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Role of central and peripheral opiate receptors in the effects of fentanyl on analgesia, ventilation and arterial blood-gas chemistry in conscious rats

Fraser Henderson^a, Walter J. May^a, Ryan B. Gruber^b, Joseph F. Discala^b, Veljko Puscovic^b, Alex P. Young^a, Santhosh M. Baby^b, and Stephen J. Lewis^{c,*}

^aPediatric Respiratory Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908, USA

^bDivision of Biology, Galleon Pharmaceuticals, Horsham, PA 19044, USA

^cDepartment of Pediatrics, Case Western Reserve University, Cleveland, OH 44106-4984, USA

Abstract

This study determined the effects of the peripherally restricted μ -opiate receptor (μ -OR) antagonist, naloxone methiodide (NLXmi) on fentanyl (25 μ g/kg, i.v.)-induced changes in (1) analgesia, (2) arterial blood gas chemistry (ABG) and alveolar-arterial gradient (A-a gradient), and (3) ventilatory parameters, in conscious rats. The fentanyl-induced increase in analgesia was minimally affected by a 1.5 mg/kg of NLXmi but was attenuated by a 5.0 mg/kg dose. Fentanyl decreased arterial blood pH, pO₂ and sO₂ and increased pCO₂ and A-a gradient. These responses were markedly diminished in NLXmi (1.5 mg/kg)-pretreated rats. Fentanyl caused ventilatory depression (e.g., decreases in tidal volume and peak inspiratory flow). Pretreatment with NLXmi (1.5 mg/kg, i.v.) antagonized the fentanyl decrease in tidal volume but minimally affected the other responses. These findings suggest that (1) the analgesia and ventilatory depression caused by fentanyl involve peripheral μ -ORs and (2) NLXmi prevents the fentanyl effects on ABG by blocking the negative actions of the opioid on tidal volume and A-a gradient.

Keywords

Fentanyl; Naloxone methiodide; Ventilation; Arterial blood gases; Analgesia; Rats

1. Introduction

The peripheral administration of opioids such as morphine elicits analgesia in humans (DeHaven-Hudkins and Dolle, 2004; Stein and Lang, 2009) and rodents (Reichert et al., 2001; Lewanowitsch and Irvine, 2002; Lewanowitsch et al., 2006; Stein et al., 2009) via activation of opiate receptors (ORs) in the central nervous system (CNS) and on peripheral

*Correspondence: Stephen J. Lewis, Department of Pediatrics, Case Western Reserve University, 0900 Euclid Avenue, Cleveland, OH 44106-4984, USA. sjl78@case.edu.

Conflicts of Interest

None

nociceptive afferents. Analgesic doses of opioids are associated with a significant incidence of ventilatory depression in humans and rodents via activation of ORs within the CNS and also key peripheral structures such as the carotid bodies and neuromuscular components of the chest-wall and diaphragm (Dahan et al., 2010). In addition, opiates increase pulmonary vascular resistance suggesting decreased perfusion of alveoli (Schurig et al., 1978; Hakim et al., 1992).

Fentanyl is a high-potency opiate that is widely prescribed to treat acute and chronic pain (Nelson and Schwaner, 2009; Johnston, 2010). Abuse or misuse lead to significant consequences, including death via depression of ventilation (Nelson and Schwaner, 2009). The mechanisms responsible for the analgesic and ventilatory depressant effects of fentanyl and analogs have been extensively investigated (Dahan et al., 1998; Sarton et al., 1999; Stein et al., 2009). Although fentanyl is thought of as a selective μ -OR agonist (Trescot et al., 2008; Hajiha et al., 2009) and has high affinity for μ -ORs (Raynor et al., 1994; Huang et al., 2001), it also activates δ - and κ -ORs with affinities/intrinsic activities of biological significance (Yeadon and Kitchen, 1990; Zhu et al., 1996; Butelman et al., 2002; Gharagozlou et al., 2006). For example, whereas fentanyl has low affinity for κ -ORs it has a remarkably high efficacy at these ORs (Gharagozlou et al., 2006).

The relative contributions of central and peripheral ORs in the analgesic and ventilatory effects of fentanyl have received little attention. One approach to evaluating these contributions is to compare the effects of centrally-penetrant and -impenetrant OR antagonists on the fentanyl-induced responses. Naloxone (NLX) is an OR antagonist that readily enters the CNS (DeHaven-Hudkins and Dolle, 2004; Stein et al., 2009). NLX is an effective antagonist of μ -, δ - and κ -ORs although it has \approx twice the affinity for μ -ORs than for δ -ORs and \approx 15 times greater affinity for μ -ORs than κ -ORs (see Lewanowitsch and Irvine, 2003). In contrast, it appears that naloxone methiodide (NLXmi) does not cross the blood-brain barrier in rodents (Lewanowitsch and Irvine, 2002; Lewanowitsch et al., 2006; Inglis et al., 2008; He et al., 2009). NLXmi has lower affinities for μ -, δ - and κ -ORs than NLX (Lewanowitsch and Irvine, 2003). For example: (1) NLXmi has \approx 20 times lower affinity for μ -ORs in rat brain membranes than NLX (Bianchetti et al., 1983; Valentino et al., 1983), (2) NLXmi had \approx 10 times lower affinity for μ -, κ - and δ -ORs in guinea pig brain homogenates than NLX (Magnan et al., 1982), and (3) binding affinities for NLX versus NLXmi in mouse brain homogenates was 15:1 for μ -, 6:1 for κ - and 330:1 for δ -ORs (Lewanowitsch and Irvine, 2003). Evidence that the analgesic and ventilatory depressant effects of morphine, methadone and heroin in mice were reversed by NLXmi (Lewanowitsch and Irvine, 2002; Lewanowitsch et al., 2006) provides evidence that peripheral ORs are involved in the effects of these opioids.

Since the relative contributions of central and peripheral ORs in the analgesic and ventilatory effects of fentanyl are not known, the aims of this study were to use conscious rats to determine (1) the effects of NLXmi (1.5 mg/kg, i.v.) on fentanyl-induced changes in analgesia status, arterial blood-gas chemistry (ABG) and Alveolar-arterial (A-a) gradient, an index of ventilation-perfusion (Stein et al., 1995; Story, 1996) and (2) the effects of 1.5 mg/kg doses of NLXmi or NLX on fentanyl-induced changes in ventilatory parameters. These studies were designed to discern which of the ventilatory responses and/or changes in

A-a gradient were responsible for the fentanyl-induced changes in ABG chemistry, and the role of peripheral ORs in these responses.

2. Methods

2.1. Rats and surgeries

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996. The protocols were approved by the Animal Care and Use Committee of the University of Virginia. Adult male Sprague-Dawley rats were purchased from Harlan (Madison, WI) with jugular vein catheters and/or femoral artery catheters (surgeries performed under ketamine-xylazine). The rats were allowed 5 days to recover from surgery and transport and were used for the blood-gas and analgesia studies. Other adult male Sprague-Dawley rats were purchased from Harlan and venous catheters were implanted under 2% isoflurane anesthesia. These rats were given 4 days to recover from surgery and were used for the plethysmography and body temperature (T_B) studies.

2.2. Protocols for blood gas measurements and determination of Arterial-alveolar gradient

Study 1: Arterial blood samples (200 μ L) were taken before and 5 and 12 min after injection of vehicle (saline, i.v.; n = 6 rats; 319 ± 3 g) or NLXmi (1.5 mg/kg, i.v.; n = 6 rats; 323 ± 4 g) and 1, 5 and 9 min after injection of fentanyl (25 μ g/kg, i.v.). **Study 2:** Arterial blood samples (200 μ L) were taken before and 5, 10 and 15 min after injection of vehicle (n = 6 rats; 312 ± 2 g) or NLXmi (1.5 mg/kg, i.v.; n = 6 rats; 310 ± 3 g) and 5, 10 and 15 min after injection of fentanyl (50 μ g/kg, i.v.). The pH, pCO₂, pO₂ and sO₂ of arterial blood samples (100 μ L) were measured by a Radiometer blood-gas machine (ABL800 FLEX). The A-a gradient measures the difference between alveolar and arterial blood concentrations of O₂ (Stein et al., 1995; Story, 1996). A decrease in PaO₂, without a change in A-a gradient is caused by hypoventilation whereas a decrease in PaO₂ with an increase in A-a gradient indicates ventilation-perfusion mismatch or shunting (Stein et al., 1995). A-a gradient = PAO₂ - PaO₂, where PAO₂ is the partial pressure of alveolar O₂ and PaO₂ is pO₂ in arterial blood. PAO₂ = [(FiO₂ × (P_{atm} - P_{H2O}) - (PaCO₂/respiratory quotient)], where FiO₂ is the fraction of O₂ in inspired air; P_{atm} is atmospheric pressure; P_{H2O} is the partial pressure of water in inspired air; PaCO₂ is pCO₂ in arterial blood; and respiratory quotient (RQ) is the ratio of CO₂ eliminated/O₂ consumed. We took FiO₂ of room-air to be 21% = 0.21, P_{atm} to be 760 mmHg, and P_{H2O} to be 47 mmHg (see Stein et al., 1995; Story, 1996). We took the RQ value of our adult male Sprague-Dawley rats to be 0.9 (Stengel et al., 2010; Chapman et al., 2012).

2.3. Antinociception protocols

Antinociception was determined by the radiant heat tail-flick (TF) assay (Lewis et al., 1991). The intensity of the light was adjusted so that baseline TF latencies were ≈ 3 s. A cutoff time of 12 s was set to minimize damage to the tail. Rats were injected with vehicle (saline, i.v.; n = 4 rats; 300 ± 1 g) or NLXmi (1.5 mg/kg, i.v.; n = 4; 290 ± 2 g) and after 15 min the rats received fentanyl (25 μ g/kg, i.v.). Other rats received vehicle (saline; n = 5 rats; 315 ± 7 g) or NLXmi (5 mg/kg, n = 5 rats; 317 ± 5 g) and after 15 min, an injection of fentanyl (25

$\mu\text{g}/\text{kg}$, i.v.). The rats were tested for antinociception at 15, 45, 60, 90 and 120 min post-fentanyl. Data are presented as TF latencies (s) and as “maximum possible effect” (%MPE) using the formula, $\%MPE = [(post\text{-}injection\ TF\ latency - baseline\ TF\ latency)/(12 - baseline\ TF\ latency)] \times 100$.

2.4. Body temperature (T_B) protocols

Changes in T_B can significantly impact the magnitude of recorded flow-related variables in plethysmography chambers (Mortola and Frappell, 1998). Although our plethysmography chambers are not equipped to continuously monitor T_B , it remains imperative to record T_B to better understand the influences of NLXmi and NLX on fentanyl-induced changes in ventilation and RQ. Adult male Sprague-Dawley rats of approximately 300 g were placed in separate open plastic boxes and allowed 60–90 min to acclimatize. T_B was recorded as described previously (Kregel et al., 1997). In brief, a thermistor probe was inserted 5–6 cm into the rectum to allow regular recording of T_B . A 2–3 in. length of the probe cable, which was connected to a telethermometer (Yellow Springs Instruments, South Burlington, Vermont), was taped to the tail. T_B was recorded every 5 min during the acclimatization period to establish baseline values. One group of rats ($n = 6$) received an injection of vehicle (saline, i.v.) whereas other groups ($n = 6$ rats per group) received either NLX (1.5 mg/kg, i.v.) or NLXmi (1.5 or 5.0 mg/kg, i.v.). After 15 min, the four groups of rats received fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.). T_B was recorded 5, 10 and 15 min after injection of vehicle or the OR antagonists, and 5, 10, 15 and 20 min after injection of fentanyl. These and all rats used in the other described studies were not fasted prior to use in the experiments.

2.5. Ventilatory protocols

Ventilatory parameters were continuously recorded in rats using a whole body plethysmography system (PLY 3223; Buxco Inc., Wilmington, NC, USA), as described previously (Kanbar et al., 2010; Young et al., 2013). The parameters were (1) frequency of breathing (f_R), (2) tidal volume (V_T), (3) minute ventilation ($V' = f_R \times V_T$), (4) inspiratory time (T_I), (5) expiratory time (T_E), (6) end inspiratory pause (EIP), time between end of inspiration and start of expiration, (7) peak inspiratory flow (PIF), and (8) peak expiratory flow (PEF). Provided software constantly corrected digitized values for changes in chamber temperature and humidity and a rejection algorithm was included in the breath-by-breath analysis to exclude nasal breathing. The rats were placed in the plethysmography chambers and allowed 45–60 min to acclimatize. One group of rats received a bolus injection of vehicle (saline, i.v.) whereas another group received an injection of NLX (1.5 mg/kg, i.v.). After 15 min, both groups of rats received an injection of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) and parameters recorded for a further 20 min. In another study, one group of rats received an injection of vehicle (saline, i.v.) and another received an injection of NLXmi (1.5 mg/kg, i.v.). After 15 min, both groups of rats received an injection of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) and parameters recorded for a further 20 min. The body weights of the four groups of rats ($n = 6$ rats per group) were 328 ± 3 , 331 ± 3 , 334 ± 3 and 329 ± 5 g, respectively ($P > 0.05$, for all between-group comparisons). Due to the closeness of these body weights, ventilatory data are presented without body weight corrections.

2.6. Statistics

All data are presented as mean \pm SEM and were analyzed by one-way or two-way analysis of variance followed by Student's modified t test with Bonferroni corrections for multiple comparisons between means using the error mean square terms from the analyses of variance (Wallenstein et al., 1980). A value of $P < 0.05$ was taken to denote statistical significance.

3. RESULTS

3.1. Tail-flick latencies

Fentanyl (25 $\mu\text{g}/\text{kg}$) elicited a robust antinociception of at least 2h in duration in vehicle-treated (VEH) rats and smaller but not statistically different responses in NLXmi (1.5 mg/kg)-treated (NLXmi) rats (Fig. 1). Pretreatment with a higher dose of NLXmi (5 mg/kg) markedly attenuated but did not abolish the antinociceptive effects of the 25 $\mu\text{g}/\text{kg}$ dose of fentanyl (Fig. 1).

3.2. Arterial blood gas chemistry

The effects of fentanyl (25 $\mu\text{g}/\text{kg}$) on ABG chemistry and A-a gradients in VEH or NLXmi (1.5 mg/kg) rats are summarized in Fig. 2. The terms F1, F5 and F9 on the x-axis refer to the values that were recorded 1, 5 and 9 min after the injection of fentanyl. Neither VEH nor NLXmi affected ABG chemistry or A-a gradients. In VEH rats, fentanyl decreased pH, pO_2 and sO_2 values whereas it elevated pCO_2 and A-a gradient values. These fentanyl responses were virtually absent in NLXmi rats. The effects of a higher dose of fentanyl (50 $\mu\text{g}/\text{kg}$) on ABG and A-a gradient in VEH or NLXmi (1.5 mg/kg) rats are summarized in Table 1. This higher dose of fentanyl elicited (1) a decrease in pH at 5, 10 and 15 min, (2) an increase in pCO_2 at 5, 10 and 15 min, (3) a decrease in pO_2 at 5, 10 and 15 min, (4) a decrease in sO_2 at 5 and 10 but not 15 min, and (5) an increase in A-a gradient at 5, 10 and 15 min. The fentanyl responses were markedly attenuated but not abolished by NLXmi.

3.3. Effects of vehicle, NLX and NLXmi on baseline ventilatory parameters

Resting T_B was similar in the four groups of rats before drug injection (Table 2). The injection of vehicle, NLX (5.0 mg/kg, i.v.) or NLXmi (1.5 or 5 mg/kg, i.v.) did not affect T_B as recorded at +15 min (Table 2). The changes in T_B elicited by fentanyl (25 $\mu\text{g}/\text{kg}$) in these rats are summarized in Fig. 3. Fentanyl elicited relatively minor changes in T_B in vehicle-treated rats over the 20 min recording period. Pretreatment with the 1.5 or 5 mg/kg doses of NLXmi did not affect these increases in T_B whereas pretreatment with NLX abolished the hyperthermic responses.

3.4. Effects of NLX or NLXMI on fentanyl-induced changes in ventilatory parameters

Resting parameters before injection of VEH, NLX or NLXmi are summarized in Table 3. There were no between-group differences in any of these parameters. As seen in Figs. 4–6, the injection of VEH elicited transient changes in some ventilatory parameters (i.e., f_R , V' , T_I , T_E , PIF and PEF) but not others (V_T or EIP). These responses resolved before the

injection of fentanyl (25 µg/kg). The injections of NLX or NLXmi elicited responses that were similar to the vehicle responses (Figs. 4–6).

3.5. Effects of NLX or NLXMI on fentanyl-induced changes in ventilatory parameters

3.5.1. f_R , V_T and V' —As seen in Fig. 4, fentanyl (25 µg/kg) elicited an increase in f_R of about 5 min in duration (F1–F5) in VEH rats, which was followed by a more sustained decrease (F6–F20). The increases in f_R were not affected by NLX whereas they were reversed to a decrease in NLXmi rats. The fentanyl-induced decreases in f_R were absent in NLX rats but were not affected by NLXmi. Fentanyl decreased V_T for about 5 min in VEH rats (F1–F5) with minor F6–F20 changes (Fig. 4). The decreases in V_T were abolished by NLX and markedly attenuated by NLXmi. The minor F6–F20 changes in V_T were not affected by NLX or NLXmi. Fentanyl decreased V_T for about 15 min in VEH rats (Fig. 4). The F1–F5 decreases in V' were converted to increases by NLX whereas the F6–F20 responses were abolished. None of the fentanyl changes in V' were affected by NLXmi. Response areas (RA, cumulative arithmetic change from pre) for the F1–F5 (Table 4) and F6–F20 (Table 5) time periods confirm that (1) the fentanyl-induced increases in f_R were attenuated by NLXmi but not NLX whereas the fentanyl decreases in f_R were attenuated by NLX but not NLXmi, (2) the fentanyl-induced decreases in V_T (F1–F6) were eliminated by NLX and markedly attenuated by NLXmi whereas the F6–F20 responses were unaffected by NLX or NLXmi, and (3) the fentanyl-induced decreases in V' in NLX rats were reversed to increases at F1–F6 and were abolished at F6–F20, and that NLXmi was without effect.

3.5.2. T_I , T_E and EIP—Fentanyl (25 µg/kg) elicited sustained increases in T_I in VEH rats (Fig. 5). The F1–F5 increases in T_I were reversed to decreases by NLX but were augmented by NLXmi. The F6–F20 increases in T_I were abolished by NLX but were unaffected by NLXmi. Fentanyl elicited minor changes in T_E in VEH or NLX rats (Fig. 5). The F1–F5 and F6–20 T_E values in NLXmi rats were higher than in VEH rats. These responses were absent in NLX rats. Fentanyl increased EIP for at least 20 min in VEH rats (Fig. 5). The increases in EIP at F1–F10 were similar whereas the F11–F20 values were smaller in NLXmi rats. RAs for the F1–F5 (Table 4) and F6–F20 (Table 5) periods confirm that (1) the fentanyl F1–F5 increases in T_I were reversed to decreases by NLX but were augmented by NLXmi, and that the F6–F20 increases in T_I were blocked by NLX only, (2) fentanyl elicited minimal effects on T_E in VEH, NLX and NLXmi rats, and (3) the fentanyl increases in EIP were eliminated by NLX and that the latter time-points were attenuated by NLXmi.

3.5.3. PIF and PEF—Fentanyl decreased PIF for at least 20 min in VEH rats (Fig. 6). The F1–F5 decreases in PIF were converted to increases in the presence of NLX whereas F6–F20 responses were abolished. These fentanyl responses were not affected by NLXmi. Fentanyl decreased in PEF for about 5 min in VEH rats (Fig. 6). The F1–F5 decreases in PEF were converted to increases by NLX but were unaffected by NLXmi. The F6–F20 values were similar in VEH or NLX rats whereas these values tended to be lower in NLXmi rats. RAs for the F1–F5 (Table 4) and F6–F20 (Table 5) periods confirm that (1) the fentanyl-induced decreases in PIF were reversed to increases at F1–F6 and were abolished at F6–F20 in NLX rats, and that NLXmi was without effect and (2) the fentanyl decreases in PEF were reversed to increases at F1–F5 in NLX rats and that NLXmi was without effect.

4. DISCUSSION

The present study provides compelling evidence that the ventilatory depressant and analgesic effects of fentanyl involve the activation of peripheral μ -ORs that were sensitive to blockade with NLXmi. On the basis of our findings with NLXmi, it was apparent that the key mechanisms responsible for the deleterious effects of fentanyl on ABG chemistry were mainly dependent on peripheral μ -OR-mediated reductions in V_T and mismatch in ventilation–perfusion.

4.1. NLXmi and fentanyl-induced changes in antinociception

A major finding of this study was that although the analgesic action of fentanyl was minimally affected by 1.5 mg/kg of NLXmi, it was markedly affected by a 5 mg/kg dose of this peripherally restricted OR antagonist. These findings support evidence that peripheral ORs participate in the analgesic actions of other opiates (Stein et al., 2009). In contrast to the analgesia, the lower dose of NLXmi virtually eliminated the effects of fentanyl on ABG chemistry and A-a gradient (see below). As such, NLXmi may have greater access to and/or greater affinity/intrinsic activity at OR sub-types mediating the ventilatory depressant effects of fentanyl than for those mediating the analgesic actions of this opioid. This scenario could arise if the relative roles of μ -, δ - and κ -ORs in the ventilatory depressant effects of 25 μ g/kg fentanyl differ from those mediating the analgesia. Indeed, μ_1 -, μ_2 - and δ -ORs play different roles in morphine-induced (Ling et al., 1985; Su et al., 1998) and sufentanil-induced (Verborgh and Meert, 1999a,b; Verborgh et al., 1997; Latach and Freye, 2002) analgesia and respiratory depression. Based on the known pharmacology of NLXmi, it could be argued that the effects of the 1.5 mg/kg dose of NLXmi are due mainly to blockade of μ -ORs, lesser blockade of δ -ORs and minimal blockade of κ -ORs. As such, if the ventilatory depressant effects of fentanyl (especially those mediating the changes in ABG and A-a gradient) are mediated primarily via μ -ORs and δ -ORs whereas the analgesic actions are primarily via μ -ORs and κ -ORs, then the 1.5 mg/kg dose of NLXmi would be expected to have relatively greater effects on the ventilatory depressant effects than the analgesic effects of fentanyl.

4.2. Effects of NLX/NLXmi on fentanyl-induced changes in T_B

The observation that the injections of NLX and NLXmi had minimal effects on resting T_B is consistent with evidence that blockade of peripheral and central μ -ORs has minimal effects on T_B in rats (Geller et al., 1983; Colman and Miller, 2002; Cao and Morrison, 2005). The administration of fentanyl elicits a gradual hyperthermia in rats with maximal responses (0.5–0.7 °C) occurring between 45 and 60 min after administration (Geller et al., 1983; Colman and Miller, 2002; Cao and Morrison, 2005; Savi Vujovi et al., 2013). The findings that the 25 μ g/kg dose of fentanyl elicited minor responses during the 20 min post-injection period is consistent with the above findings. Moreover, our finding that this minor hyperthermia was blocked by NLX but not NLXmi (and therefore likely to be of central origin) is also consistent with previous findings (Geller et al., 1983; Colman and Miller, 2002; Cao and Morrison, 2005). It is important to note that the minor changes in T_B observed in this study (about 0.25 °C) are likely to have a minimal impact (less than 2%) effect on the flow-dependent variables (e.g., V_T , PIF, and PEF) recorded in the

plethysmography chambers (Mortola and Frappell, 1998). Moreover, these minor changes in TB would not be expected to have major effects on RQ (van Klinken et al., 2013).

4.3. Effects of fentanyl on ventilatory parameters, ABG chemistry and A-a gradient

The 25 µg/kg dose of fentanyl elicited (1) a decrease in \dot{V} via reductions in f_R and V_T , (2) increases in T_I and EIP with no effect on T_E , and (3) decreases in PIF and PEF (see Table 6). The decreases in V_T and PEF were relatively transient whereas the decreases in f_R and therefore \dot{V} were more sustained. The increases in T_I , EIP and PIF lasted for at least 20 min. As such, it is evident that this dose of fentanyl diminished \dot{V} by a combination of reduced rate and efficiency of breathing. The finding that fentanyl markedly affected T_I and PIF whereas it elicited relatively minor effects on T_E and PEF suggests that fentanyl primarily affected active inspiration rather than passive expiration. In addition, the 25 and 50 µg/kg doses of fentanyl produced changes in ABG chemistry and A-a gradient that were consistent with hypoventilation and mismatch of ventilation-perfusion. Specifically, fentanyl decreased pH, increased pCO_2 , decreased pO_2 and sO_2 , and increased A-a gradient. The above effects of fentanyl are likely to involve activation of ORs on neurons in ventilatory control regions of the brainstem and spinal cord (Laferriere et al., 1999; Wang et al., 2002; Haji et al., 2003; Lonergan et al., 2003a; Lonergan et al., 2003b) and peripheral tissues such as the carotid bodies, pulmonary arteries and neuromuscular components of the chest-wall and diaphragm (Schurig et al., 1978; Shook et al., 1990; Hakim et al., 1992; Haji et al., 2000; Dahan et al., 2010).

The novel finding that fentanyl increased A-a gradient suggests that it increased pulmonary vascular resistance and/or exacerbated the hypoxic pulmonary vasoconstriction due to the fentanyl-induced reduction in ventilation and the concomitant decreases in arterial blood pO_2 . These findings are consistent with evidence that other opiates increase pulmonary vascular resistance in humans (e.g., Popio et al., 1978; Mitaka et al., 1985) and animals (Schurig et al., 1978; Gentil et al., 1989; Hakim et al., 1992). ORs exist on alveolar walls, smooth muscle of the trachea and bronchi, and on autonomic nerve terminals and vagal afferents in the lungs (Cabot et al., 1996; Zebraski et al., 2000; Groneberg and Fischer, 2001). As such, the increase in A-a gradient elicited by fentanyl is also likely to involve increases in upper and/or lower airways resistance thereby diminishing O_2 delivery to alveoli. Indeed, fentanyl increases airway resistance in rats (Willette et al., 1982, 1983, 1987; Bennett et al., 1997) and in rabbits (Taguchi et al., 1986). In humans, fentanyl and sufentanil increase overall airway resistance (Cohendy et al., 1992; Ruiz Neto and Auler Júnior, 1992) via tracheal constriction (Yasuda et al., 1978) and bronchoconstriction (Ruiz Neto and Auler Júnior, 1992). Moreover, fentanyl increases the tendency for airway obstruction at glottic and supraglottic levels and produces rigidity of chest wall and abdominal musculature (Niedhart et al., 1989; Bennett et al., 1997; Bowdle, 1998).

4.4. Effects of NLX/NLXmi on fentanyl changes in ventilation, ABG chemistry, and A-a gradient

The finding that NLX and NLXmi did not elicit ventilatory responses or changes in ABG chemistry or A-a gradients that were different to those of VEH, is consistent with evidence that blockade of endogenous $\mu_{1,2}$ -OR-dependent pathways does not have major effects on

these parameters (Trescot et al., 2008; Stein and Lang, 2009; Stein et al., 2009; Dahan et al., 2010). As expected, pretreatment with NLX blocked the effects of fentanyl on ABG chemistry and A-a gradient. NLX also blocked some of the ventilatory depressant effects of fentanyl (i.e., the decreases in f_R and increases in EIP) whereas it actually reversed (i.e., decrease converted to an increase) many of the depressant effects of fentanyl (i.e., uncovered a facilitatory response) including V_T , T_I , PIF and PEF. Since NLXmi did not uncover facilitatory response to fentanyl, the ability of NLX to elicit such responses to fentanyl suggests that blockade of central μ -ORs allows κ - and δ -ORs, ORL1, and/or non-OR-dependent mechanisms to facilitate ventilation (see below).

A key conclusion from the NLXmi studies is that the major mechanisms responsible for fentanyl-induced disturbances in ABG chemistry are the peripheral OR-mediated reductions in V_T and the mismatch in ventilation–perfusion (as denoted by the increase in A-a gradient). More specifically, our study demonstrated that the ability of the low dose of NLXmi (1.5 mg/kg) to attenuate the effects of fentanyl on ABG chemistry was correlated with attenuation of the negative effects of fentanyl on (1) A-a gradient, and (2) V_T specifically, since NLXmi did not blunt the effects of fentanyl on the other ventilatory parameters except for a minor effect on EIP (Table 6). This raises the possibility that the primary mechanisms by which fentanyl disturbs ABG chemistry is via activation of peripheral ORs on structures regulating V_T including the carotid bodies and neuromuscular elements of the chest wall and diaphragm, and ORs within the lungs and pulmonary vasculature (decreased blood flow to alveoli and an increase in upper/lower airways resistance). The findings that NLXmi did not uncover ventilatory excitatory responses to fentanyl as effectively as NLX gives confidence to ascribing the effects of fentanyl to central and peripheral ORs and further supports evidence that NLXmi is peripherally restricted in the rat.

It should be noted that the accurate estimation of A-a gradient would normally require a detailed analysis of the changes in RQ. This quotient can be affected by numerous factors (e.g., changes in T_B , hypoxia as elicited by opioids) that directly or indirectly affect metabolic rate and carbohydrate and lipid metabolism (see Mendoza et al., 2013). A switch to carbohydrate metabolism would increase RQ whereas a switch to lipid metabolism would decrease RQ (see Mendoza et al., 2013; van Klinken et al., 2013). The minor changes in BT observed in this study would minimally affect RQ. In addition, the majority of relevant studies provide compelling evidence that fentanyl in similar or higher doses used in the present study have minimal direct effects on carbohydrate and lipid metabolism, which is a major determinant of RQ (van Klinken et al., 2013). For example, (1) fentanyl (50 and 100 $\mu\text{g}/\text{kg}$, i.v.) or sufentanil (10–200 $\mu\text{g}/\text{kg}$, i.v.) had minimal effect on cerebral metabolism/energy status in dogs (Milde et al., 1989, 1990), (2) high-dose fentanyl (100 $\mu\text{g}/\text{kg}$, i.v. loading dose followed by an infusion of 200 $\mu\text{g}/\text{kg}/\text{h}$, i.v.), did not alter glucose, lactate or high-energy metabolite levels in the brains of rats (Keykhah et al., 1988), (3) intra-coronary fentanyl produced minor effects on left-ventricular mechanoenergetics in dog hearts over a wide range of blood concentration (Kohno et al., 1997), and (4) fentanyl was devoid of major effects on myocardial metabolism in isolated rat hearts (Blaise et al., 1990). Although several studies have provided evidence that morphine can either enhance or decrease lipid metabolism (see Mendoza et al., 2013). There is little direct evidence as to the effects of

fentanyl on lipid metabolism. However, Reneman and Van der Vusse (1982) reported that fentanyl (25 µg/kg, i.v.) decreased fatty acid metabolism slightly in the ischemic myocardium of open-chest dogs. A decrease in fat metabolism, would shift RQ to a value greater than 0.9, which would increase the calculated A-a gradient (van Klinken et al., 2013). As such, keeping to an RQ of 0.9 in the calculations of A-a gradient, would underestimate the negative effects of morphine on gas exchange in the lungs.

5. Summary

This study provided evidence that the fentanyl-induced changes in ABG chemistry are closely related to the temporal decreases in V_T and the increases in A-a gradient. The finding that the ability of NLXmi to block the effects of fentanyl on ABG chemistry was strongly correlated with its ability to diminish the fentanyl-induced decrease in V_T and the increase in A-a gradient, provides novel evidence that fentanyl exerts detrimental ventilatory responses via activation of peripheral $\mu_{1,2}$ -ORs. The importance of peripheral $\mu_{1,2}$ -ORs in the ventilatory depressant effects of fentanyl in our rats is consistent with evidence that NLXmi attenuates the inhibitory effects of morphine in mice (Lewanowitsch and Irvine, 2002; Lewanowitsch et al., 2006). It is tempting to assume that the ability of NLXmi to blunt some of the ventilatory effects of fentanyl (i.e., depression of V_T and increase in EIP) is due to blockade of μ -ORs in peripheral structures such as the carotid bodies, pulmonary circulation and the chest and diaphragm. However, an extensive search of the literature could not confirm that direct evidence (e.g., analyses of brain levels after peripheral administration) exists as to whether NLXmi actually does enter the brain. In addition, NLXmi has at least 10 times lower affinity for opioid receptors than NLX (e.g., Lewanowitsch and Irvine, 2003) and we have not found any studies that compare the effects of, for example, a 1.5 mg/kg a dose of intravenous NLX with a 10 times higher dose of NLXmi to provide more definitive evidence as to whether NLXmi is centrally-active. Moreover, the ventilatory effects of systemically injected opiates may involve ORs in brain structures devoid of a blood–brain barrier, sites that would be readily accessible to NLXmi. These circumventricular organs of the brain, which include the area postrema (Johnson and Gross, 1993), express μ -, δ - and κ -ORs (Pert et al., 1976; Atweh and Kuhar, 1977; Guan et al., 1999), and direct injections of OR agonists into these structures elicit physiological responses (Hattori et al., 1991; Bhandari et al., 1992; Mosqueda-Garcia et al., 1993).

Although fentanyl inhibits the carotid chemoreceptor reflex in conscious dogs (Mayer et al., 1989), it is not known whether this involves activation of $\mu_{1,2}$ -ORs in the carotid bodies or whether fentanyl depresses the responses of carotid body chemoafferents upon exposure to hypoxia and/or hypercapnia. The relative contribution of central and peripheral ORs in the analgesic and ventilatory depressant effects of opiates in humans is still open to question. However, peripherally restricted μ -OR antagonists (e.g., methyl-naltrexone) attenuate opiate-induced constipation and pruritus while preserving (the presumably) centrally mediated analgesia (Moss and Rosow, 2008). Of interest is that these antagonists also attenuate opiate-induced nausea, vomiting and urinary retention, which are traditionally thought to be due to central actions of opiates (Moss and Rosow, 2008).

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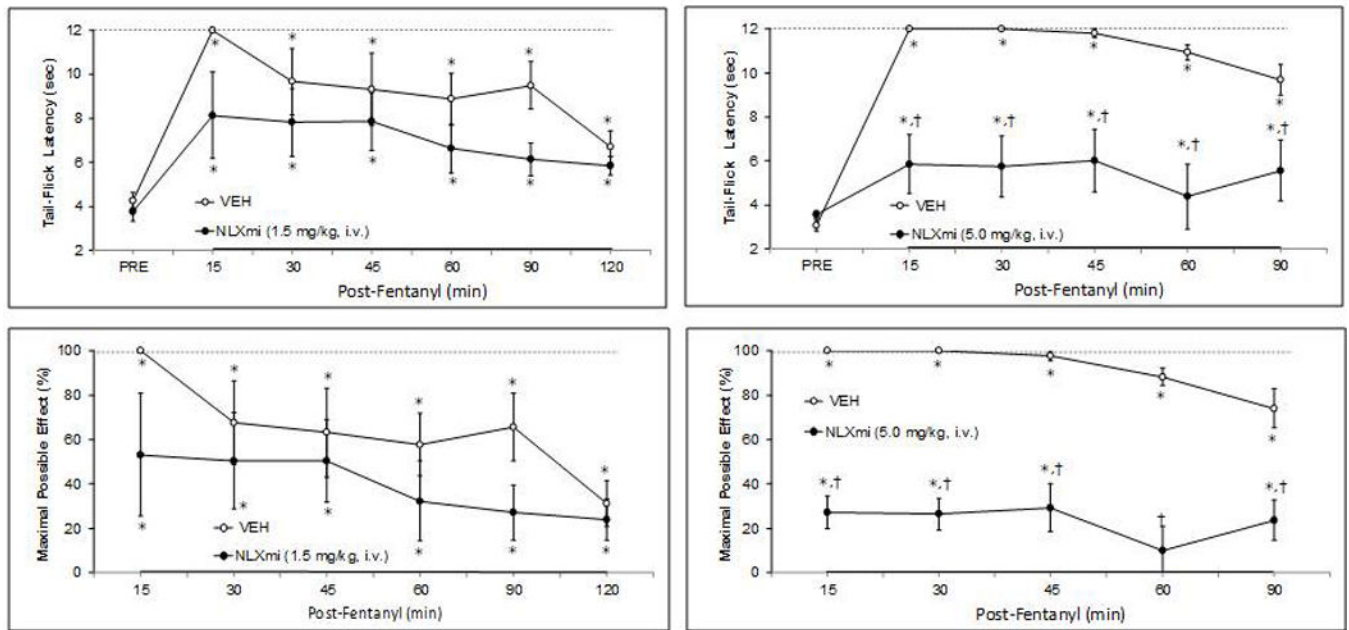


Fig. 1.

Effects of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) on Tail-Flick latencies in rats pretreated with (a) vehicle (n=4) or naloxone methiodide (NLXmi; 1.5 mg/kg, i.v., n=4) (upper-left panel) or (b) vehicle (n=5) or naloxone methiodide (NLXmi; 5.0 mg/kg, i.v., n=5) (upper-right panel). The data are also expressed as maximal possible effect (%MPE) (lower panels). All data are presented as mean \pm SEM. * $P < 0.05$, significant change from Post-Drug value. † $P < 0.05$, NLXmi *versus* vehicle.

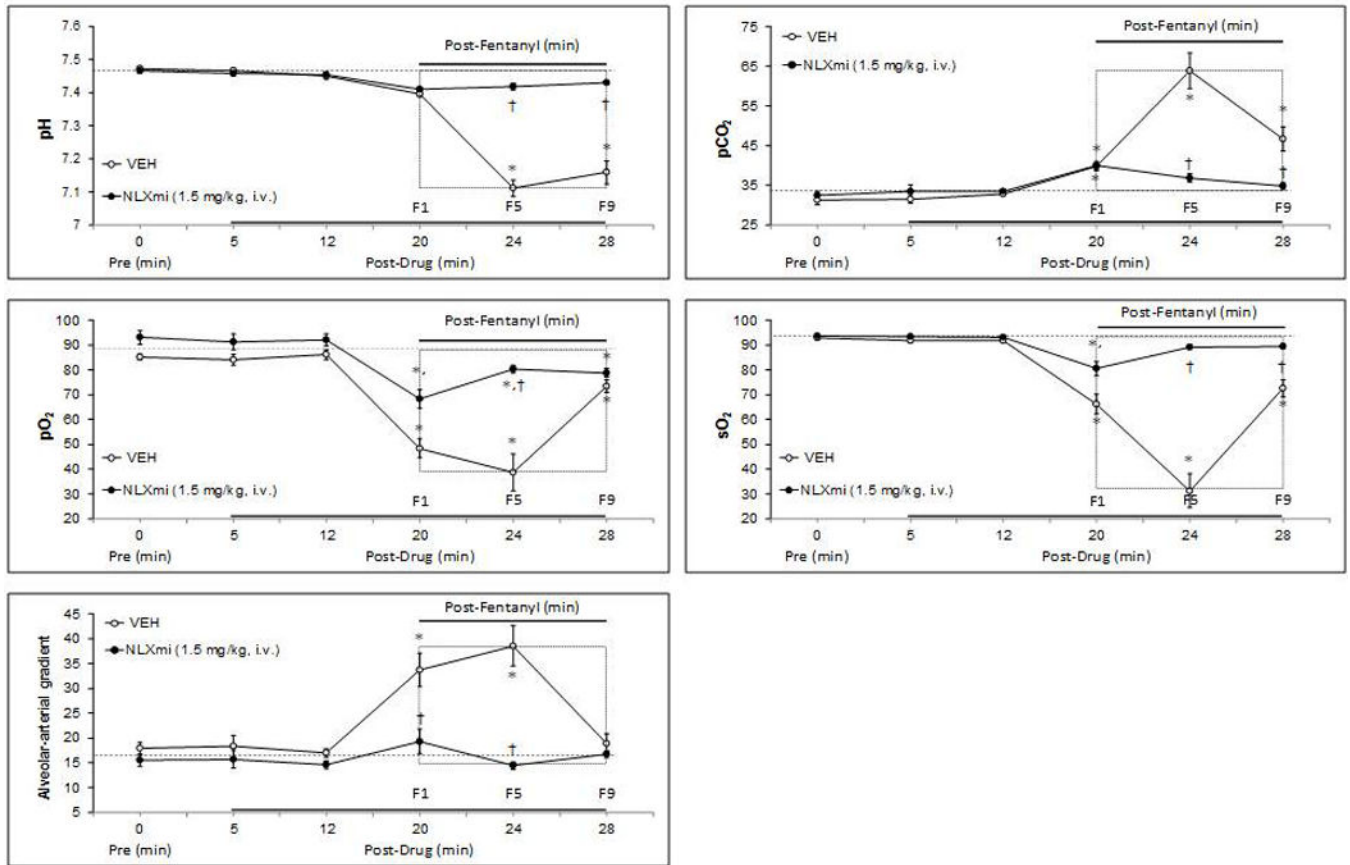


Fig. 2. Effects of fentanyl (25 µg/kg, i.v.) on arterial blood pH, pO₂, pCO₂ and sO₂ values and Alveolar-arterial gradients in conscious rats pretreated with vehicle (saline) or naloxone methiodide (NLXmi; 1.5 mg/kg, i.v.). The terms F1, F5 and F9 refer to values recorded 1, 5 and 9 min after administration of fentanyl. The data are presented as mean ± SEM. There were 6 rats in each group. * $P < 0.05$, significant change from post-drug value. † $P < 0.05$, NLXmi versus vehicle.

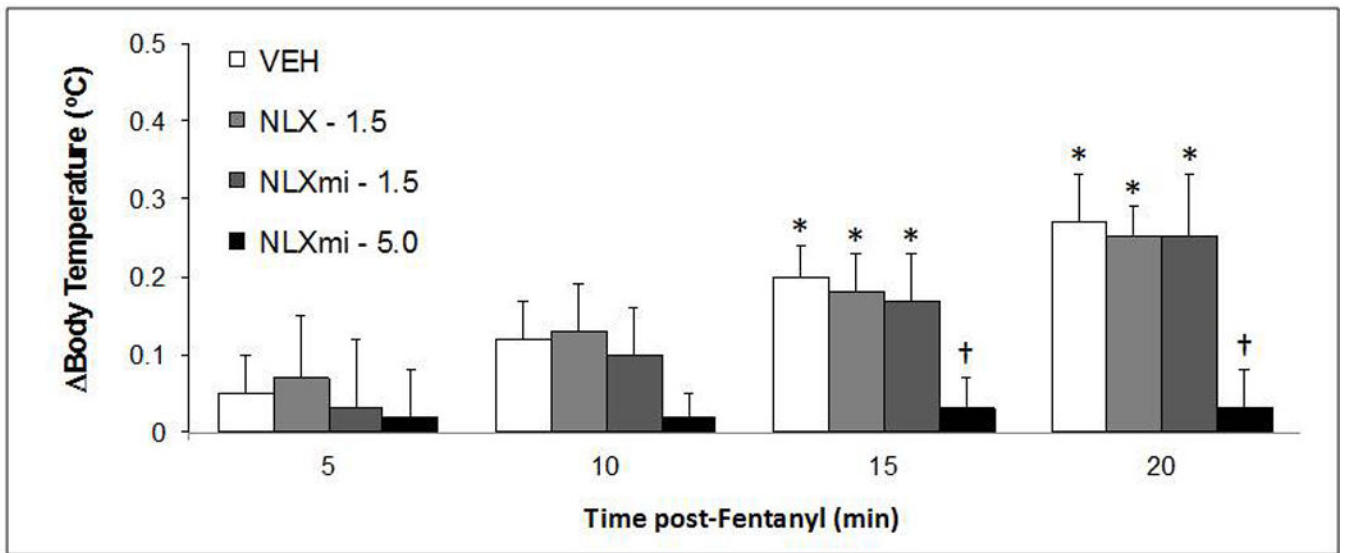


Fig. 3. Effects of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) on body temperatures (arithmetic change) in conscious rats pretreated with vehicle (saline), naloxone methiodide (NLXmi; 1.5 or 5.0 mg/kg, i.v.) or naloxone (NLX, 1.5 mg/kg, i.v.). The data are presented as mean \pm SEM. There were 6 rats in each group. * $P < 0.05$, significant change from post-drug value. † $P < 0.05$, NLXmi or NLX versus vehicle.

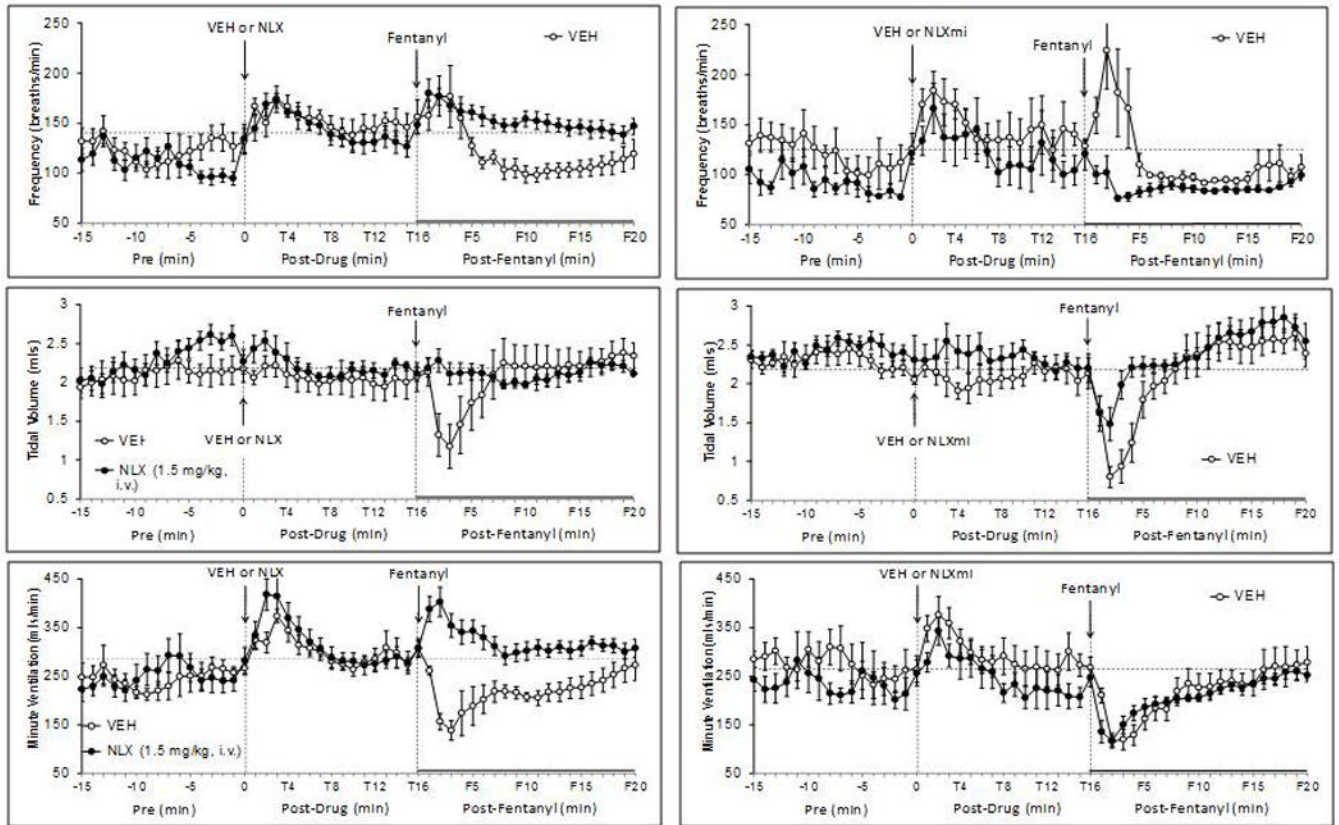


Fig. 4. Effects of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) on frequency of breathing (top panels), tidal volume (middle panels) and minute volume (bottom panels) in rats pretreated with either vehicle or naloxone (NLX; 1.5 mg/kg, i.v.) (left-hand panels) or vehicle or naloxone methiodide (NLXmi; 1.5 mg/kg, i.v.) (right-hand panels). The stippled horizontal line denotes average resting values immediately before injection of fentanyl. The data are presented as mean \pm SEM. There were 6 rats in each group.

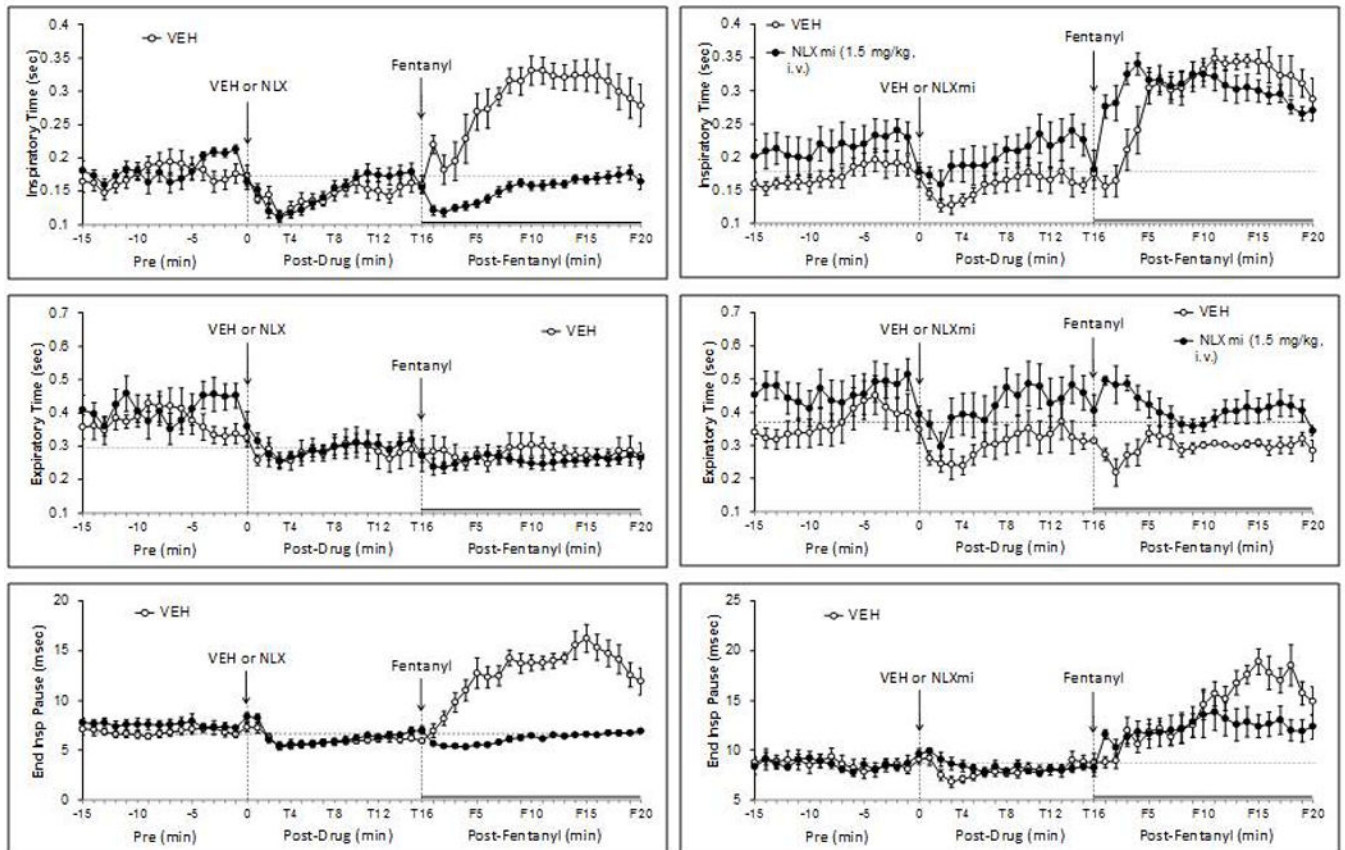


Fig. 5. Effects of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) on inspiratory time (top panels), expiratory time (middle panels) and end inspiratory pause (bottom panels) in rats pretreated with either vehicle or naloxone (NLX; 1.5 mg/kg, i.v.) (left-hand panels) or vehicle or naloxone methiodide (NLXmi; 1.5 mg/kg, i.v.) (right-hand panels). The stippled horizontal line denotes average resting values immediately before injection of fentanyl. The data are presented as mean \pm SEM. There were 6 rats in each group.

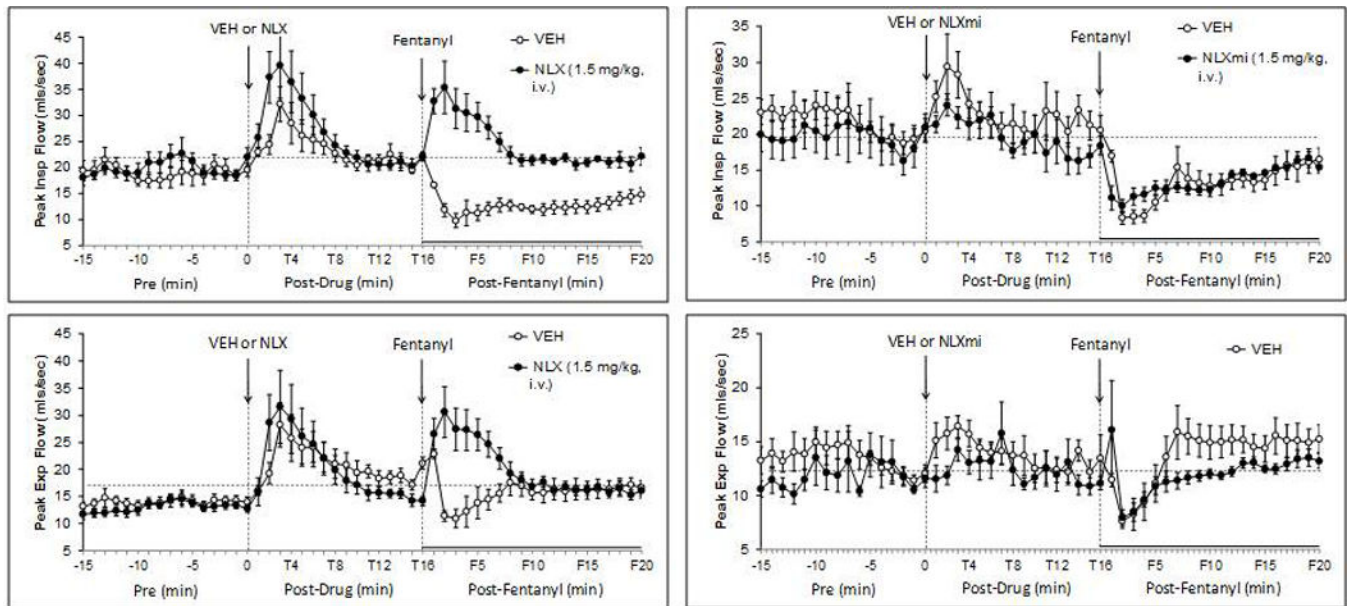


Fig. 6.

Effects of fentanyl (25 $\mu\text{g}/\text{kg}$, i.v.) on peak inspiratory flow (top panels) and peak expiratory flow (bottom panels) in rats pretreated with either vehicle or naloxone (NLX; 1.5 mg/kg, i.v.) (left-hand panels) or vehicle or naloxone methiodide (NLXmi; 1.5 mg/kg, i.v.) (right-hand panels). The stippled horizontal line denotes average resting values immediately before injection of fentanyl. The data are presented as mean \pm SEM. There were 6 rats in each group.

Effects of fentanyl on arterial blood gas values and Arterial-alveolar (A-a) gradients in rats pretreated with vehicle (saline) or naloxone methiodide

Table 1

Parameter	Group	Pre	Post-Treatment (min)					Post-Fentanyl (min)				
			T5	T10	T15	F5	F10	F15				
pH	Vehicle	7.45 ± 0.01	7.46 ± 0.01	7.45 ± 0.02	7.46 ± 0.02	7.46 ± 0.02	7.15 ± 0.02*	7.21 ± 0.02*	7.33 ± 0.01*			
	NLXmi	7.46 ± 0.02	7.43 ± 0.04	7.45 ± 0.02	7.45 ± 0.02	7.35 ± 0.01*†	7.38 ± 0.02†	7.44 ± 0.01†				
pCO ₂	Vehicle	35.9 ± 0.5	36.3 ± 0.5	36.1 ± 0.5	35.8 ± 0.7	61.2 ± 2.5*	49.6 ± 1.8*	43.3 ± 1.4*				
	NLXmi	36.2 ± 0.6	36.5 ± 0.5	36.4 ± 0.4	36.1 ± 0.5	38.0 ± 0.8†	36.0 ± 0.4†	35.5 ± 0.7†				
pO ₂	Vehicle	94.3 ± 1.2	92.6 ± 1.1	94.2 ± 1.5	94.1 ± 1.2	52.2 ± 1.2*	59.7 ± 1.8*	76.1 ± 1.0*				
	NLXmi	93.0 ± 0.5	92.2 ± 1.6	92.8 ± 0.8	92.4 ± 1.6	88.7 ± 2.2†	93.3 ± 1.0†	95.6 ± 1.1†				
sO ₂	Vehicle	99.2 ± 1.0	99.5 ± 1.3	98.3 ± 0.7	99.0 ± 1.0	55.8 ± 1.4*	70.8 ± 2.9*	92.5 ± 2.2				
	NLXmi	98.7 ± 0.8	99.7 ± 0.9	99.2 ± 0.7	99.3 ± 0.9	90.2 ± 1.7*†	94.3 ± 2.1†	98.8 ± 1.8				
A-a gradient	Vehicle	15.5 ± 2.6	16.8 ± 2.5	15.4 ± 3.2	15.9 ± 2.8	29.5 ± 4.3*	34.9 ± 4.4*	25.5 ± 2.6*				
	NLXmi	16.5 ± 1.5	17.0 ± 1.1	16.5 ± 1.9	17.2 ± 1.5	18.8 ± 3.3†	16.4 ± 2.2†	14.7 ± 1.6†				

Data are shown as mean ± SEM. There were 6 rats in each group. The dose of fentanyl was 50 µg/kg, i.v. The dose of naloxone methiodide (NLXmi) was 1.5 mg/kg, i.v.). The terms T5, T10 and T15 refer to values taken 5, 10 and 15 min after administration of vehicle or NLXmi. The terms F5, F10 and F20 refer to values taken 5, 10 and 20 min after administration of fentanyl.

Table 2

Effects of treatments on body temperatures.

Group	Body weight (g)	Body temperature (°C)		
		Pre	Post	Change (°C)
Vehicle	332 ± 3	37.8 ± 0.1	37.8 ± 0.1	+0.02 ± 0.09
NLX – 1.5 mg/kg	330 ± 4	37.9 ± 0.1	37.8 ± 0.1	-0.05 ± 0.08
NLXmi – 1.5 mg/kg	328 ± 3	37.7 ± 0.1	37.8 ± 0.1	+0.04 ± 0.10
NLXmi – 1.5 mg/kg	334 ± 2	37.8 ± 0.1	37.8 ± 0.1	+0.03 ± 0.06

Data are presented as mean ± SEM. NLX, naloxone. NLXmi, naloxone methiodide. There were 6 rats in each group. There were no between-group differences in body weights or body temperatures ($P > 0.05$, for all comparisons). None of the treatments affected baseline body temperatures ($P > 0.05$, for all comparisons).

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Table 3

Baseline values in study groups prior to injection of vehicle, naloxone or naloxone methiodide

Parameter	Naloxone Study		Naloxone methiodide Study	
	Vehicle	NLX	Vehicle	NLXmi
Frequency (breaths/min)	124 ± 13	112 ± 10	122 ± 17	94 ± 11
Tidal Volume (mls)	2.11 ± 0.19	2.29 ± 0.14	2.29 ± 0.13	2.45 ± 0.12
Minute Ventilation (mls/min)	246 ± 23	252 ± 27	274 ± 31	235 ± 34
Inspiratory Time (sec)	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.21 ± 0.03
Expiratory Time (sec)	0.37 ± 0.04	0.40 ± 0.04	0.37 ± 0.05	0.46 ± 0.05
End Inspiratory Pause (msec)	6.9 ± 0.4	7.6 ± 0.5	8.7 ± 0.7	8.6 ± 0.5
Peak Inspiratory Flow (mls/sec)	19.1 ± 1.7	19.9 ± 1.5	21.7 ± 1.9	19.7 ± 3.1
Peak Expiratory Flow (mls/sec)	14.0 ± 1.0	13.0 ± 0.8	13.4 ± 1.2	11.8 ± 1.4

Data are shown as mean ± SEM. NLX, naloxone. NLXmi, naloxone methiodide. There were 6 rats in each group. There were no significant between-group differences for any parameter ($P > 0.05$, for all comparisons).

Table 4

Cumulative responses recorded 1–5 min after injection of fentanyl in vehicle-, naloxone- or naloxone-methiodide-treated rats

Parameter	NLX Study		NLXmi Study	
	Vehicle	NLX	Vehicle	NLXmi
Frequency (breaths/min) x min	88 ± 18*	196 ± 19*, †	200 ± 39*	-164 ± 18*, †
Tidal Volume (mls) x min	-2.53 ± 0.43*	0.26 ± 0.14†	-4.27 ± 0.52*	-1.49 ± 0.30*, †
Minute Ventilation (mls/min) x min	-454 ± 58*	441 ± 36*, †	-604 ± 73*	-471 ± 66*
Inspiratory Time (sec) x min	0.30 ± 0.08	-0.27 ± 0.03*, †	0.26 ± 0.06*	0.68 ± 0.09*, †
Expiratory Time (sec) x min	-0.00 ± 0.06	-0.12 ± 0.06	-0.19 ± 0.08	0.20 ± 0.13
End Inspiratory Pause (msec) x min	19 ± 3*	-8 ± 3†	13 ± 1	16 ± 2
Peak Inspiratory Flow (mls/sec) x min	-50 ± 5*	56 ± 9*, †	-53 ± 6*	-38 ± 5*
Peak Expiratory Flow (mls/sec) x min	-23 ± 8	68 ± 10*, †	-19 ± 7	-16 ± 8

Data are presented as mean ± SEM. NLX, naloxone. NLXmi, naloxone methiodide. There were 6 rats in each group.

* $P < 0.05$, significant Response Area.

† $P < 0.05$, NLX or NLXmi *versus* relevant vehicle.

Table 5

Cumulative responses recorded 6–20 min after injection of fentanyl in vehicle-, naloxone- or naloxone-methiodide-treated rats

Parameter	Naloxone Study		Naloxone methiodide Study	
	Vehicle	NLX	Vehicle	NLXmi
Frequency (breaths/min) x min	-582 ± 69*	246 ± 38*, †	-435 ± 51*	-502 ± 60*
Tidal Volume (mls) x min	2.14 ± 0.56	-0.13 ± 0.18	4.06 ± 0.52*	5.04 ± 0.58*
Minute Ventilation (mls/min) x min	-679 ± 73*	455 ± 67*, †	-444 ± 53*	-337 ± 46*
Inspiratory Time (sec) x min	2.27 ± 0.36*	-0.25 ± 0.08†	2.25 ± 0.37*	1.80 ± 0.29*
Expiratory Time (sec) x min	0.13 ± 0.06	-0.16 ± 0.08	-0.15 ± 0.06	-0.20 ± 0.07
End Inspiratory Pause (msec) x min	121 ± 17*	-9 ± 6	109 ± 15*	66 ± 9*, †
Peak Inspiratory Flow (mls/sec) x min	-141 ± 13*	20 ± 3*, †	-91 ± 9*	-78 ± 9*
Peak Expiratory Flow (mls/sec) x min	24 ± 3*	1 ± 6*, †	28 ± 4*	18 ± 4*

Data are presented as mean ± SEM. NLX, naloxone. NLXmi, Naloxone methiodide. There were 6 rats in each group. $P < 0.05$, significant Response Area.

† $P < 0.05$, NLX or NLXmi versus relevant vehicle.

Table 6

Summary of the effects of naloxone and naloxone methiodide on fentanyl-induced responses

Parameter	Fentanyl Responses		Effects of Antagonists	
	Direction	Duration (min)	NLX	NLXmi
Frequency	↑*↓	1–5, 6–20	+++	0
Tidal Volume	↓	5	+++	++
Minute Ventilation	↓	20	+++	0
Inspiratory Time	↑	20	+++	0
Expiratory Time	≈0	n.a.	n.a.	n.a.
End Inspiratory Pause	↑	> 20	+++	+
Peak Inspiratory Flow	↓	20	+++	0
Peak Expiratory Flow	↓	4	+++	0

NLX, naloxone. NLXmi, naloxone methiodide. +++ to +, complete to partial blockade; 0, no effect; n.a., not applicable.