (Fig. 2)

Figure 2 shows the results from control rats that breathed medical air, demonstrating absence of variation of their NT throughout the experiment, i.e., D_2 to D_7. Naloxone 1 mg kg^{-1} *sc* administration on D_7 had no effect on the NT value in naive rats (Student's *t* test, $P = 1$).

In the presence of sevoflurane 1.0%, rats that had not received fentanyl had increased values of their NT throughout the gas exposure. When sevoflurane was switched to air after a 4.5 h exposure, NT remained higher than corresponding values in the air control group (Dunnett's test, $P = 0.001$) but quickly decreased to the basal values after 30 min. In both groups that received air or sevoflurane, but no fentanyl on D_0, the NT remained stable for the ensuing 7 days. Naloxone administration on D_7 had no effect on the NT (Student's *t* test, $P = 0.41$).

The administration of four subcutaneous injections of fentanyl (4×60 µg kg^{-1}) in rats breathing medical air induced a large increase of the NT for 3.5 h ($P < 0.001$ for each point). This increase was followed by a decrease of the NT below the basal value for 2.5 h (Dunnett's test for five hyperalgesic points for 2.5 h: $P = 0.002$, $P = 0.006$, $P < 0.001$, $P < 0.001$, $P = 0.04$, respectively). On the following three days, the NT values were diminished compared to the basal value (Dunnett's tests, $P < 0.001$ on D_1, $P < 0.001$ on D_2, and $P = 0.002$ on D_3). When the NT returned to the basal value, naloxone administration on D_7, induced a significant decrease in the NT compared to basal value (Student's *t* test, $P = 0.0013$) and saline rats (Dunnett's test, $P = 0.003$).

The association of fentanyl and sevoflurane 1% treatments induced an early increase of the NT that lasted throughout the sevoflurane exposure. After sevoflurane was discontinued, no decrease below the basal value was observed in rats treated by both fentanyl and sevoflurane,

Table 1 Nociceptive threshold on day D_0 (g; mean ± SD)

	D_0 basal value	Before stopping gas administration (H_4)	$H_4 + 30$ min	$H_4 + 60$ min	$H_4 + 90$ min	$H_4 + 120$ min	$H_4 + 150$ min	$H_4 + 180$ min	$H_4 + 210$ min	$H_4 + 240$ min
Group air + subcutaneous saline	287 ± 24	307 ± 24	295 ± 24	285 ± 21	295 ± 26	297 ± 23	277 ± 21	282 ± 20	292 ± 20	292 ± 15
Group air + subcutaneous fentanyl	289 ± 13	364 ± 35	291 ± 28	233 ± 27	236 ± 31	221 ± 33	216 ± 31	246 ± 27	257 ± 20	272 ± 43
	NS*	$P = 0.03$*	NS*	$P < 0.001$*	$P = 0.003$*	$P = 0.001$*	$P = 0.009$*	$P = 0.02$*	NS*	NS*
Group sevoflurane + subcutaneous saline	295 ± 17	583 ± 26	362 ± 42	317 ± 46	305 ± 25	295 ± 17	308 ± 13	292 ± 21	288 ± 23	295 ± 14
Group sevoflurane + subcutaneous fentanyl	285 ± 15	583 ± 26	573 ± 46	347 ± 48	318 ± 45	282 ± 40	273 ± 36	268 ± 26	275 ± 29	285 ± 29
	NS$	$P < 0.001$$	$P < 0.001$$	$P < 0.001$$	$P < 0.001$$	$P = 0.004$$	$P = 0.003$$	$P = 0.009$$	NS$	NS$

Nociceptive thresholds (NT) at the left hind paw level on day (D_0): NT basal values, NT before discontinuing the anesthetic, and NT for several hours after gas discontinuation in four groups of rats (see groups from Fig. 2) are reported.

* Dunnett's test for comparison between groups: air + subcutaneous saline vs. air + subcutaneous fentanyl

$ Dunnett's test for comparison between groups: air + fentanyl vs. sevoflurane 1.0% + fentanyl

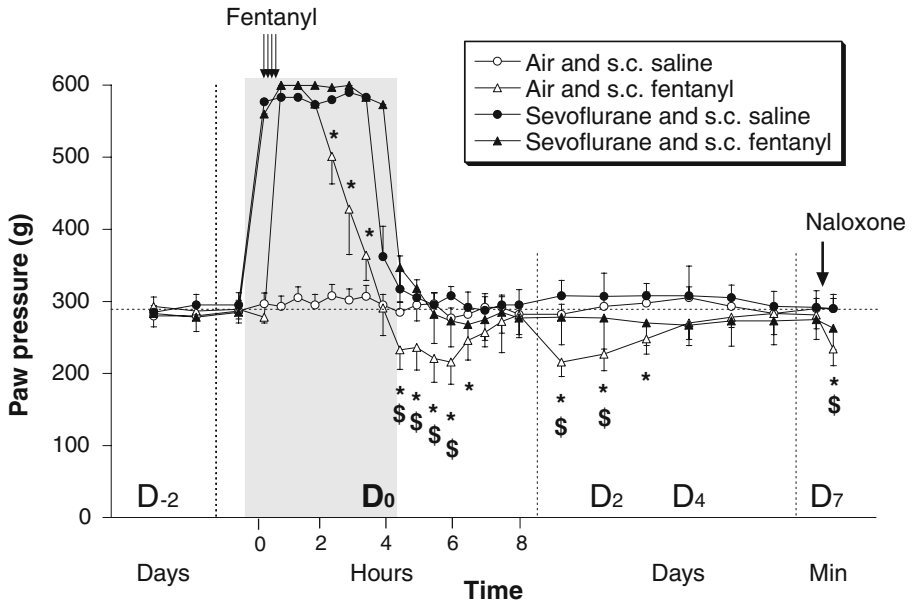

Fig. 2 Effects of sevoflurane 1% on the nociceptive threshold in saline- or fentanyl-treated rats. Four fentanyl (4×60 μg kg^{-1}) or subcutaneous saline injections were administered on D_0 every 15 min. D_0 was the day for drug administration: subcutaneous fentanyl or saline, inhaled medical air or sevoflurane. The nociceptive threshold was evaluated on D_2, D_1 and D_0, then every 30 min on D_0 for 8 h, and finally once daily until D_7. On D_7, a subcutaneous naloxone injection was administered and the nociceptive threshold was evaluated 5 min later. *Open circles* subcutaneous saline-treated rats and breathing air for 4.5 h on D_0 (gas exposure = *grey plot* on D_0); *filled circles* subcutaneous saline-treated rats and breathing sevoflurane 1.0% concentration for 4.5 h on D_0; *open triangles* fentanyl 4×60 μg kg^{-1} *sc* treated rats breathing air for 4.5 h on D_0; *filled triangles* fentanyl 4×60 μg kg^{-1} *sc* treated rats breathing sevoflurane 1.0% concentration for 4.5 h on D_0. *Dunnett's test, $P < 0.05$ for comparison between groups: air + saline vs. air + fentanyl; $^{\$}$Dunnett's test, $P < 0.05$ for comparison between groups: air + fentanyl vs. sevoflurane 1.0% + fentanyl

compared to rats treated with fentanyl only. Differences were observed in NT between air-fentanyl-treated rats and sevoflurane 1.0%-fentanyl-treated rats from 4.5 to 6 h after fentanyl injections (Dunnett's test for each point from 4.5 to 6 h after gas discontinuation, $P < 0.001$, $P < 0.001$, $P = 0.004$, $P = 0.003$, and $P = 0.009$, respectively), with higher NT values for the group sevoflurane 1.0%-fentanyl. Differences in the NT were observed between groups for 2 days (Dunnett's test, $P < 0.001$ on D_1 and $P = 0.034$ on D_2), and the NT was lower in the air-fentanyl group compared to the sevoflurane 1.0%-fentanyl group. When fentanyl-treated rats breathed air rather than sevoflurane on D_0, the reduction in the NT was greater after naloxone injection on D_7 (Dunnett's test, $P = 0.031$).

Effects of sevoflurane 1.0% on NT in fentanyl- and carrageenan-treated rats (Fig. 3a)

Both fentanyl subcutaneous administration (in the lower back part of the neck) and carrageenan injection (in one hind paw) on D_0 induced a large increase of the NT value in the rats breathing medical air (control group) for the 2 h after the first fentanyl injection, showing an analgesic effect of fentanyl (Fig. 3a). Measurements at 6 and 8 h after the first fentanyl administration showed a NT decrease below the D_0 basal value (Dunnett's test, $P = 0.009$ and $P = 0.008$, respectively). The nociceptive threshold remained below the basal value for 3 days (Dunnett's test, $P < 0.001$ for the three days). When the NT had returned to the basal value, naloxone administration on D_7 was associated with a decrease in the NT (Student's t test, $P < 0.001$).

Rats treated with fentanyl and carrageenan and breathing sevoflurane 1.0% showed an increase of NT that lasted for 4 h. At 6 and 8 h after the first fentanyl injection, NT values were lower than the basal value on D_0 (Dunnett's test, $P < 0.001$ for the two NT values), but were not statistically different from the medical air-treated group (Dunnett's test, $P = 0.7$). For air-fentanyl rats, the nociceptive threshold remained below the basal value for 3 days after D_0 and for 2 days for the sevoflurane-fentanyl rats (Dunnett's test, $P < 0.001$ and $P = 0.009$, respectively). From hour six on D_0 to D_7, no differences were observed between the two groups before naloxone administration. Naloxone administration on D_7 did not induce a decrease in the NT in the sevoflurane-fentanyl rats (Student's t test, $P = 0.061$). Naloxone-induced hyperalgesia on D_7 was significantly greater in rats treated with air on D_0 compared to those treated with sevoflurane 1.0% concentration (Dunett's test, $P = 0.0058$).

Effects of sevoflurane 1.5% on NT in fentanyl- and carrageenan-treated rats (Fig. 3b)

Sevoflurane 1.5% did not differ from the medical air group from hour six to D_7. The only difference between these groups was observed on D_7 after naloxone administration, when the NT decrease occurred in air-treated rats but not in sevoflurane-treated rats (Dunett's test, $P < 0.001$).

Discussion

There are two key findings from this study. First, in opioid-naive rats without any inflammation, sevoflurane 1.0% prevented hyperalgesia induced by large doses of fentanyl. Second, sevoflurane 1.0% or 1.5% did not antagonize hyperalgesia in rats following the combination of inflammation with carrageenan and administration of subcutaneous high doses of fentanyl.

In the first set of experiments, the experimental protocol evaluated the effects of sevoflurane 1.0% on the NT in naive rats. At this concentration, sevoflurane induced sedation with hypotonia. Thereafter, rats breathing sevoflurane 1.0% developed a NT that increased progressively until high values close to the pre-determined cut-off value (600 g) were achieved; this increase lasted throughout the period of exposure to the inhaled anesthetic. This increase in NT must be interpreted cautiously. It has been reported that halogenated anesthetics, such as isoflurane, administered at concentrations close to or above the minimum alveolar concentration (MAC), could elicit analgesic effects.[26,29] In our study, sevoflurane appears to demonstrate analgesic effects, per se, as seen with the increase in NT during exposure to the gas. Nevertheless, sedative and motor effects must also be taken into account to explain this apparent analgesic effect. Indeed, halogenated anesthetics have been widely reported to elicit direct spinal motor effects in addition to their sedative effects.[30] For this reason, we did not evaluate paw withdrawal, as this is a reflex response to measure NT. Instead, we chose to evaluate paw-pressure vocalization, as it reflects a more integrated response.

This study was not designed to evaluate the analgesic effects of sevoflurane during routine exposure, but rather to determine the ability of sevoflurane to antagonize hyperalgesia after fentanyl administration and/or inflammatory-induced pain several hours or days after exposure to the inhaled anesthetic, as previously described with other anti-NMDA pharmacological agents.[7,20]

When sevoflurane was discontinued, the rats quickly awoke and NT values decreased within 30 to 60 min to respective basal values. The nociceptive thresholds remained stable for several days until D_7. During the period

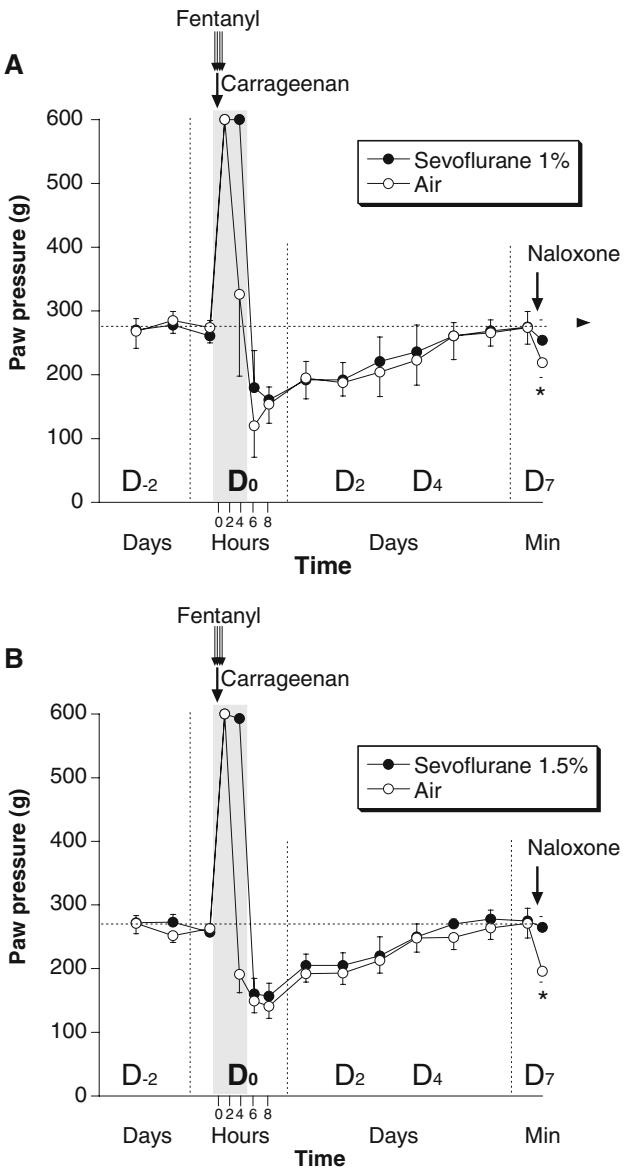

Fig. 3 Effects of sevoflurane 1.0 and 1.5% on nociceptive thresholds in fentanyl- and carrageenan-treated rats. Four fentanyl (4×60 µg kg^{-1} *sc*) injections were administered in the lower back part of the neck on D_0 every 15 min. D_0 was the day for drug administration: subcutaneous fentanyl or saline, inhaled medical air or sevoflurane. Carrageenan was injected into the plantar aspect of the left hind paw on D_0 15 min after the first fentanyl injection. Nociceptive thresholds were evaluated on D_2, D_1 and D_0, then every 2 h on D_0 for 8 h, and thereafter once daily until D_7. On D_7, subcutaneous naloxone was injected and the nociceptive threshold was assessed 5 min later. **a** Sevoflurane 1.0% inhaled concentration: *open circles* subcutaneous fentanyl- and carrageenan-treated rats and breathing air for 4.5 h on D_0 (gas exposure = *grey plot* on D_0); *filled circles* subcutaneous fentanyl- and carrageenan-treated rats and breathing sevoflurane 1% concentration for 4.5 h on D_0. **b** Sevoflurane 1.5% inhaled concentration: *open circles* subcutaneous fentanyl- and carrageenan-treated rats and breathing air for 4.5 h on D_0 (gas exposure = *grey plot* on D_0); *filled circles* subcutaneous fentanyl- and carrageenan-treated rats and breathing sevoflurane 1% concentration for 4.5 h on D_0. *Dunnett's test, $P < 0.05$ for comparison between groups: air + fentanyl + carrageenan vs. sevoflurane + fentanyl + carrageenan

of awakening, the sevoflurane concentration decreased below the MAC value for rats.[26] Our experimental data did not replicate what others have reported regarding hyperalgesia induced by low concentrations of halothane or isoflurane.[26] The concentration of sevoflurane used in the current study was below MAC values in rats, which is reported to range between 2.3 and 3.0%, according to the age of the rat. In our experiment, we worked with spontaneous breathing rats. No endotracheal tubes were used and no tracheotomies were administered, which may explain why we continued to work with "low" sevoflurane concentrations at 1.0 and 1.5% maximum. Furthermore, the investigation of sevoflurane with relatively high doses of fentanyl (all rats received 4×60 µg kg^{-1} fentanyl *sc*) required the use of moderate sevoflurane concentrations to prevent profound respiratory depression that could have proven fatal.

The data from experiment 1 show that sevoflurane can completely antagonize the development of long-term hyperalgesia induced by large doses of fentanyl. The 4.5 h duration of exposure to sevoflurane was chosen to correspond with the duration of fentanyl analgesia that may be responsible for fentanyl-induced hyperalgesia, which may persist for several days after fentanyl is discontinued. There is some evidence that analgesic effects, due to repeated opioid dosing, may result in the development of hyperalgesia, the magnitude and duration of which is dose-dependent.[6,12,19,31] Further evidence of this phenomenon originates from experimental studies that report hyperalgesia lasting for several days after heroin or fentanyl administration.[32,33] The concept of *general balanced anesthesia* is defined by the co-administration of several pharmacological agents, so as to enhance their beneficial effects while reducing the side effects of individual drugs administered in high doses.[34] Clinical studies have reported that the reduction of intraoperative opioid doses is associated with diminished postoperative pain and reduced morphine consumption.[10,11]

In the present study, repeated fentanyl administrations every 15 min (4×60 µg kg^{-1}) on D_0 induced an early (lasting 4 h) and delayed and long-lasting hyperalgesia (lasting 3 days). Sevoflurane exposure at 1.0% for 4.5 h on D_0 prevented the development of hyperalgesia. This protective effect of sevoflurane could be analogous to that observed with NMDA antagonists (e.g., MK-801, ketamine) in rats[7,8,33] and in humans.[9] Moreover, several in vitro electrophysiological and pharmacological studies have demonstrated sevoflurane's anti-NMDA properties.[21,23] The findings from our study suggest that sevoflurane, when administered at concentrations even below the MAC value, might play an anti-hyperalgesic role via an anti-NMDA action.

Our results have led us to evaluate a potential preventive role of sevoflurane to antagonize hyperalgesia after inflammatory pain in fentanyl-treated rats, which more closely mimics the clinical situation in patients undergoing anesthesia and surgery. From a medical viewpoint, we can consider that postoperative pain consists of two components.[35] The first component follows inflammation, incision, and neuropathy and depends on the duration of the nociceptive stimulation. The second component is a consequence of the central sensitization induced by nociceptive inputs and facilitated by intraoperative use of large doses of opioid analgesics.[7] The inflammatory pain model with a plantar injection of carrageenan was previously used to evaluate such postoperative pain hypersensitivity leading to long-lasting hyperalgesia. It has been shown that fentanyl injections dose-dependently enhance the long-lasting hyperalgesia induced by inflammatory pain for several days after the carrageenan injection. Such a hyperalgesic enhancement was prevented by ketamine administration in rats.[8] More recent studies have shown that nitrous oxide also presented anti-NMDA properties and prevented the enhancement of hyperalgesia induced by fentanyl in this inflammatory pain model.[7] For these reasons, we decided to test sevoflurane in fentanyl-treated rats with inflammatory pain. Despite the positive effects of sevoflurane for preventing hyperalgesia induced by opioids in rats without pain, this gas exposure at 1.0% did not prevent the long-lasting hyperalgesia observed after fentanyl treatment in rats experiencing pain. Increasing the sevoflurane concentration to 1.5% did not induce greater effects for preventing inflammation and opioid-related hyperalgesia. These results suggest that, at clinical concentrations, the anti-hyperalgesic properties of sevoflurane are not sufficiently potent to prevent hyperalgesia induced by both nociceptive inputs and high-doses of fentanyl. Similar findings have been reported for halothane and isoflurane regarding the lack of effectiveness of these gases to reduce neuronal hyperexcitability in the dorsal horn after surgery in rats.[22] Other studies reported differential effects of halogenous anesthetics on windup phenomena and neuronal excitability in the dorsal horn[36] or the effects of halogenous anesthetics on wide-dynamic range neuronal activity in the dorsal horn.[37]

One limitation of this work stems from a pharmacological viewpoint, that it would have been justified to have evaluated the effects of higher concentrations of sevoflurane. But, as previously reported, it would have been impossible to administer higher concentrations of sevoflurane concomitant with high dose fentanyl in spontaneously breathing rats, without the risk of profound respiratory depression and death that would terminate the experiment.

Our results also indicated that sevoflurane administration (1.0 or 1.5%) in rats, with or without pain, prevented hyperalgesia induced by naloxone administration on D_7, once the rats' NT had returned to basal values. It has been

suggested that hyperalgesia, induced by naloxone in animals previously treated with opioids on D_0, could be explained by the development of a new state in terms of pain sensitivity. This new state would be called "latent pain sensitization" or "pain vulnerability". In the rat model for such experiments, this pain vulnerability developed after the exposure to high doses of opioids could be revealed by naloxone administration, whereas NT did eventually normalize.[13,32] In the present study, sevoflurane demonstrated potentially beneficial properties that could decrease naloxone-induced hyperalgesia.

It warrants further investigation whether this development could translate into a potential reduction in the risk for developing chronic pain.

Our results may help us better understand the benefits of balanced anesthesia. Some clinical studies have reported an increase in pain scores and higher morphine consumption after anesthesia involving high doses of opioids.[10,11,38] These studies compared two anesthetic strategies—the first was based on high opioid doses, and the second was based on concentrations of inhaled anesthetics. Patients who received higher concentrations of inhaled anesthetics reported lower pain scores and had reduced morphine consumption compared to the high-dose opioid group, although it was not possible to elucidate the mechanism.

In conclusion, our results suggest that prolonged exposure to sevoflurane may antagonize opioid-induced hyperalgesia; however, below MAC sevoflurane concentrations of 1.0 and 1.5% in a rat model are inadequate to prevent hyperalgesia induced by both large doses of opioids and an inflammatory painful process, as previously demonstrated with other anti-NMDA receptors such as ketamine or nitrous oxide. Nevertheless, by reducing opioid requirements during surgery, sevoflurane has the potential to reduce postoperative pain and hyperalgesia.

Acknowledgments This work was supported by the Université Victor Segalen Bordeaux 2, Bordeaux, France, the Ministère de l'Education nationale, de l'Enseignement supérieur et de la Recherche, Paris, France, the Conseil Régional d'Aquitaine, Bordeaux, France.

Conflicts of interest None declared.

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