Respiratory Physiology & Neurobiology 191 (2014) 95–105

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

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

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ARTICLE INFO

Article history:
Accepted 18 November 2013

Keywords:
Fentanyl
Naloxone methiodide
Ventilation
Arterial blood gases
Analgesia, Rats

ABSTRACT

This study determined the effects of the peripherally restricted μ-opiate receptor (μ-OR) antagonist, naloxone methiodide (NLXmi) on fentanyl (25 μg/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, pO_2 and sO_2 and increased pCO_2 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.

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 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 approximately twice the affinity for μ-ORs than for δ-ORs and ≈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

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http://dx.doi.org/10.1016/j.resp.2013.11.005

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 ≈20 times lower affinity for μ-ORs in rat brain membranes than NLX (Bianchetti et al., 1983; Valentino et al., 1983), (2) NLXmi had ≈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 μL) 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 μg/kg, i.v.). **Study 2:** Arterial blood samples (200 μL) 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 μg/kg, i.v.). The pH, pCO_2, pO_2 and sO_2 of arterial blood samples (100 μL) were measured by a Radiometer blood-gas machine (ABL800 FLEX). The A-a gradient measures the difference between alveolar and arterial blood concentrations of O_2 (Stein et al., 1995; Story, 1996). A decrease in PaO_2, without a change in A-a gradient is caused by hypoventilation whereas a decrease in PaO_2 with an increase in A-a gradient indicates ventilation–perfusion mismatch or shunting (Stein et al., 1995). A-a gradient = $PAO_2 - PaO_2$, where PAO_2 is the partial pressure of alveolar O_2 and PaO_2 is pO_2 in arterial blood. $PAO_2 = [(FiO_2 \times (P_{atm} - P_{H_2O}) - (PaCO_2/\text{respiratory quotient})]$, where FiO_2 is the fraction of O_2 in inspired air; P_{atm} is atmospheric pressure, P_{H_2O} is the partial pressure of water in inspired air; $PaCO_2$ is pCO_2 in arterial blood; and respiratory quotient (RQ) is the ratio of CO_2 eliminated/O_2 consumed. We took FiO_2 of room-air to be 21% = 0.21, P_{atm} to be 760 mmHg, and P_{H_2O} 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 μg/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 μg/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-injection TF latency − baseline TF latency)/(12 − baseline TF latency)] × 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 μg/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 ($\dot{V} = f_R \times V_T$), (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