Introduction

In daily clinical practice, opioid drugs are often used for analgesia after surgery and for control of cancer pain, but they are also used as a part of balanced and total intravenous anesthesia (1). Despite their favorable analgesic effects, opioid-induced respiratory depression remains an important clinical problem (2). Remifentanil is a novel opioid drug, potent µ-agonist, approved for clinical use in 1996, developed to meet the need for an ultrashort-acting opioid (1). It has a rapid onset and short latency to peak effect and it is rapidly inactivated by esterases in both blood and tissues, resulting in a very short duration of action, even in patients with renal and hepatic failure (3). The context-sensitive half-time is very short (3 to 4 minutes), independent of the duration of infusion. These characteristics allow titration of dose effect and usage of very high doses without prolonging recovery from its effects (3). Remifentanil also leads to respiratory depression in humans (4), as well as in other species, such as rats (5). Previously, it has been proven that opioids can abolish hypoxic ventilatory response acting at µ-opioid receptors (6).

Acute intermittent hypoxia (AIH) is known to be a strong respiratory stimulus that could be used in induction of a specific form of respiratory plasticity, such as long term facilitation (LTF) (7, 8). LTF is a serotonin-dependent increase in respiratory motor output elicited by AIH that persists long after hypoxic stimulus has ceased (9). It is manifested as an increase in phrenic nerve amplitude (PNA), burst frequency (f) and breathing rhythm parameters (T1, T2, Ttot) were analyzed during 5 hypoxias and at 15, 30, and 60 minutes after the final hypoxia, and compared to baseline values. At the end of the experiment, the infusion of remifentanil was stopped and phrenic nerve activity was compared to baseline values prior to remifentanil infusion. In conclusion, the short acting µ-opioid receptor agonist, remifentanil, reversibly abolished phrenic long term facilitation in urethane anesthetized rats.

Key words: long term facilitation, intermittent hypoxia, remifentanil, phrenic nerve, opioids, long term potentiation, mean arterial blood pressure

Remifentanil reversibly abolished phrenic long term facilitation in rats subjected to acute intermittent hypoxia

B. IVANCEV1, M. CAREV1, R. PECOTIC2, M. VALIC2, I. PAVLINAC DODIG2, N. KARANOVIC1, Z. DOGAS2

1Department of Anesthesiology and Intensive Care, University Hospital Split, Split, Croatia;
2Department of Neuroscience, University of Split School of Medicine, Split, Croatia

The aim was to investigate whether intravenous infusion of remifentanil would depress phrenic long term facilitation (pLTF) evoked by acute intermittent hypoxia (AIH) in adult, male, urethane anaesthetized Sprague-Dawley rats, bilaterally vagotomized, paralyzed and mechanically ventilated. The experimental group received a remifentanil infusion (0.5 µg/kg/min i.v., n=12), whereas the control group (n=6) received saline. Rats were exposed to AIH protocol. Phrenic nerve amplitude (PNA), burst frequency (f) and breathing rhythm parameters (T1, T2, Ttot) were analyzed during 5 hypoxias and at 15, 30, and 60 minutes after the final hypoxia, and compared to baseline values. At the end of the experiment, the infusion of remifentanil was stopped and phrenic nerve activity was compared to baseline values prior to remifentanil infusion. In the control group, peak phrenic nerve activity (pPNA) significantly increased at 60 min (T60, increase by 138.8±28.3%, p=0.006) after the last hypoxic episode compared to baseline values, i.e. pLTF was induced. In remifentanil treated rats, there were no significant changes in peak phrenic nerve activity at T60 compared to baseline values (decrease by 5.3±16.5%, p>0.05), i.e. pLTF was abolished. Fifteen minutes following cessation of remifentanil infusion, pPNA increased by 93.2±40.2% (p<0.05) and remained increased compared to pre-remifentanil-infusion values for more than 30 minutes, i.e. pLTF could be observed after cessation of the remifentanil infusion. In conclusion, the short acting µ-opioid receptor agonist, remifentanil, reversibly abolished phrenic long term facilitation in urethane anesthetized rats.
MATERIALS AND METHODS

The protocol for this study was approved by the Biomedical Research Committee of the University of Split School of Medicine, Split, Croatia. All experiments were carried out in accordance with the National Research Council's guide for the care and use of laboratory animals (Institute of Laboratory Animal Research, 1996).

Experimental preparation

Experiments were conducted on adult male Sprague-Dawley rats weighting 300–340 g (Animal Facility of the University of Split School of Medicine). Anesthesia was performed with urethane, and the adequacy of anesthesia was frequently assessed by observing the absence of a withdrawal reflex to a noxious paw pinch, as well as registering changes in blood pressure. The trachea was cannulated through a midline ventral neck incision. The animals were artificially ventilated (volume controlled ventilation) through a tracheostomy using a respirator (CWE Inc, Ardmore, USA) in order to maintain stable parameters of ventilation. Small positive end-expiratory pressure (2–3 cm H₂O) was applied to all animals.

The rats were paralyzed with pancuronium bromide 1 mg/kg intravenously (Pavulon, Organon, Netherlands) to prevent spontaneous breathing efforts. Bilateral femoral arterial and venous catheterizations were made in order to continuously measure blood pressure, obtain blood samples, and deliver intravenous infusions. After venous cannulation, an infusion of saline (1.7 ml kg⁻¹h⁻¹) was started in order to maintain arterial blood pressure and to improve the viability of the preparation due to extensive surgery. The rats were placed in a prone position in a stereotaxic instrument (Lab Standard, Stoeling, USA). The right phrenic nerve was dissected using dorsal approach at the level of C5 nerve rootlet, mounted on a bipolar silver wire electrode and covered with silicone gel to prevent it from drying. The obtained electrophysiological signal was then amplified (Super Z & System 1000 Modular Instrumentation, CWE Inc., Ardmore, USA).

End-tidal CO₂ (PETCO₂) was continuously monitored with GEMINI respiratory gas analyzer (CWE Inc, Ardmore, USA) and adjusted by changing the respiratory rate (RR) and inspiratory time/pressure. Arterial blood samples (0.2 ml) were drawn from the catheterized femoral artery to determine blood gases and pH (RapidLab 348, Bayer Diagnostics, Sudbery, UK). Rectal temperature was monitored by digital thermometer and maintained between 37 and 38.5°C by means of an external heating pad (FST, Heidelberg, Germany).

Experimental groups

The animals were divided into two groups: control group (urethane-anesthetized, 20% urethane Sigma Aldrich, St. Louis, USA, 1.2 g/kg intraperitoneally, with supplemental doses of 0.2 g/kg given as needed; n=6) and remifentanil group (urethane anesthetized + continuous remifentanil infusion, Ultiva, Glaxo Wellcome, Middlesex, UK, 0.5 µg kg⁻¹min⁻¹; 1.7 ml kg⁻¹h⁻¹; n=12). Remifentanil was reconstituted in saline according to the manufacturer's recommended list of diluents for remifentanil (Ultiva, Glaxo Wellcome, Middlesex, UK). The continuous remifentanil infusion was delivered by means of a special perfusor for small laboratory animals (SP 200iZ Syringe Pump, World Precision Instruments, Sarasota, USA).

Experimental protocol

After completion of the phrenic nerve surgery, a minimum of 30 minutes was allowed for the nerve signal to stabilize at FIO₂=0.5. Baseline phrenic nerve activity was set at PaCO₂, ~2–3 mmHg above CO₂ apneic threshold, and was achieved by manipulating ventilator parameters until PNA attained a low but stable level of activity. After achieving a stable phrenic nerve signal, the control blood sample was taken.

In the control group, basic nerve activity was recorded followed by five 3-min episodes of isocapnic hypoxia (FIO₂ =0.09), separated by 3 minutes of recovery (FIO₂ =0.5). Blood samples were taken in three posthypoxic periods, at 15, 30 and 60 min (T15, T30, and T60) after the termination of the last hypoxic episode. The experimental protocol of acute intermittent hypoxia is shown in Fig. 1.

In the remifentanil group, after achieving a stable phrenic nerve signal, remifentanil infusion (0.5 µg kg⁻¹min⁻¹) was started and 30 min were allowed for remifentanil to achieve its effect. An initial blood sample was taken and the experimental protocol was started. Sixty minutes after the termination of the last hypoxic episode, the remifentanil infusion was stopped (Fig. 1). Approximately 45 min after cessation of the remifentanil infusion, animals were euthanized with an overdose of anaesthetics and an intravenous potassium-chloride injection. Relative isocapnia was maintained throughout the protocol in order to exclude influences of PaCO₂ changes on breathing.

Time control

Due to the relative long duration of the experimental protocol, we devised two time-control experimental groups to ensure that the changes in phrenic nerve activity were not related to time-dependent factors as follows:

Group 1: remifentanil sham-treated rats (n=5). Rats received remifentanil diluted in saline (infusion rate 1.7 ml kg⁻¹h⁻¹) with the exception that the animals were not exposed to intermittent hypoxic episodes, but in which the same number of blood samples was taken. We found no time-dependent changes in phrenic nerve activity (PNA) for up to 130 minutes, indicating the stability of the experimental preparation during this time.

Group 2: vehicle sham-treated rats (n=3) were performed in order to prove that the vehicle (saline) for remifentanil does not block phrenic LTF and does not cause any time-dependent changes in phrenic nerve activity. Rats received saline infusion (infusion rate of 1.7 ml kg⁻¹h⁻¹) with the exception that the animals were not exposed to intermittent hypoxic episodes, but in which the same number of blood samples was taken.

Data acquisition and analysis

Phrenic nerve activity was amplified, filtered (bandpass 300 Hz to 10 kHz) and rectified. The moving time average of phrenic nerve activity was obtained using MA-1000 Moving Averager, System 1000 Modular Instrumentation (CWE Inc., Ardmore, USA) with a 50 ms time constant. The processed signal - phrenic neurogram (PNG) was recorded using PowerLab software, Chart for Windows version 5.4.2 and Scope for Windows version 3.8 (ADI Instruments, Castle Hill, Australia). Analysis of recorded PNA and blood pressure data was performed by sampling averaged nerve activity and blood pressure over 20 s periods at predetermined time points. In the control group, there were nine pre-determined time points for data analysis: before the first hypoxia
(T0), during hypoxic episodes (TH1-TH5), and 15 (T15), 30 (T30), and 60 (T60) minutes after the end of the last hypoxia. In the remifentanil group, two additional time points were analyzed: 30 min after starting the remifentanil infusion (RON) and 15 min after cessation of the remifentanil infusion (R OFF). From the digital record of phrenic nerve activity, five parameters were analyzed: peak phrenic nerve activity (pPNA), phrenic burst frequency (f), inspiratory duration (Ti), expiratory duration (Te), and respiratory cycle duration (Ttot). Minimum 20 s of signal with stable response was required for successful analysis. The average number of breaths was between 11 and 20 in a 20 s period.

Statistical analysis

Averaged amplitude data were normalized as a percentage change from baseline activity prior to remifentanil infusion (T0) and following remifentanil infusion (RON). The results were analyzed using MedCalc (MedCalc Software, Mariakerke, Belgium) with two-way repeated measures ANOVA (within and between groups) followed by Bonferroni post hoc correction. Two-way repeated measures ANOVA and post hoc analyses were used to provide information for all pair-wise comparisons of interest, both against baseline, and between groups at the same time points during the protocol. Data were presented as mean ±S.E.M. Statistical significance was set at p<0.05.

RESULTS

Systemic administration of the µ-opioid receptor agonist remifentanil, abolished pLTF in response to five 3-min episodes of hypoxic exposures. However, after cessation of the remifentanil infusion pLTF could be observed.

The steady state respiratory response obtained by continuous infusion of remifentanil was reached approximately 30 minutes after the infusion had started (at time point RON), and on average no significant changes in peak phrenic nerve activity - compared with T0 - were observed. Remifentanil elicited changes in the respiratory rhythm primarily through an alteration in inspiratory time, that increased from 0.51±0.04 s to 0.84±0.06 s following systemic administration of remifentanil (p<0.05). However, duration of expiratory time decreased from 0.90±0.09 s to 0.71±0.08 s (p<0.05), resulting in a non-significant increase of total respiratory cycle duration from 1.41±0.06 s to 1.55±0.06 s following remifentanil infusion (Table 1, p>0.05). A hypoxic ventilatory response was preserved under remifentanil infusion during all five (TH1-TH5) hypoxic episodes, i.e. in all five time points there was a significant increase in pPNA compared with both baseline phrenic nerve activity before remifentanil infusion (time point T0), and with remifentanil baseline (time point RON) (p<0.001, Fig. 2A). However, this HVR under remifentanil infusion was significantly lower than in the control group of animals (p<0.02, Fig. 2A). In the remifentanil treated group, there was a slight but not significant increase in phrenic burst frequency during five hypoxic episodes (Fig. 2B), whereas in the control group of animals there were significant increases in the phrenic burst frequency during all hypoxic episodes compared to baseline values (Fig. 2B).

In the control group of animals, there was a significant increase in peak phrenic nerve activity at 60 minutes after the last hypoxic episode (increase by 138.8±33.5%, p=0.006), compared to baseline values before the first hypoxic episode (Fig. 2A and Fig. 3), i.e. pLTF was induced. However, at 15 and 30 minutes following the end of the last hypoxic episode (T15 and T30), peak phrenic nerve activity was continually increasing but did not reach a level of significance (increase by 73.4±35.4% and 86.8±35.3%, respectively, p<0.05, Fig. 2A).

In the remifentanil treated rats, there were no significant changes in peak phrenic nerve activity at T15 and T30 compared with the values before the first hypoxic episode (increase by 40.9±56.0%, and decrease by 11.3±12.4%, respectively, p>0.05, Fig. 2A and Fig 3). Also, in the remifentanil treated rats, pLTF could not be induced 60 minutes after the end of the last hypoxic episode (Fig. 3). Peak phrenic nerve activity at one hour post-hypoxia increased by 11.8±23.8% compared to the values before

Fig. 1. The acute intermittent hypoxia (AIH) protocol used in this experiment in both and the control and remifentanil group. There were five, 3-min episodes of isocapnic hypoxia (FiO2=0.09), separated by 3-min episodes of recovery (FiO2=0.5). The control group of animals was urethane anesthetized and received a saline infusion throughout the experimental protocol, whereas the remifentanil group was urethane anesthetized and received a continuous remifentanil infusion. T0: time point immediately before the first hypoxia; RON: baseline value after onset of remifentanil infusion; TH1-5: hypoxic episodes 1-5; T15: 15 min after the last hypoxic episode; T30: 30 min after the last hypoxic episode; T60: 60 min after the last hypoxic episode; ROFF: 15 min following cessation of remifentanil infusion; BG: arterial blood gas sample.
the first hypoxia (p>0.05, Fig. 2A) and was significantly lower compared to the control group of animals (Fig. 2A, p<0.001). However, fifteen minutes following cessation of the remifentanil infusion, pPNA increased by 93.2±40.2% (p<0.05) and remained high compared to the pre-remifentanil-injection values for more than 30 minutes, i.e. pLTF could be observed after cessation of remifentanil infusion.

At time points T15 and T30, acute intermittent hypoxia did not elicit significant changes in phrenic burst frequency and respiratory rhythm parameters (Ti, Te, and Ttot) in both, remifentanil treated and the control group of animals (Table 1). There were no significant changes in either group of animals in PaCO₂ and PaO₂ values obtained before the first hypoxia, as well as at T15, T30, and T60 (Table 2). Mean arterial pressure (MAP) was significantly decreased during all hypoxic exposures in both groups compared to baseline values (Table 3). There were no statistically significant differences in MAP between the control and remifentanil group at any time point throughout the experimental protocol.

**DISCUSSION**

In this study, the pLTF was observed in control (urethane-anesthetized) rats 60 minutes after the last hypoxic episode exposure, and it was expressed mainly as amplitude, and not frequency pLTF. On the contrary, the intravenous infusion of remifentanil abolished pLTF in urethane-anesthetized rats.
following AIH protocol. However, after cessation of remifentanil infusion, pLTF could be observed.

Opioids may influence some form of synaptic plasticity, such as long term potentiation (LTP) and long term depression (LTD) (14-16), but this is the first study that investigates the potential influence of opioids on respiratory plasticity known as LTF. Remifentanil was suitable for this investigation because of its pharmacokinetic characteristics: it reaches steady state plasma concentration in less than 10 minutes and has an effective site elimination half-life of less than 4 minutes once the infusion is stopped (3). Therefore, 15 minutes after cessation of remifentanil infusion we were able to monitor recovery, and compare monitored values with baseline values prior to the remifentanil infusion. In some previous studies the remifentanil dosage varied, from 0.03 to 4 µg kg⁻¹min⁻¹, depending on the animal model and concomitant anesthetics used (18-20). In our study, the highest remifentanil dose with a stable phrenic nerve signal was 0.5 µg kg⁻¹min⁻¹, because in animals receiving higher doses of remifentanil, bursts of phrenic nerve activity were completely abolished, which was not suitable for our experimental protocol. It is well known that the type of anesthetic used can modulate the expression of pLTF. The most

\[ \text{Fig. 3. Changes in phrenic motor output following episodic hypoxia in one control rat (upper panel). There was an increase in pPNA observed at T60, indicating pLTF in the urethane-anaesthetized rat (control). Changes in phrenic motor output following episodic hypoxia in one remifentanil treated rat (lower panel). Remifentanil abolished development of the pLTF at 60 (T60) minutes after the last hypoxia. Fifteen minutes (ROFF) following cessation of the remifentanil infusion, there was an increase of pPNA, accompanied by prolongation of Ttot. Integrated (IPNA) and raw phrenic nerve activity (RPNA) are shown during baseline conditions (T0), at onset of remifentanil infusion (RON), first hypoxia (TH1), and at 15 (T15), 30 (T30), and 60 (T60) minutes after the last hypoxia.} \]

| Table 1. Relative changes in Ti, Te and Ttot during the experimental protocol in control (urethane-anesthetized) and remifentanil treated rats. Data are expressed in seconds (mean ±S.E.M). |
|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Control                  |                 |                 |                 |                 |                 |
| Ti                       | 0.45±0.05       | 0.48±0.04       | 0.48±0.05       | 0.40±0.01       |                 |
| Te                       | 1.13±0.06       | 1.07±0.10       | 0.96±0.11       | 0.96±0.03       |                 |
| Ttot                     | 1.56±0.09       | 1.55±0.10       | 1.44±0.09       | 1.36±0.04       | *               |
| Remifentanil             |                 |                 |                 |                 |                 |
| Ti                       | 0.51±0.04       | 0.84±0.06       | 0.76±0.07       | 0.75±0.04       | 0.91±0.12; ‡    |
| Te                       | 0.90±0.09       | 0.71±0.08       | 0.73±0.10       | 0.76±0.07       | 0.79±0.09       | §               |
| Ttot                     | 1.41±0.06       | 1.55±0.06       | 1.49±0.07       | 1.52±0.06       | 1.71±0.12; ‖    |

Ti: inspiratory time; Te: expiratory time; Ttot: total respiratory time; T0: baseline value before the first hypoxia; RON: baseline value after beginning of remifentanil infusion; T15: 15 min after the last hypoxic episode; T30: 30 min after the last hypoxic episode; T60: 60 min after the last hypoxic episode; ROFF: 15 min following cessation of remifentanil infusion. (* significantly different from baseline value, p= 0.032; † significantly different from T60 value, p=0.009; ‡ significantly different from baseline value before the first hypoxia, p=0.013; § significantly different from T60 value, p = 0.028; ‖ significantly different from baseline value, p=0.008).
commonly used anesthetic in animal LTF studies is urethane due to its ability to anesthetize with minimal effects on variables related to hemodynamic and respiratory mechanics (21).

It is generally accepted that opioids depress the hypoxic ventilatory response (HVR). Stimulation of carotid body receptors is important in the initiation of HVR (22), but Bailey et al. demonstrated that direct action in the brainstem cannot be excluded (23). In addition, hypoxia may have an important role in the initiation of LTF; even in chemodenervated anesthetized rats, possibly through direct CNS mechanisms (24, 25). The results from our study indicate that HVR was preserved during all five hypoxic episodes, but was later in rats receiving continuous infusion of remifentanil. However, one might speculate whether reduced hypoxic phrenic nerve response of the remifentanil treated rats may have been caused by differences in blood gases between the control group and remifentanil treated rats (26). Similarly to our results, administration of μ-opioids reduced HVR (6), and at clinical relevant doses, leads to bradypnea (2, 27). In our previous study, we discussed the potential significance of HVR during the first hypoxic episode in predicting the amplitude pLTF based on a previously described prediction model (12). In the present study, there was an increase in phrenic nerve activity of 452.4% during first hypoxia in remifentanil treated rats. Therefore, according to the prediction of an increase in peak amplitude of phrenic nerve activity 60 minutes post hypoxia, one could presume that with the obtained increase in the first hypoxic episode in remifentanil treated rats, the increase in the amplitude pLTF 60 min post hypoxia should be at least 152.9% (13). However, in remifentanil treated rats, pLTF was abolished, but after cessation of the remifentanil infusion, pLTF could be observed. From our experiments, we cannot determine the precise mechanisms and specific site of action following systemic application of remifentanil that could be responsible for the increase in amplitude of pLTF after withdrawal of the remifentanil infusion.

In different animal species, relative effects of remifentanil on central respiratory activity have been assessed by measurement of phrenic nerve activity, which is the main index of central respiratory activity in this type of study (20). Additionally, in a similar model of paralyzed, mechanically ventilated animals, remifentanil evoked greater depression of phrenic nerve activity compared to cardiovascular responses, even if animals were not exposed to acute intermittent hypoxia protocol (20). Opioid receptors are present in multiple non-respiratory sites throughout the CNS and in respiratory control centers located in the brainstem and higher centers. Additionally they are located in the carotid bodies and in the vagal nerves. Mechanosensory receptors located in epithelial, submucosal and muscular layers of the airways, express opioid receptors and relay mechanical and sensory information from the lungs to the higher centers. Since bilateral vagotomy was performed in our study, we can rule out the potential influence of remifentanil on mechanoreceptors (2).

Based on previous studies that investigated the effects of systemic and local modulation of μ-opioid receptors in the medullary raphe region after hypoxic exposures (28, 29), we can speculate whether systemic administration of remifentanil could affect medullary raphe region neurons since they express abundant μ-opioid receptors and participate in HVR (30). Remifentanil induces respiratory depression possibly affecting μ-opioid receptors located at sites upstream from the respiratory premotor neurons (27). The sensitivity of neurons to low

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### Table 2. Partial pressures of carbon dioxide (PaCO₂), and oxygen (PaO₂) in arterial blood registered in two groups of animals at different time points. Data are presented as mean ±S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>PaCO₂ (mmHg)</th>
<th>PaO₂ (mmHg)</th>
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<tbody>
<tr>
<td>T0</td>
<td>R_ON</td>
<td>T15</td>
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<tr>
<td></td>
<td>Control</td>
<td>Remifentanil</td>
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<tr>
<td></td>
<td>44.35±0.8</td>
<td>46.55±1.1</td>
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<td></td>
<td>110.67±6.3</td>
<td>237.20±9.6</td>
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<td></td>
<td>73.77±6.5</td>
<td>229.83±13.0</td>
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T0: baseline value before the first hypoxia; R_ON: baseline value after beginning of remifentanil infusion; T15: 15 min after the last hypoxic episode; T30: 30 min after the last hypoxic episode; T60: 60 min after the last hypoxic episode; R_OFF: 15 min following cessation of remifentanil infusion.

### Table 3. Mean arterial blood pressure (MAP) in both groups of animals during all time points. Data are presented as mean ±S.E.M.

<table>
<thead>
<tr>
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<th>MAP (mmHg)</th>
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<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>T0</td>
<td>R_ON</td>
</tr>
<tr>
<td></td>
<td>110.67±6.3</td>
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<td>96.59±10.2</td>
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T0: baseline value before the first hypoxia; R_ON: baseline value after beginning of remifentanil infusion; TH1-5: hypoxic episodes 1-5; T15: 15 min after the last hypoxic episode; T30: 30 min after the last hypoxic episode; T60: 60 min after the last hypoxic episode; R_OFF: 15 min following cessation of remifentanil infusion. (* significantly different from baseline values, p <0.001; † significantly different from baseline values, p=0.021)
concentrations of opioids following systemic administration might be related to the number, density and location of µ-opioid receptors, assuming that affecting receptors primarily located on axon terminals profoundly alters neurotransmitter release (27). It has been well accepted that serotonin plays an important role in different forms of synaptic plasticity (31), mainly respiratory plasticity, acting via 5-HT1 and 5-HT2A receptors (7, 8, 32, 33), thus relaxing on activity of serotonergic neurons located at the spinal (9, 34) and supraspinal level, in the caudal raphe region (7, 8). Interaction between the serotonergic and opioid systems was investigated by Wang et al., who demonstrated that activation of specific serotonergic 5HT1A and 5HT2 receptors may prevent opioid-evoked cardiorespiratory depression (30). The role of opioids in modulation of synaptic plasticity remains to be established but it may prove to be important under a variety of conditions. In a model of slice preparation from rat lumbar spinal horn it has been shown that abrupt withdrawal from α-opioid receptor agonists induces other forms of synaptic plasticity, LTP at the first synapse in pain pathways by postsynaptic G protein activation and NMDA receptor-dependent Ca2+ signaling (14). It remains to be established whether, similar to the pain pathway, withdrawal from the short acting µ-opioid receptor agonist, remifentanil, could induce respiratory plasticity known as pLTF after exposures to AHI in a model of a urethane anesthetized rat.

Lately, plausible clinical importance of long term facilitation in humans has received increasing attention, but the relevance of LTF in humans and its potential role in respiratory physiology remains to be established. The majority of LTF research has been done using animal models that resulted in the discovery of various neuromodulators and cellular pathways that mediate this phenomenon. Although, the acute intermittent hypoxia protocol has been widely used in investigating mechanisms of respiratory plasticity, some studies also report that continuous hypercapnia is more effective in eliciting long term depression on respiratory motor output than episodic hypercapnia (35). In addition, in an animal model of working heart brainstem preparation hypercapnia has been shown to reversibly increase phrenic nerve frequency (36). Combined hypoxia and hypercapnia has been shown to have a synergistic effect on ventilation and on sympathetic nerve activity in humans (37, 38). Therefore, basic animal research can provide important insights into natural compensatory mechanisms such as respiratory plasticity, and how this phenomenon affects breathing instability experienced in individuals suffering from increased upper airway resistance (39). Lastly, some agents that are routinely used in a daily clinical practice have been shown to modulate manifestation of pLTF in a model of an anesthetized rat as highlighted by Carev et al. who showed that pLTF was attenuated under propofol anesthesia (12). In addition, results of our study suggest that systemic application of short acting µ-opioid receptor agonist remifentanil also attenuates pLTF in a model of anesthetized rat. In clinical practice, it has been shown that perioperative complications and adverse events are emphasized in individuals suffering from compromised upper airway flow (40). Furthermore, surgical patients who have been diagnosed with obstructive sleep apnea, because of repetitive exposures to hypoxic episodes, may be vulnerable to sedative, analgesic, and anesthetic drugs (40). In general, opioids and other drugs that have central respiratory and sedating effects should be administered with caution perioperatively in patients with obstructive sleep apnea (41).

However, the question remains whether results obtained using this animal model and protocol could be directly translated to healthy human physiology or specific breathing disorders related to increased upper airway resistance. Still, we believe that results obtained in animal model studies could offer some useful insights concerning the choice of sedatives, anesthetics and analgesics used in the perioperative management plan for patients with breathing disorders.

In conclusion, we confirmed the hypothesis that remifentanil depressed pLTF in anesthetized rats in a model of AHI protocol, but the suppression of pLTF was reversible following cessation of remifentanil infusion.

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