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Activation of Opioid μ-Receptors, but not δ- or κ-Receptors, Switches Pulmonary C-Fiber-Mediated Rapid Shallow Breathing into An Apnea in Anesthetized Rats

Zhenxiong Zhang, PhD [Associate Research Scientist], **Cancan Zhang, BS [Technician]**, **Moxi Zhou [summer intern]**, and **Fadi Xu, MD*** **[Senior Scientist]**
Pathophysiology Program, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive, SE, Albuquerque, NM 87108

Abstract

Rapid shallow breathing (RSB) is mainly mediated by bronchopulmonary C-fibers (PCFs). We asked whether this RSB could be modulated by opioid. In anesthetized rats right atrial bolus injection of phenylbiguanide (PBG) to evoke RSB was repeated after: 1) intravenously giving fentanyl (μ-receptor agonist), DPDPE (δ-receptor agonist), or U-50488H (κ-receptor agonist); 2) fentanyl (iv) following naloxone methiodide, a peripheral opioid receptor antagonist; 3) bilateral microinjection of fentanyl into the nodose ganglia; 4) fentanyl (iv) with pre-blocking histamine H_1 and H_2 receptors by diphenhydramine and ranitidine. Systemic fentanyl challenge, but not DPDPE or U-50488H, switched the PBG-induced RSB to a long lasting apnea. This switch was blocked by naloxone methiodide rather than diphenhydramine and ranitidine. After microinjecting fentanyl into the nodose ganglia, PBG also produced an apnea. Our results suggest that activating μ-receptors is capable of turning the PCF-mediated RSB into an apnea, at least partly, via facilitating PCFs' activity and this switching effect appears independent of the released histamine.

1. Introduction

Tachypnea, especially RSB, can occur in patients suffering from pulmonary inflammation, congestion, infection, and edema (Churchill & Cope, 1929; Hatridge *et al.*, 1989; Roussos & Koutsoukou, 2003). RSB is thought to result mainly from activating bronchopulmonary C-fibers (PCFs) under the pulmonary disorders (see reviews (Coleridge & Coleridge, 1994; Kubin *et al.*, 2006)) although rapidly adapting receptors (Coleridge & Coleridge, 1986) or high threshold delta fibers in the airways (Yu *et al.*, 2007; Lin *et al.*, 2011) may be also involved. PCFs innervating the lungs and airways constitute approximately 75% of the sensory fibers in the pulmonary branches of the vagus nerve and play an important role in modulating the respiratory rhythm (see the review (Lee *et al.*, 2003)).

Interestingly, opioid receptors are expressed in both vagal nerves (Chang, 2005) and the nucleus tractus solitarius (NTS) (Haber & Elde, 1982; Carter & Lightman, 1985; Ding *et al.*, 1996) where PCFs terminate. Opioids are widely used in clinic as analgesics. There are at least three distinct opioid receptors including mu (μ), kappa (κ), and delta (δ) (Santiago &

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*Corresponding Author: Fadi Xu, MD, Lovelace Respiratory Research Institute, Pathophysiology Program, 2425 Ridgecrest Dr. SE, Albuquerque, NM 87108, Phone: (505) 348-9565, Fax: (505) 348-8567, fxu@lrri.org.

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Edelman, 1985). It is unknown whether systemic activation of these opioid receptors is able to modulate the PCF-mediated RSB. Opioids are capable of producing a naloxone-preventable excitation of unmyelinated C-type neural activity in the nodose ganglion of rabbits (Higashi *et al.*, 1982; Crain & Shen, 1990; Huang, 1992) although they generally inhibit central neural activities (Werz & Macdonald, 1983; Pan *et al.*, 1990). In agreement, bolus injection of opioids into the right atrium can excite PCFs in vivo (Willette & Sapru, 1982). Thus, we tested to what extent the modulatory effect of systemic opioid challenge on the PCF-mediated RSB was dependent on activating peripheral opioid receptors. We further determined whether local activation of opioid receptors on nodose ganglia neurons, cell bodies of vagus nerves afferent fibers, could modulate the PCF-mediated RSB, similar to the result from systemic opioid challenge.

Vagal nerves contain histamine receptors (Sampson & Vidruk, 1975). Opioids can act on lung mast cells to promote the pulmonary release of histamine (Barke & Hough, 1993) that is capable of sensitizing PCFs (Lee & Morton, 1993; Undem & Weinreich, 1993). Thus, it is possible that opioids may be able to indirectly change the PCFs-mediated RSB via promoting pulmonary mast cells' release of histamine. In fact, exposure to aerosol histamine in baboons has been shown to cause a rapid breathing by acting on histamine H_1 or H_2 receptors of pulmonary sensory fibers (Yeates & Hameister, 1992). Therefore, our overall hypothesis was that systemic administration of opioids could affect the PCF-mediated RSB, and this effect was triggered directly by acting on PCF opioid receptors and/or indirectly by the opioid-induced release of histamine.

2. Materials and Methods

Fifty pathogen-free Sprague-Dawley male rats (400–500 g) were purchased from Charles River Laboratories, Inc. (Wilmington, MA), housed in the animal facility at Lovelace Respiratory Research Institute (LRRI) in filter top cages, and provided with water and food ad libitum. The room was constantly ventilated and the temperature was kept at 23°C. The experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by LRRI's Institutional Animal Care and Use Committee, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, USA.

2.1 General Animal Preparation

The rats were anesthetized with urethane [1200 mg/kg, intraperitoneal (ip)]. As needed, supplemental urethane (300 mg/kg, ip) was administered to completely eliminate eye-blink and limb-withdrawal reflex throughout the experiment. The general animal preparation was the same as we previously reported in rats (Zhang *et al.*, 2007). Briefly, the right femoral vein was cannulated for solution infusion and the right femoral artery cannulated for monitoring of arterial blood pressure (BP) and heart rate (HR). The right jugular vein was also cannulated for the bolus injection of phenylbiguanide (PBG) into the pulmonary circulation with the catheter tip placed just above right atrium. The trachea below the larynx was exposed through a midline incision, tracheotomized, and cannulated. The tracheal cannula was connected to a pneumotachograph to record airflow. End-tidal pressure of carbon dioxide ($P_{ET}CO_2$) was measured via a carbon dioxide analyzer (MicroCapStar end-tidal carbon dioxide analyzer, Model 15–10000; CWE, Inc. Ardmore, PA) connected to a side-port of the tracheal cannula. In those paralyzed preparations, the animal received an intravenous infusion of pancuronium (0.1–0.3 mg kg^{-1} for induction and 0.1 mg kg^{-1} h^{-1} for maintenance). For phrenic nerve recording, the left cervical phrenic nerve was isolated, cut, and its central end recorded as detailed in our previous reports (Xu *et al.*, 1995; Xu & Frazier, 1997) while the tracheal pressure (Pt) was measured via a side port of the tracheal cannula. The animal was exposed to 50% O_2 in nitrogen throughout the experiment and the

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core temperature was monitored with a rectal probe and maintained at 36.5–37.5°C by a water heating pad and radiant heat lamp.

2.2. Drugs preparation

All drugs were purchased from Sigma Corporation and prepared in saline. Stock solutions of PBG (1 mg/ml) and fentanyl (1 mg/ml) were made and the desired concentrations prepared daily based on the animal's body weight.

2.3. PCFs' Stimulation

PCFs were stimulated to induce RSB by a bolus injection of PBG (3–6 μg/kg), a PCF selective stimulant (Wilson & Bonham, 1997), into the right atrium. PBG (0.05 ml) was first slowly loaded into the catheter with a volume of 0.08 ml and then quickly flushed into the right atrium with 0.12 ml saline, which was the same for the following studies. A previous study has shown that 3 μg/kg of PBG leads to a maximal tachypnea (Dutta & Deshpande, 2010). Therefore, this dose was chosen as the initial dose to evoke the RSB in this study. If 3 μg/kg of PBG failed to cause RSB, 4.5 μg/kg or 6.0 μg/kg (if 4.5 μg/kg still failed) was utilized.

2.4. Microinjection of fentanyl or vehicle into nodose ganglia

In some rats, bilateral nodose ganglia were exposed through side approach. As reported previously (Hermann *et al.*, 2005), a glass micropipette (broken tip with diameter of ~50 μm) was filled with fentanyl (1 μg/ml, 1 μl) or the same volume of vehicle. The micropipette was inserted into the nodose ganglion under a stereomicroscope and agents were injected slowly (within 5 min) using a computerized microinfusion pump (Model 55–1111, Harvard Apparatus, USA).

2.5. Experiment Protocol

Study Series I was designed to test whether systemic administration of μ-, δ- or κ-receptor agonist could modulate the PBG-induced RSB in 26 rats. To this end, PBG was injected into the right atrium to evoke RSB in anesthetized and spontaneously breathing rats. This protocol was repeated in three groups of rats (n = 7/group) 5 min after systemic administration of fentanyl (μ-receptor agonist, 8 μg/kg (Brookes *et al.*, 2006)), DPDPE (δ-receptor agonist, 2 mg/kg (Yilmaz *et al.*, 1998)), or U-50488H (κ-receptor agonist, 4 mg/kg (Pei *et al.*, 2006)). Owing to that fentanyl rather than DPDPE or U-50488H was capable of switching the RSB into an apnea in our pilot studies, three-fold higher concentrations of DPDPE and U-50488H were applied in two other rats to ensure no effect of them. Activating μ-receptor could contribute to airway obstruction (McCrimmon & Alheid, 2003). Because the upper airway was cannulated in our preparation, the fentanyl-induced apnea we observed is likely central but not obstructive. To further confirm this view, the same protocols (PBG alone and coupled with fentanyl) were carried out while the phrenic nerve activity was recorded in three paralyzed and artificially ventilated rats as reported before (Xu *et al.*, 2001).

Study Series II was carried to test whether fentanyl exerted this switch depending on activating peripheral μ-receptors (n = 6). The procedure was similar to *Study Series I* with the exception that naloxone methiodide (0.1 mg/kg, iv), a selective peripheral opioid receptor antagonist (Hayashida *et al.*, 2004), was administered 5 min prior to fentanyl.

Study series III was performed to determine whether locally activating μ-receptors on nodose ganglia neurons could also make a similar switch. Intra-atrium injection of PBG was performed before and 5 min after bilateral microinjection of fentanyl into the nodose ganglia

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in 6 rats. In addition, microinjection of vehicle was conducted in five other rats to serve as a sham-operation control.

Study series IV was conducted to test whether fentanyl switched the PBG-induced RSB into an apnea via promoting the release of histamine, i.e., an indirect effect of fentanyl. The same intra-atrium injection of PBG was administered (n = 7) before and after intravenously giving combined histamine H_1 and H_2 receptor antagonists diphenhydramine (DPH, 1 mg/kg) and ranitidine (RTD, 5 mg/kg), followed by fentanyl (iv). The same dose of DPH and RTD has been used previously to block histamine H_1 or H_2 receptors in rats (Zochodne & Ho, 1993). We chose to block H_1 or H_2 receptors because they are mainly responsible for the lung and airway responses to histamine (Braude *et al.*, 1984; Bryce *et al.*, 2006).

2.6. Data Acquisition and Analysis

Raw data of the airflow, mean arterial blood pressure (MBP), HR, phrenic neurogram, Pt, and rectal temperature were digitized, monitored, and recorded using a computer-based data acquisition and analysis system (PowerLab/8sp; AD Instruments Inc., Colorado Springs, CO) with Chart 5 software. The airflow signals were integrated to generate expiratory duration (T_E), tidal volume (V_T), respiratory frequency (f), and minute ventilatory volume (V_E). The baseline cardiorespiratory variables were collected for 1 min immediately before and 5 min after agents administration. These variables were expressed as absolute values. With respect to the responses to PBG, baseline T_E and associated cardiovascular values were averaged 1 min before PBG administration as controls, and the values from the PBG-evoked four fast breaths (before fentanyl) and the apneic response (after fentanyl) were measured as the responses. These responses were presented as percentage changes from the control (Δ%). In the present study, the V_T decrease > 15% and f increase > 15% compared with control were defined as RSB, while a T_E value that was three-fold longer than the control defined as an apnea (Peng *et al.*, 2007). Paired-t test was used to test the effects of a given treatment on the baseline cardiorespiratory variables, while repeated one-way ANOVA was employed to detect significant changes in the evoked cardiorespiratory responses to PBG and significant effect of a given treatment on the responses. If an overall test was significant, Tukey test was used for specific comparisons between individual groups. The software Statistica 6.0 (StatSoft, Inc., Tulsa, OK) was used for statistical analysis. All data are presented as means ± standard error (SE). The difference was considered significant at a P value < 0.05.

3. Results

3.1. Fentanyl, rather than DPDPE and U-50488H, switches the RSB into an apnea

We first tested whether systemically activating μ, δ, or κ receptors could affect the PCF-mediated RSB. As shown in Fig. 1, right atrial bolus injection of PBG induced RSB associated with bradycardia and hypotension, consistent with a previous report in rats (Dutta & Deshpande, 2010). The cardiorespiratory responses to PBG in this study were characterized by: 1) a shortened T_E (−35%, from 0.40 ± 0.05 s to 0.26 ± 0.03 s, P < 0.01) with a latency of 1.88 ± 0.03 s; 2) a decreased MBP (−38%, from 88 ± 5 to 54 ± 6 mmHg, P < 0.01) and 3) a lowed HR (−40%, from 336 ± 23 to 201 ± 21 beats/min, P < 0.05). Fentanyl (8 μg/kg, iv) significantly inhibited V_E, f and V_T; moderately prolonged T_E; increased $P_{ET}CO2$ from 35 ± 2 to 39 ± 3 mmHg (P < 0.05); and elevated MBP without effect on HR (Table 1). After systemic fentanyl administration, the same dose of PBG produced a long-lasting apnea with T_E 6.5-fold longer than the control (increasing from 0.52 ± 0.06 s to 3.9 ± 0.63 s, P < 0.01). Furthermore, the PBG-induced hypotension (−38%) and bradycardia (−40%) were markedly aggravated after giving fentanyl (−52% and −63%, respectively, P < 0.01). The modulatory impact of fentanyl on the PBG-induced cardiorespiratory responses

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usually disappeared 2.5 h later. In contrast, intravenous injection of vehicle did not significantly alter the PBG-induced RSB.

We also examined the influences of δ- or κ-receptor agonists (DPDPE or U-50488H) on the PBG-induced RSB, respectively. Different from μ-receptor agonist fentanyl, DPDPE and U-50488H neither switched the PBG-induced RSB into an apnea nor altered the PBG-induced BP and HR responses (Fig. 2). In fact, the same results were also observed in the two rats when triple-dosed DPDPE or U-50488H was injected, in which the PBG-induced T_E was shorten by −37% and−38% before and after DPDPE; and by−40% and−41% before and after U-50488H ($P > 0.05$). Additionally, DPDPE had no significant effect on cardiorespiratory baseline variables, while U-50488H decreased V_E via lowering V_T without change in cardiovascular activity (Table 2).

3.2. Fentanyl switches the PBG-induced RSB into a central apnea

We recorded the phrenic nerve firing in response to the same PBG before and after fentanyl (iv) to verify that the apnea induced by the switching effect of fentanyl is central but not obstructive. As shown in Fig. 3, fentanyl switched the PBG-induced RSB into an apnea denoted on the phrenic nerve recording. In three rats tested, T_E was shortened by PBG from 0.76 ± 0.01 s to 0.52 ± 0.12 s, indicating the PBG-induced RSB. The PBG-induced RSB became an apnea after fentanyl, i.e., the T_E was profoundly prolonged by 6.1-fold (from 0.77 ± 0.01 s to 4.68 ± 0.61 s). PBG injection did not change Pt either before fentanyl (4.97 ± 0.23 vs. 5.01 ± 0.21 cmH_2O) or after fentanyl (5.03 ± 0.24 vs. 5.02 ± 0.24 cmH_2O).

3.3. Fentanyl-mediated switch is dependent on peripheral μ-receptors

To test whether fentanyl (iv) triggered the switch via acting peripheral μ-receptors, naloxone methiodide, a peripheral μ-receptor antagonist, was administered 5 min prior to fentanyl. This pretreatment with naloxone methiodide failed to alter: 1) the baseline respiratory activities (Table 2), and 2) the fentanyl-induced cardiorespiratory changes including inhibition of V_E, f and V_T, moderate prolongation of T_E, and increase in BP (Table 1). However, this pretreatment prevented: 1) the switching effect of fentanyl although the T_E of the PBG-induced RSB after fentanyl was slightly prolonged (Fig. 4), and 2) fentanyl's aggravating effect on the PBG-induced hypotension and bradycardia response (Fig. 4).

3.4. Microinjecting fentanyl into the nodose ganglia turns on the switch

After confirming that peripherally activating μ-receptors is necessary for fentanyl to switch the PBG-induced RSB into an apnea, we further examined whether selectively activating μ-receptors in the nodose ganglia where the cell bodies of vagal sensory fibers reside could produce a similar switch. Local application of fentanyl decreased baseline V_E significantly ($P_{ET}CO2$ changed from 35 ± 2 to 38 ± 3 mmHg, $P < 0.05$) although this inhibition of V_E was less than that induced by systemic fentanyl challenge (−11 ± 3% vs. −33 ± 5%, $P < 0.05$) (Table 1). As illustrated in Fig 5, this local fentanyl treatment also switched the PBG-induced RSB into an apnea (3.1-fold prolongation of T_E from 0.48 ± 0.03 s to 1.49 ± 0.26 s) though the latter was significantly shorter than that induced by systemically injected (6.5-fold prolongation). Different from systemic administration, local fentanyl failed to change PBG-induced decrease in MBP and HR (Fig. 5). As a sham-operation control, local microinjection of vehicle into the nodose ganglia neither changed the baseline cardiorespiratory values (Table 2) nor produced such switching effect (−39 ± 4% vs. −34 ± 6% for T_E response to PBG, $P > 0.05$).

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3.5. Fentanyl-mediated switch is independent of histamine H_1 and H_2receptors

The data mentioned above showed a relatively smaller switching effects induced by local fentanyl injection compared with the result from systemic administration of fentanyl, suggesting an involvement of other mechanisms in full expression of the systemic fentanyl-induced switch. Here we sought to define the possible role of the indirect effect of fentanyl via promoting histamine release in the switch. We tested whether fentanyl failed to switch the PBG-induced RSB into an apnea after blocking both histamine H_1 and H_2 receptors. As a result, similar to fentanyl alone, fentanyl following blockade of both types of histamine receptors by combination of DPH and RTD still evoked a similar RSB-apnea switch and aggravated hypotension and bradycardia (Fig. 6). DPH/RTD per se slightly increased the baseline V_E by 8% due to the elevation of f, and increased MBP with no change of HR (Table 2). After DPH/RTD, fentanyl still depressed V_E by 34% ($P_{ET}CO2$ changed from 34 ± 2 to 39 ± 3 mmHg, $P < 0.05$) due to the inhibition of both V_T and f, and increased MBP (Table 1).

4. Discussion

The major finding of this study is that systemically activating μ-receptors, but not δ- and κ-receptors, is able to switch the PCF-mediated RSB into a long-lasting apnea. RSB commonly occurs in patients with pulmonary inflammation (infection), congestion, and edema (Churchill & Cope, 1929; Hatridge *et al.*, 1989; Roussos & Koutsoukou, 2003). These patients are more vulnerable to suffering from respiratory depression and even respiratory failure than normal subjects when opioids are administered as analgesics, mainly due to acting on μ-receptors, in the clinical setting (Gruber & Tschernko, 2003; Horton & Barber, 2009). However, the reason for this vulnerability is not clear. PCFs can be activated and/or sensitized in patients by pulmonary inflammation (infection), congestion, and edema (Churchill & Cope, 1929; Hatridge *et al.*, 1989; Tepper *et al.*, 1990; Roussos & Koutsoukou, 2003), and these fibers are thought to be the main factor in generating RSB under the pulmonary disorders (Widdicombe, 1982; Coleridge & Coleridge, 1994; Kubin *et al.*, 2006). Therefore, our result that fentanyl could switch the PCFs-mediated RSB into an apnea may benefit our understanding why opioids are much more depressant to respiration in these patients though several other mechanisms may also be involved.

An important finding of this study is that the activation of peripheral μ-receptors, most likely those on PCFs, is the prerequisite for this switch. As shown in Fig. 4, switching the PBG-induced RSB into a long lasting apnea by intravenous administration of fentanyl was fully prevented after using naloxone methiodide, a peripheral opioid receptor antagonist. One of the most likely peripheral mechanisms for this switching is that fentanyl directly facilitate PCFs' activity to significantly augment PBG-induced PCF excitation, and consequently, turn the PBG-evoked RSB to an apnea. This assumption is supported by several lines of evidence. First, the production of RSB or an apnea is dependent on the stimulating intensity of PCFs. The RSB was induced by intra-atrial injection of a low dose of PBG while an apnea was also evoked if a high dose of PBG was used, and both types of responses disappeared after bilateral vagotomy (Coleridge & Coleridge, 1994; Moreira *et al.*, 2007; Dutta & Deshpande, 2010). Second, μ-receptor agonists can excite unmyelinated C type neurons in the nodose ganglion of rabbits (Higashi *et al.*, 1982; Crain & Shen, 1990) in vitro and stimulate PCFs to induce an apnea in vivo (Willette & Sapru, 1982). Third, more importantly, our data showed that fentanyl microinjected into nodose ganglia also switched the RSB to an apnea. It is worthy to note that local fentanyl treatment-induced 3.1-fold prolongation of T_E is shorter than that induced by systemic administration (6.5-fold T_E prolongation). This discrepancy may be due to an insufficient stimulation of all PCFs by the pretreatment of nodose ganglia with fentanyl in this study. On the other hand, it is also possible that the central μ-receptors may contribute to the switch response to systemic

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administration of fentanyl. μ-receptors are abundant in the central pathways underlying the PCF-mediated respiratory responses (Kubin *et al.*, 2006), including those in the nucleus tractus solitarius and pre-Botzinger complex (Ding *et al.*, 1996; Haji *et al.*, 2003). In fact, the result that after naloxone methiodide, fentanyl failed to induce the switch but still attenuated the PBG-induced RSB (Fig. 4B) points to an involvement of central μ-receptors in this modulation. Nevertheless, the two lines of results from blocking peripheral μ receptors and local injection of fentanyl into the nodose ganglia indicate that peripheral μ receptors, especially those on PCFs, are necessary in triggering this switch.

Opioids including fentanyl and its derivatives can promote the pulmonary release of histamine (Kaye *et al.*, 2006a; Kaye *et al.*, 2006b), which was reported to sensitize PCFs (Lee & Morton, 1993; Undem & Weinreich, 1993). Furthermore, inhaling histamine causes a rapid breathing by activating histamine H_1 or H_2 receptors of pulmonary sensory fibers in baboons (Yeates & Hameister, 1992). Dutta and Deshpande (Dutta & Deshpande, 2011) have also shown the involvement of histaminergic system to the augmentation of PBG reflexes by scorpion venom. These data, together with a relative shorter PBG-induced apnea after fentanyl locally injected into the nodose ganglion, suggest a possible involvement of secondarily released histamine by systemic fentanyl in the switch. We compared the fentanyl effect on the PBG-induced RSB with and without blocking both H_1 and H_2 receptors. As the result, switching the RSB to the apnea by systemic fentanyl was not significantly affected by blocking both histamine receptors, not supporting a histamine H_1/H_2 receptors' involvement in this switch. Because fentanyl may also release other mediators stimulatory to PCFs, such as adenosine (Gu *et al.*, 2003), our data in this study can not rule out their involvement in the switch.

Another interesting finding in the present study was that systemic fentanyl augmented the PBG-induced bradycardia and hypotension that could be prevented by the blockage of peripheral opioid receptors. Intra-atrium injection of PBG induced a transient hypotension and bradycardia as reported previously (Dutta & Deshpande, 2010). Fentanyl augmented the cardiovascular response to PBG seemingly not through acting on vagal μ-receptors because the local treatment of nodose ganglia with fentanyl failed to exhibit such augmenting effect. In our study systemic administration of fentanyl increased BP, which may be due to its easy access to the central nervous system and its central impact on pressor (Bellet *et al.*, 1980). This centrally mediated hypertension was related to activation of sympathetic tone as it was abolished after bilateral adrenalectomy and application of ganglion blocking agents (Bellet *et al.*, 1980).

In summary, our results allow us to conclude that the systemic activation of μ-receptors rather than δ- or κ-receptors is capable of switching the PCF-mediated RSB into a central apnea in anesthetized rats. Moreover, this switching effect is triggered by acting peripheral μ-receptors, at least partially, through acting PCF μ-receptors.

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- Fentanyl switched pulmonary C-fibers (PCFs)-mediated rapid shallow breathing (RSB) into an apnea in anesthetized rats.
- The switching effect of fentanyl could be prevented by blocking peripheral μ-receptors.
- Microinjection of fentanyl into nodose ganglia also switched PCFs-mediated RSB into an apnea.
- Fentanyl's switching effect was not dependent on the histamine receptors

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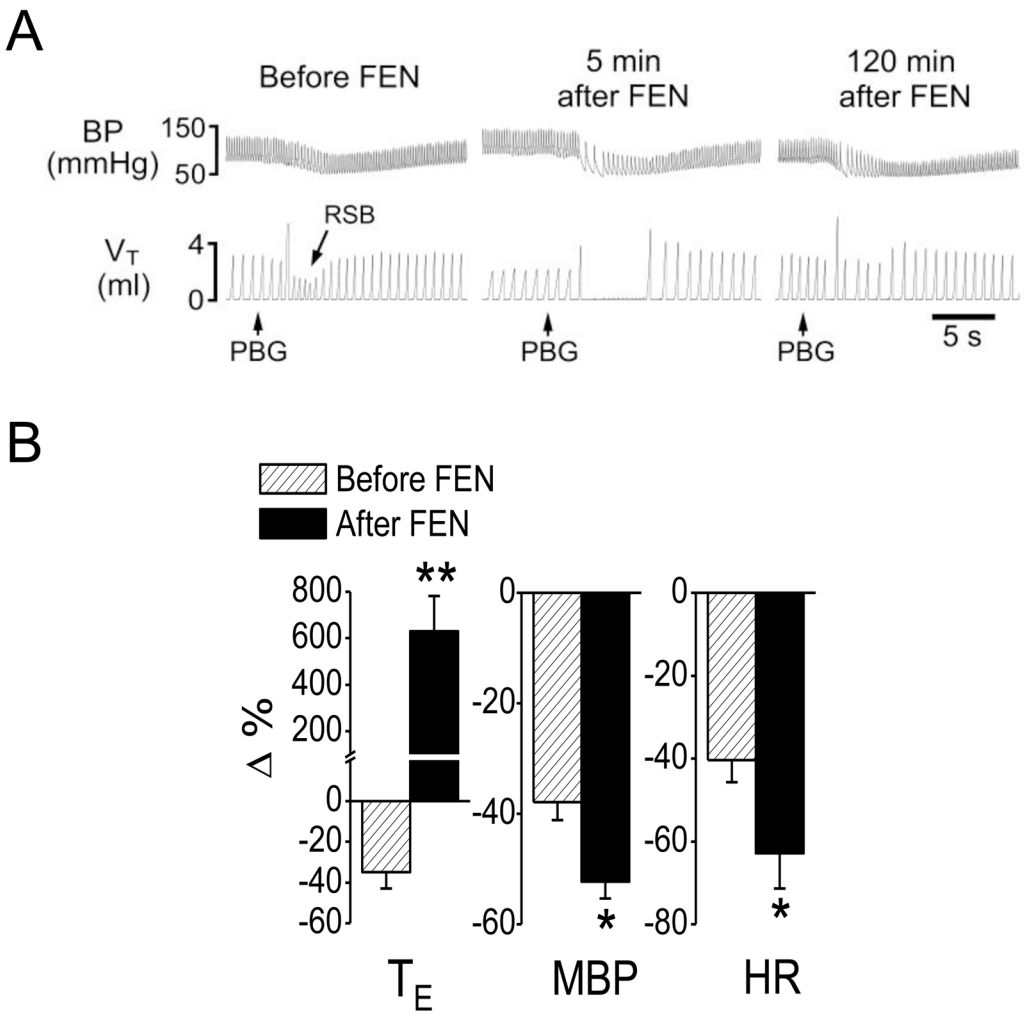

Fig 1.

Fentanyl-induced changes in cardiorespiratory responses to PBG. A: A representative recording showing that fentanyl (FEN, 8 μg/kg, iv) converted the PBG (4.5 μg/kg)-induced RSB (left) into a long-lasting apnea (middle) and this facilitating effect disappeared 2 h later (right). The traces from the top to bottom are arterial blood pressure (BP) and tidal volume (V_T). B: Group data of the effects of FEN on the cardiorespiratory responses to PBG. N = 7; mean ± SE. Note: all the cardiorespiratory responses to PBG were significant ($P < 0.01$). * $P < 0.05$ and ** $P < 0.01$ compared with before fentanyl. T_E, expiratory duration; MBP, mean arterial blood pressure; HR, heart rate.

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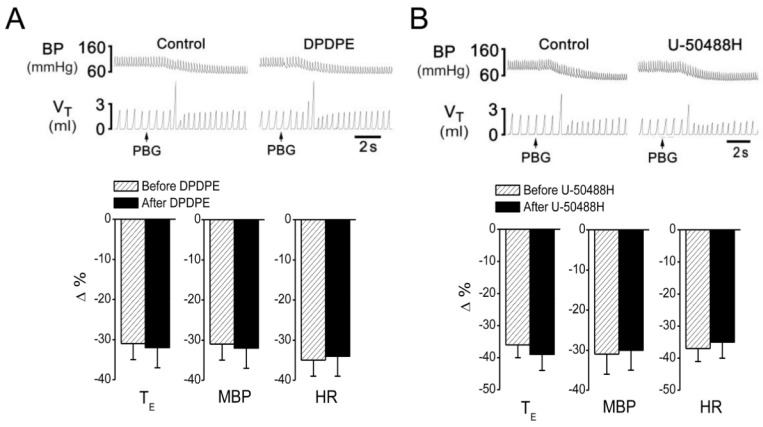

Fig 2.

Opioid δ- (A) and κ-receptor agonist (B) failed to convert the PBG-induced RSB into an apnea. The top panes present representative recordings showing that fentanyl (FEN, 8 μg/kg, iv) failed to converted the PBG (4.5 μg/kg)-induced RSB into a apnea and the bottom panes are the corresponding group data. In the top panel, the traces are arterial blood pressure (BP) and tidal volume (V_T). N = 7 for each agonist; mean ± SE; Note:all the cardiorespiratory responses to PBG were significant ($P < 0.01$). T_E, expiratory duration; MBP, mean arterial blood pressure; and HR, heart rate.

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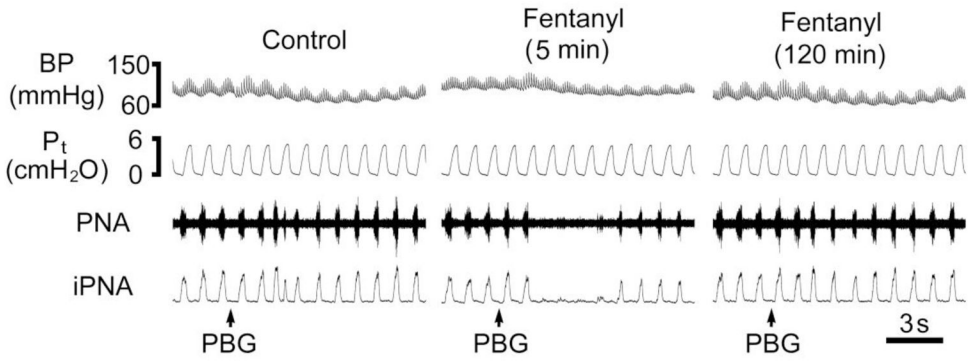

Fig 3.

A representative phrenic nerve recording showing that fentanyl (8 μg/kg, iv) turns the PBG (3 μg/kg)-induced RSB into an apnea without changing the tracheal pressure and the partial recovery from the apnea in a paralyzed and ventilated rat. The traces from the top to bottom are arterial blood pressure (ABP), tracheal pressure (Pt), phrenic nerve activity (PNA) and integrated PNA (iPNA).

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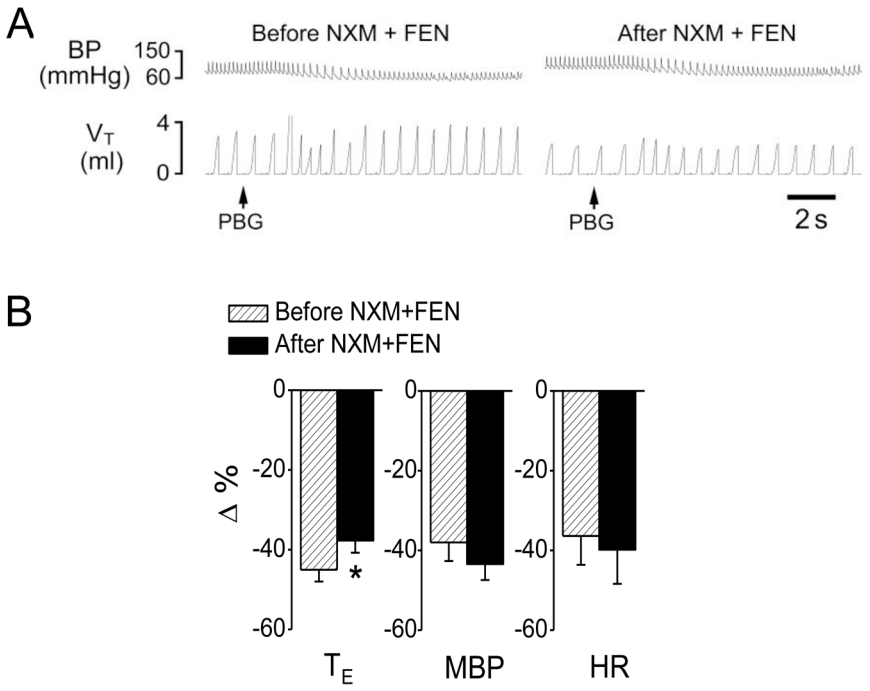

Fig 4.

The effect of peripheral opioid receptor antagonist naloxone methiodide (NXM) and fentanyl (FEN) on the cardiorespiratory responses to PBG. A, a representative recording showing that fentanyl (8 μg/kg, iv) failed to convert the PBG (6 μg/kg)-induced RSB into an apnea after NXM was administered. The traces from the top to bottom are arterial blood pressure (BP) and tidal volume (V_T). B, the corresponding group data. N = 6; mean ± SE. Note: all the cardiorespiratory responses to PBG were significant ($P < 0.01$). * $P < 0.05$ compared with before fentanyl. T_E, expiratory duration; MBP, mean arterial blood pressure; and HR, heart rate.

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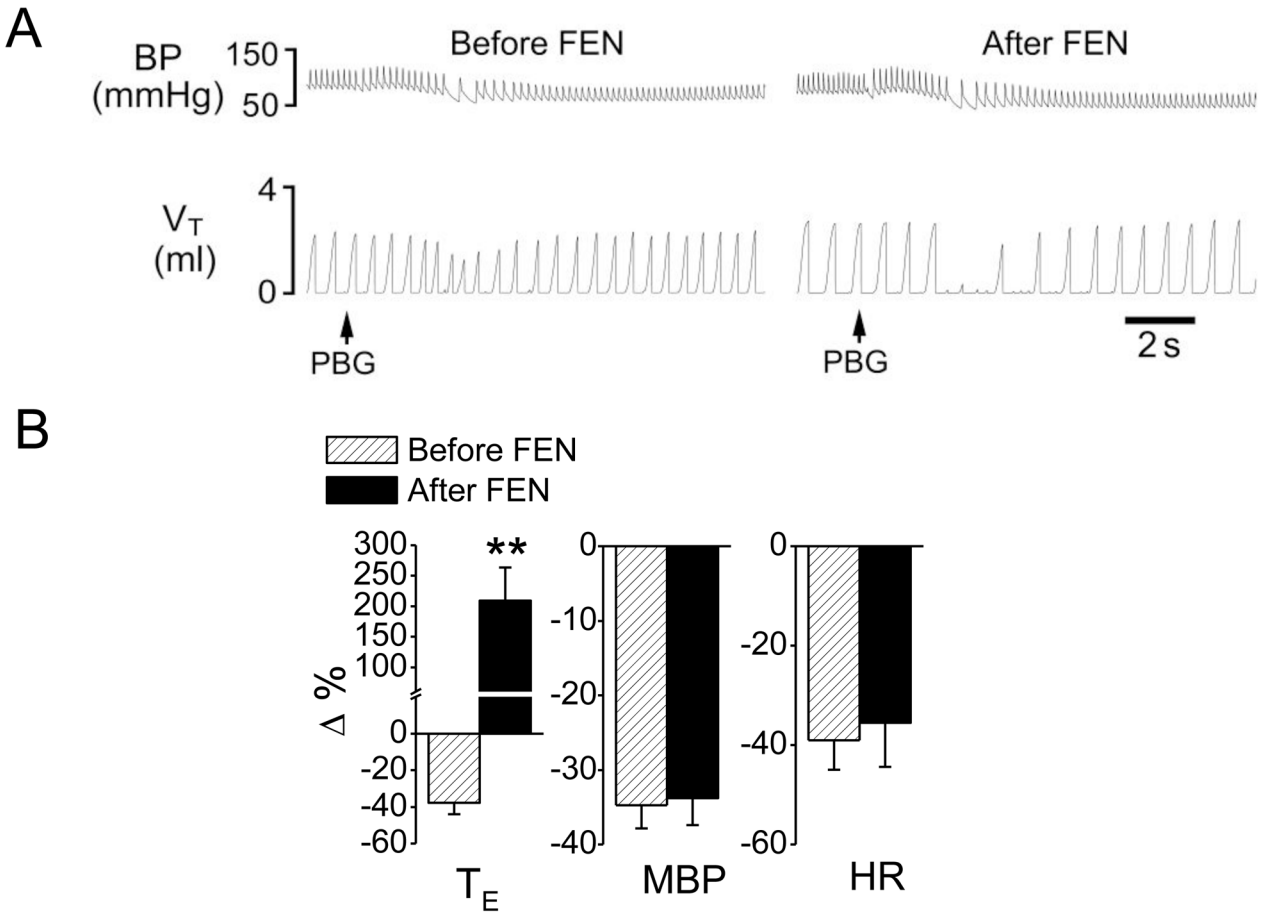

Fig 5.

The effect of intra-nodose ganglia microinjection of fentanyl (FEN) on cardiorespiratory responses to PBG. A, a representative recording showing that fentanyl microinjected into the nodose ganglia converted the PBG (4.5 μg/kg)-induced RSB into an apnea. The traces from the top to bottom are arterial blood pressure (BP) and tidal volume (V_T). B, the corresponding group data. N = 6; mean ± SE. Note: all the cardiorespiratory responses to PBG were significant ($P < 0.01$). ** $P < 0.01$ compared with before fentanyl. T_E, expiratory duration; MBP, mean arterial blood pressure; and HR, heart rate.

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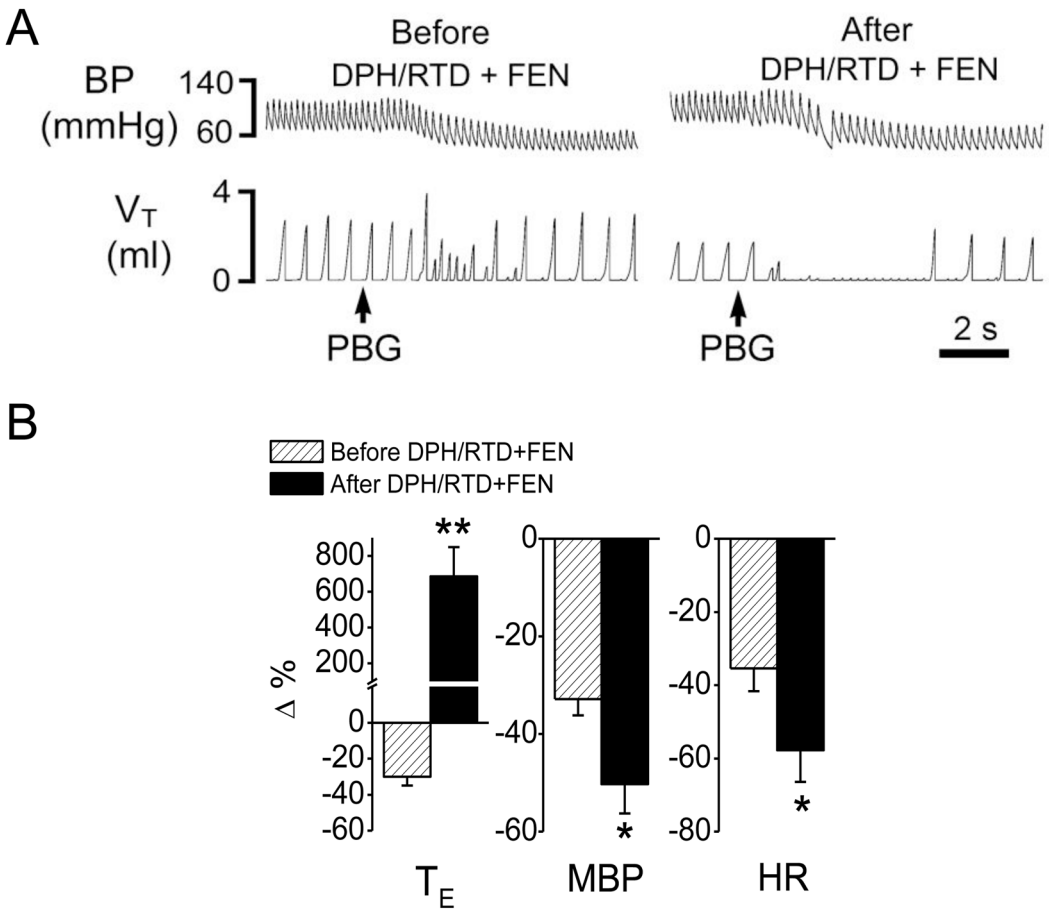

Fig 6.

The effect of fentanyl (FEN) on cardiorespiratory responses to PBG after blocking histamine H_1 and H_2 receptors. A, a representative recording showing that fentanyl still converted the PBG (4.5 μg/kg)-induced RSB into an apnea after the blockage of histamine H_1 and H_2 receptors by diphenhydramine and ranitidine (DPH/RTD). The traces from the top to bottom are arterial blood pressure (BP) and tidal volume (V_T). B, the corresponding group data. N = 7; mean ± SE. Note: all the cardiorespiratory responses to PBG were significant ($P < 0.01$). * $P < 0.05$ and ** $P < 0.01$ compared with before fentanyl. HR, heart rate; MBP, mean arterial blood pressure; T_E, expiratory duration.

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Table 1

Effect of fentanyl alone or coupled with a given pretreatment on baseline cardiorespiratory variables

		V_E (ml/min)	f (breaths/min)	V_T (ml)	T_E (s)	MBP (mmHg)	HR (beats/min)
FEN (iv) (n = 7)	Before	249 ± 30	101 ± 5	2.5 ± 0.2	0.40 ± 0.05	87 ± 6	336 ± 23
	After	167 ± 25 **	77 ± 5 **	2.1 ± 0.3 **	0.52 ± 0.06 **	104 ± 5 **	315 ± 26
NXM+FEN (iv) (n = 6)	Before	256 ± 21	102 ± 7	2.5 ± 0.3	0.36 ± 0.05	90 ± 7	332 ± 9
	After	176 ± 27 **	86 ± 9 **	2.1 ± 0.3 **	0.45 ± 0.07 *	109 ± 5 **	325 ± 11
DPH/RTD+FEN (iv) (n = 7)	Before	235 ± 30	97 ± 7	2.4 ± 0.2	0.38 ± 0.03	92 ± 7	356 ± 29
	After	154 ± 26 **	73 ± 6 **	2.1 ± 0.2 **	0.51 ± 0.04 **	111 ± 7 **	350 ± 26
FEN (local) (n = 6)	Before	215 ± 19	105 ± 5	2.1 ± 0.2	0.36 ± 0.03	92 ± 7	375 ± 10
	After	192 ± 21 *	83 ± 6 *	2.3 ± 0.3	0.48 ± 0.05 *	90 ± 9	402 ± 25 *

Data are shown as mean ± SE;

* $P < 0.05$,

** $P < 0.01$ compared with "before". DPH/RTD, diphenhydramine/ranitidine; FEN, fentanyl; iv, intravenous; local, injection into the nodose ganglia; f, respiratory frequency; HR, heart rate; MBP, mean arterial blood pressure; NXM, naloxone methiodide; T_E, expiratory duration; V_E, minute ventilation; V_T, tidal volume.

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Table 2

Comparison of baseline cardiorespiratory variables before and after intravenous DPDPE, U-50488H, histamine blockers, peripheral opioid receptor blocker and local injecting vehicle into the nodose ganglia.

		V_E (ml/min)	f (breaths/min)	V_T (ml)	T_E (s)	MBP (mmHg)	HR (beats/min)
DPDPE (iv) (n = 7)	Before	267 ± 34	97 ± 7	2.8 ± 0.2	0.39 ± 0.04	96 ± 7	315 ± 22
	After	270 ± 39	103 ± 8	2.6 ± 0.3	0.38 ± 0.05	91 ± 8	311 ± 18
U-50488H (iv) (n = 7)	Before	238 ± 11	92 ± 8	2.6 ± 0.2	0.42 ± 0.04	94 ± 9	331 ± 17
	After	210 ± 22 **	97 ± 8	2.1 ± 0.2 **	0.40 ± 0.05	96 ± 10	330 ± 9
NXM (iv) (n = 6)	Before	251 ± 19	98 ± 6	2.5 ± 0.3	0.39 ± 0.04	93 ± 6	329 ± 7
	After	256 ± 21	102 ± 7	2.5 ± 0.3	0.36 ± 0.05	90 ± 7	332 ± 9
DPH/RTD (iv) (n = 6)	Before	216 ± 11	87 ± 6	2.5 ± 0.3	0.45 ± 0.05	82 ± 7	363 ± 19
	After	235 ± 30 *	97 ± 7 *	2.4 ± 0.2	0.38 ± 0.03 **	92 ± 7 *	356 ± 29
Vehicle (local) (n = 5)	Before	231 ± 14	98 ± 7	2.4 ± 0.3	0.40 ± 0.05	98 ± 7	343 ± 17
	After	226 ± 21	93 ± 9	2.4 ± 0.3	0.42 ± 0.05	97 ± 8	341 ± 25

Data are shown as mean ± SE;

* P < 0.05,

** P < 0.01 compared with “before”.

DPH/RTD, diphenhydramine/ranitidine; iv, intravenous; local, injection into the nodose ganglia; f, respiratory frequency; HR, heart rate; MBP, mean arterial blood pressure; NXM, naloxone methiodide; T_E, expiratory duration; V_E, minute ventilation; V_T, tidal volume.

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