The “other” respiratory effect of opioids: suppression of spontaneous augmented (“sigh”) breaths

Harold J. Bell, Elizabeth Azubike, and Philippe Haouzi

Division of Pulmonary and Critical Care, Department of Medicine, Penn State University College of Medicine, Hershey, Pennsylvania

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Bell HJ, Azubike E, Haouzi P. The “other” respiratory effect of opioids: suppression of spontaneous augmented (“sigh”) breaths. J Appl Physiol 111: 1296–1303, 2011. First published August 25, 2011; doi:10.1152/japplphysiol.00335.2011.—The purpose of this study was to examine the effects of a clinically relevant opioid on the production of augmented breaths (ABs) in unanesthetized animals breathing normal room air, using a dosage which does not depress breathing. To do this we monitored breathing noninvasively, in unrestrained animals before and after subcutaneous injection of either morphine, or a saline control. The effect of ketamine/xylazine was also studied to determine the potential effect of an alternative sedative agent. Last, the effect of naloxone was studied to determine the potential influence of endogenous opioids in regulating the normal incidence of ABs. Morphine (5 mg/kg) had no depressive effect on breathing, but completely eliminated ABs in all animals in room air (P = 0.027). However, when animals breathed hypoxic air (10% O₂), animals did express ABs, although their incidence was still reduced by morphine (P < 0.001). This was not a result of sedation per se, as ABs continued at their normal rate in room air during sedation with ketamine. Naloxone had no effect on breathing or AB production, and so endogenous opioids are not likely involved in regulating their rate of production under normal conditions. Our results show that in unanesthetized animals breathing normal room air, a clinically relevant opioid eliminates ABs, even at a dose that does not cause respiratory depression. Despite this, hypoxia-induced stimulation of breathing can facilitate the production of ABs even with the systemic opioid present, indicating that peripheral chemoreceptor stimulation provides a potential means of overcoming the opioid-induced suppression of these respiratory events.

Control of breathing: opioids; side effect

One deleterious respiratory side effect of opioids may be a suppression of the endogenous mechanisms triggering spontaneous augmented or “sigh” breaths. Two previous studies in human patients suggest this may be the case, but these investigations were complicated by the fact that subjects had undergone major abdominal surgery immediately prior to having the opioid administered for routine pain management (15, 44). There are two previous animal studies that also support the possibility that opioids suppress augmented breaths (ABs), but these studies either provide limited data, or interpretation is complicated by the research models used and the background conditions in which the animals were studied. In an early study by Schmidt and Harer (53) there are no data or discussion provided regarding the effect of morphine on the incidence of ABs, other than what appears in a representative trace. Moreover, the cat studied was anesthetized with ether, and had undergone other complicating experimental interventions. A study by Zhang et al. (72) was more specifically focused on studying ABs, and showed that systemic administration of a synthetic µ-opioid receptor agonist (DAMGO; Ala²-MePhe⁴-Glyol⁵-Enkephalin) decreased the expression of ABs during exposure to hypoxia (10% O₂) or hypercapnia (7% CO₂). Their use of urethane anesthesia meant that they were unable to examine the effect of opioid administration in normal room air breathing (see Discussion for further details). Zhang et al. also purposely used a dose of DAMGO that caused significant respiratory depression, potentially limiting the relevance of their findings to clinical scenarios wherein respiratory depression is avoided.

Presently, it is not known whether or not the systemic administration of a clinically relevant opioid will suppress the spontaneous production of ABs during normal room air breathing, and further whether or not this effect might occur in the absence of respiratory depression. It is also not known whether opioids suppress the hypoxia-induced facilitation of ABs in the absence of background urethane anesthesia. These are essential questions to address to determine whether the opioid-induced suppression of ABs is a reproducible and physiologically relevant respiratory side effect that is pertinent to opioid use in conditions more typical of human patient care.

This possible side effect of opioid treatment has received surprisingly limited attention considering that ABs are essential, protective respiratory mechanisms; they help to maintain normal lung mechanics (13, 50), promote beneficial surfactant release (42, 43), prevent gradual reductions in functional residual capacity (46, 47), and prevent the development of significant atelectasis and related ventilation/perfusion mismatching (9, 22, 35, 40). The tendency for opioids to suppress the production of spontaneous ABs means that active protective mechanisms preventing hypoxemia and reductions in lung volume are no longer present in the breathing rhythm, and this should be considered a significant risk related to their use in the management of pain in spontaneously breathing humans.

The aim of the present study was therefore to examine breathing and the incidence of ABs before and again after administering the prototypical µ-opioid receptor agonist morphine, in an unanesthetized, unrestrained and noninvasively monitored animal model. We sought to determine if any opioid-induced suppression of ABs could be observed during normal room air breathing in the absence of respiratory depression and/or blunted chemoreflex responses secondary to sedation. We also sought to determine whether in the absence of urethane anesthesia opioid administration affects the production of ABs during hypocapnic hypoxia, a unique condition that we have previously demonstrated greatly increases the propensity to generate ABs (6–8). Since one of the most...
common side effects of opioids is sedation, we examined the effect of an alternative general sedative agent (ketamine/xylazine) on breathing and the production of augmented breaths. Finally, we examined the role of endogenous opioids in regulating the normal prevalence of augmented breaths by systemic administration of naloxone, a potent competitive antagonist of opioid receptors.

METHODS

Experiments were performed using six adult male, Sprague-Dawley rats (Charles River). All procedures were approved by the Institutional Animal Care and Use Committee of the Penn State College of Medicine. Animals were housed by the Animal Resource Services at the Penn State College of Medicine, which conforms to the requirements of the US Department of Agriculture and the Department of Health and Human Services. The Animal Resource Program of the Pennsylvania State University College of Medicine is accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care.

The experiment used a partially randomized, crossover design, where each animal was observed during two measurement sessions performed on each of four separate experimental days. Respiratory measurements were made while the animals were unrestrained and noninvasively monitored during exposures to both room air (~21% O2, balance N2) and hypoxia (~10% O2, balance N2). On each of the four experimental days, measurement sessions were performed immediately before and again 30 min after a subcutaneous (sc) injection of one of four possible interventions: isotonic saline (control injection), morphine, ketamine/xylazine, or naloxone. Only one intervention was performed on a given experimental day. The order of these interventions was partially randomized by generating an arbitrary sequence for each animal. Experimental days were separated by at least 48 h, but no more than 5 days.

Morphine sulfate (10 mg/ml) was administered at 5 mg/kg; ketamine/xylazine was administered at 80 and 10 mg/kg, respectively; naloxone hydrochloride dehydrate (N7758, Sigma-Aldrich) prepared in isotonic saline was injected at a dose of 10 mg/kg; saline injections (0.9% NaCl) were volume matched to the morphine injections. All injections were <0.5 ml.

The equipment and techniques used to monitor/analyze breathing activity were the same as those we have used and described previously in detail (8). Briefly, each animal was individually monitored in an animal chamber that was connected to an air control circuit allowing us to perform unrestrained whole body open-flow plethysmography. We used this form of plethysmography because it allowed us to noninvasively monitor animals repeatedly across several separate days of experimental interventions, and it allows continuous observation of breathing over several hours of monitoring. The technique is, however, subject to the limitation that it does not provide a direct measurement of tidal volume, or therefore minute ventilation. Rather it provides a pneumogram that results from a complex set of respiratory related changes that occur during the inspiration and expiration of respired gases. The technical aspects of this methodology and related techniques have been critically discussed in detail elsewhere (31, 38, 49, 59). As such, our determinations of respiratory indexes of tidal volume and minute ventilation are semiquantitative and represented throughout the manuscript as estVt and est, respectively, where “est” is estimated, to clearly reflect this fact.

The animal-monitoring chamber was a leak-proof acrylic cylinder with air inlet and outlet ports on opposite ends of the chamber. Gas mixtures were supplied to the chamber at a regulated bias flow rate of ~2.5 l/min. This flow rate allowed chamber gas composition to be minimally affected by the metabolic production/consumption of CO2/O2, but at the same time allowed excellent resolution of the respiratory-related component of the flow signal. Gas mixtures were drawn through the animal chamber using a regulated vacuum system. Valve controls allowed the experimenter to switch between supplying the animal chamber with either normoxic air (~21% O2, balance N2) or hypoxic air (~10% O2, balance N2). The hypoxic air was temporarily stored in compliant 60-liter mylar bags that were filled immediately before the experiment using a commercially prepared compressed gas mixture (GTS-Welco, Allentown, PA). The fresh gas stream to the animal chamber first passed through a Fleisch pneumotachograph (000, Phipps and Bird, Richmond, VA) before entering the chamber through an internal diffuser that distributed the gas evenly over the cross-sectional area. Mixed respired gases were exhausted from the chamber through an outlet port that vented into the building vacuum supply.

CO2 and O2 levels in air leaving the chamber air were continuously measured (model no. 17630 infrared, and no. 17518A paramagnetic analyzers respectively, Vacumed, Ventura, CA). The pneumotach was interfaced to a pressure transducer (DP45–14 Validyne Engineering, Northridge, CA) and an electronic demodulator unit (CD-15, Validyne Engineering, Northridge, CA). The output signal from the demodulator provided constant monitoring of the flow of fresh gas to the animal chamber and the respiratory tracing. Temperature in the animal chamber (Tc) was continuously monitored via a fast responding thermocouple (Thermalert TH5, Physiostemp, Clifton, NJ) that was verified for accuracy against a high-resolution mercury thermometer. Body core temperature was directly assessed to ± 0.1°C via rectal probe (model 524928, Becton Dickinson, Franklin Lakes, NJ) immediately before and again following each monitoring session.

Animals were monitored on four separate experimental days. For any given animal, the four monitoring sessions and the drug or control injections were conducted at the same time of day, with experiments taking place between 10:00 am and 4:00 pm. These procedures were repeated on separate days until each animal had all been monitored in all of the four conditions (i.e., saline, morphine, ketamine/xylazine, naloxone).

Analog signals representing flow to the chamber, %CO2 and % O2 signals, and box temperature were fed into a 14-bit analog-to-digital (A/D) converter (USB6009, National Instruments, Austin, TX), which was interfaced with an Intel/Windows Vista based computer system (Compaq 8510w, Hewlett Packard, Palo Alto, CA) running custom-written data-acquisition software (LabView, National Instruments, Austin, TX; source code available upon request). Analog signals were sampled at 200 Hz and displayed in raw form while being stored for subsequent analysis. ASCII data files were imported for visualization and analysis using Chart software (version 5.5.4, ADInstruments, Colorado Springs, CO).

To extract respiratory variables from the raw flow trace, we used identical analysis procedures as published in our earlier studies (6–8).

Each testing session provided data from animals monitored in two separate background conditions, normoxia (~21% O2, balance N2) and hypoxia (~10% O2, balance N2). In each condition the animals were monitored for ~20 min. From this 20-min trace, a 5-min window was used to determine estVt, breathing frequency (fB), estVt, and the number of augmented breaths. The default interval used for analysis was t = 10–15 min. If there was obvious disruption in the respiratory trace that totaled more than 15–20 s (~10% of the observation window), then the 5-min window was deleted. In no case did the interval used for analysis start any earlier than 5 min after the beginning of a given exposure, or any later than 15 min after the start of any given exposure.

Augmented breaths were easily identified in the open-flow plethysmograms as we have described previously (6–8), and one example is shown in the expanded trace in Fig. 1. Our objective criterion for determination of augmented breaths was any spontaneous large breath with an amplitude ≥3 times that of the background rhythmic breathing pattern. This simple criterion easily discriminated augmented breaths from other large eupneic breaths even during exposure to hypoxia where tidal volume was stimulated.
MORPHINE ELIMINATES AUGMENTED BREATHS

RESULTS

Two independent factors were considered for statistical analysis using two-factor repeated-measures ANOVA testing procedures (SigmasStat V3.5). These two factors were oxygen status (2 conditions: normoxia, hypoxia) and injection status (2 conditions: preinjection, postinjection). The a priori value for acceptability of a type I (α) error in any statistical comparison was set to 0.05. Post hoc testing was performed using the Holm-Sidak method for multiple comparisons. All data are reported as means ± SD.

Six animals were used in this study, and averaged 372 ± 88, 358 ± 101, 377 ± 96, and 403 ± 61 g across the 4 experimental days wherein animals received saline, morphine, ketamine/xylazine, or naloxone injections, respectively. Table 1 summarizes the mean values of all outcome measurements across all experimental conditions.

Saline (control). The control saline injection did not alter the incidence of augmented breaths (ABs) in room air conditions (Fig. 2), which averaged 1.8 ± 1.0 AB/5 min across pre/post status. Hypoxia caused an expected dramatic increase in the incidence of ABs both before (Δ = 15.7 ± 4.1 AB/5 min, P < 0.001) and following (Δ = 12.3 ± 5.2 AB/5 min, P < 0.001) saline injections. Moreover, the control injections did not significantly change estVe (P = 0.724), fB (P = 0.732), or estVT (P = 0.476).

Morphine. In room air conditions, ABs were eliminated following morphine injection (Fig. 3), averaging 2.5 ± 1.2 and 0.0 ± 0.0 AB/5 min, pre- vs. post-morphine, respectively (P = 0.027). In hypoxia, ABs were observed following morphine injection, although they occurred much less frequently compared with pre-morphine hypoxia condition (7.2 ± 2.1 vs. 16.5 ± 4.2 AB/5 min, P < 0.001).

Morphine administration did not independently affect the level of estVe, and there was no interaction effect between factors. Morphine did decrease fB, but only during exposures to hypoxia. fB in hypoxia preinjection was 194 ± 22 breaths/min, and after morphine injection the breathing frequency achieved was 123 ± 13 breaths/min during hypoxia (P = 0.004). Morphine had the opposite effect on estVe; however there was no interaction effect between factors (P = 0.069). When averaged across room air and hypoxia conditions, estVT was 2.3 ± 0.5 ml before morphine injection, and 3.1 ± 0.1 ml following injection (P = 0.002).

Ketamine/xylazine. The incidence of ABs in room air conditions was not affected by ketamine/xylazine injection (Fig. 4). However, the incidence of ABs during exposure to hypoxia was significantly lower after ketamine/xylazine administration (P < 0.001).

Ketamine/xylazine significantly lowered estVe (58 ± 10 ml·min⁻¹·100 g⁻¹) compared with preinjection (78 ± 18 ml·min⁻¹·100 g⁻¹), P = 0.014. The level of estVe achieved during exposure to hypoxia was significantly lower following injection (83 ± 15 ml·min⁻¹·100 g⁻¹) compared with preinjection (146 ± 27 ml·min⁻¹·100 g⁻¹), P < 0.001. fB was generally lower following ketamine/xylazine injection (87 ± 20 breaths/min), compared with preinjection (173 ± 49 breaths/min). estVT was significantly increased following ketamine/xylazine injection (3.0 ± 0.5 ml) compared with preinjection (2.4 ± 0.6 ml), independently of oxygen status (P < 0.001).

Naloxone. Naloxone injection did not affect the incidence of ABs (P = 0.221), and there was no significant interaction effect. When averaged across pre/post injection conditions, the incidence of ABs averaged 2.4 ± 1.1 AB/5 min in room air, and increased significantly to 14.1 ± 5.7 AB/5 min in hypoxia. estVT was increased slightly but significantly following naloxone injection independent of oxygen status (averaging 2.7 ± 0.5 vs. 3.0 ± 0.7 ml pre/post injection P = 0.014); however, neither estVe nor fB were significantly affected (Fig. 5).

DISCUSSION

Morphine causes a complete suppression of ABs during room air breathing. To our knowledge, this is the first controlled animal study to demonstrate that morphine administration eliminates ABs in a normal physiological respiratory background. We found that the prototypical μ-opioid receptor agonist (60) caused a very consistent and complete suppression of ABs while animals were breathing in normal room air.
conditions (see Fig. 6). In no instance did any animal display a single AB during room air monitoring following systemic morphine injection.

The only other well-controlled study examining the effect of opioids on the production of ABs is that by Zhang et al. (72). However, they were unable to report on what happened to the occurrence of ABs in normal physiological conditions. This is because the authors acknowledged that they rarely ever observed ABs when the animals were breathing normal room air. In recent experiments (unpublished observations), we have confirmed that this is a confounding effect of urethane anesthesia, which they used in their experiments.

Morphine suppresses ABs without a depression of breathing. Opioid treatment in human patients is normally tailored to avoid respiratory depression, and so our opioid dose was chosen so that it did not depress breathing. By contrast, the dose of DAMGO that was used by Zhang et al. was specifically chosen because they had previously shown that it significantly depressed breathing (73, 74) and decreased the hypoxic (74) and hypercapnic (73) ventilatory responses; indeed, this was their marker of systemic effectiveness. Our study has shown that there is a powerful suppression of ABs even in an absence of respiratory depression.

Peripheral chemoreflex stimulation can trigger ABs despite their morphine-induced elimination in normal room air breathing. Hypoxia is a well-described physiological stimulus for the generation of ABs (5, 20, 34), and we have previously shown that in awake animals, respiratory alkalosis is a key factor in this facilitation (6–8). Our present study shows that chemosensory stimulation of the carotid body chemoreceptors is capable of triggering ABs despite the fact that morphine was systemically present and had completely eliminated their occurrence in normal room air breathing.

Endogenous opioids do not influence the generation of spontaneous ABs. Considering the widespread presence of opioid receptor expression in neuronal populations of the brain stem, including respiratory centers, and the presence of endogenous opioids in these regions [see Lalley (26) for a detailed review of this topic], it is a significant finding that general competitive blockade of these receptors using naltrexone (33) does not affect the endogenous production of ABs in unanesthetized animals. The dose we used (5 mg/kg) is considered a high dose, but doses in this range are believed to be necessary to reliably block endogenous opioid signaling pathways (11, 17).

Table 1. Summary of results

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Morphine</th>
<th>Ketamine/xylazine</th>
<th>Naloxone</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>RA #ABs/5 min</td>
<td>1.5 ± 1.0</td>
<td>2.2 ± 1.0</td>
<td>2.5 ± 1.2</td>
<td>0 ± 0</td>
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<tr>
<td>RA est(\dot{V})E, ml·min(^{-1})·100 g(^{-1})</td>
<td>17.2 ± 3.5</td>
<td>14.5 ± 4.5</td>
<td>16.5 ± 4.2</td>
<td>7.2 ± 2.1</td>
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<tr>
<td>RA fB, breaths/min</td>
<td>86 ± 34</td>
<td>89 ± 24</td>
<td>86 ± 31</td>
<td>104 ± 11</td>
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<tr>
<td>RA est(\dot{V})T</td>
<td>147 ± 37</td>
<td>150 ± 30</td>
<td>146 ± 23</td>
<td>132 ± 26</td>
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<tr>
<td>RA fB, breaths/min</td>
<td>143 ± 33</td>
<td>139 ± 38</td>
<td>149 ± 63</td>
<td>151 ± 23</td>
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<tr>
<td>RA est(\dot{V})E</td>
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<td>190 ± 17</td>
<td>194 ± 22</td>
<td>123 ± 13</td>
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<td>RA fB, breaths/min</td>
<td>2.1 ± 0.5</td>
<td>2.3 ± 0.5</td>
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<td>RA est(\dot{V})T</td>
<td>2.9 ± 0.7</td>
<td>2.9 ± 0.5</td>
<td>2.6 ± 0.4</td>
<td>3.8 ± 1.0</td>
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<td>RA fB, breaths/min</td>
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<td>2.7 ± 0.8</td>
<td>2.2 ± 1.5</td>
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<tr>
<td>RA est(\dot{V})E</td>
<td>0.4 ± 2.5</td>
<td>0.8 ± 3.6</td>
<td>1.0 ± 2.5</td>
<td>1.2 ± 0.5</td>
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<td>RA fB, breaths/min</td>
<td>2.1 ± 0.5</td>
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<td>RA est(\dot{V})T</td>
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Values are means SD for the incidence of augmented breaths (#ABs), estimated minute ventilation (est\(\dot{V}\)E), breathing frequency (fB), and estimated tidal volume (est\(\dot{V}\)T), averaged across all animals in all conditions tested. RA, room air; HX, hypoxia; Pre, preinjection; Post, postinjection. See RESULTS and Figs. 2–5 for outcomes of statistical comparisons.

Fig. 2. The effects of saline (control) injections on respiratory measures during exposure to both normoxic room air (open bars) and hypoxic (closed bars) conditions. Shown are the % changes (post–pre) in the number of augmented breaths observed in the 5 min observation window (#ABs); the estimated minute ventilation (est\(\dot{V}\)E), breathing frequency (fB), and estimated tidal volume (est\(\dot{V}\)T).

Fig. 3. The effects of morphine injections on respiratory measures during exposure to both normoxic room air (open bars) and hypoxic (closed bars) conditions. Shown are the % changes (post–pre) in the number of augmented breaths observed in the 5 min observation window (#ABs); est\(\dot{V}\)E, fB, and est\(\dot{V}\)T. *Significant change within condition. ‡Significant effect of injection independent of background oxygen status (i.e., no interaction effect).
The morphine-induced suppression of ABs is not a common effect of general sedation. One of the most common side effects of therapeutic opioid use is general sedation (54). Numerous studies have proposed that the triggering of spontaneous ABs in normal room air breathing may be influenced by complex psychological factors including changes in behavioral state (21), anxiety (63–65, 68), stress and relief (56, 64). As a control intervention for this possible confounding factor of sedation, we examined the effect of a commonly used laboratory sedative ketamine/xylazine. The dose of ketamine/xylazine we used is recommended to provide a surgical plane of anesthesia in rats (58), and the sedation achieved in the ketamine condition was most certainly deeper than that present in the morphine condition. Despite this deep sedation, ketamine/xylazine did not cause any suppression or elimination of ABs in normal room air breathing. While ketamine/xylazine sedation did not affect ABs in room air breathing, it did subdue the hypoxia-induced facilitation of ABs. This is also a novel observation, and we propose that this was due to a decreased ventilatory response to hypoxia compared with control conditions, and therefore a less pronounced respiratory alkalosis (6–8).

How does morphine suppress or eliminate the presence of ABs? Hypocapnia/alkalosis is an essential factor in promoting the generation of ABs during hypoxia, though the precise mechanism through which this occurs is not yet known (6–8). We have proposed that hypocapnia/alkalosis may promote the generation of ABs in the presence of hypoxia via a number of possible pathways including decreased brain blood flow, central-peripheral chemoreceptor interactions, or increased excitability of neuronal elements involved in the central generation of ABs. Since our indexes of breathing in the present study suggest that respiratory drive was not affected by morphine, changes in brain blood flow and/or central peripheral chemoreflex interactions were unlikely to have been involved in the opioid-induced suppression of ABs. However, based on our current understanding, it remains possible that morphine causes a decrease in the sensitivity toward hypocapnia/alkalosis of the central neuronal elements regulating AB production, an effect that ultimately eliminates them in room air breathing, and dramatically suppresses them in response to a given hypoxic/hypocapnic stimulus.

Rhythmogenic neurons in the pre-Bötzinger complex (55) are powerfully suppressed by opioids (23), and neurokinin-1
receptor-expressing neurons in this region have been proposed to be essential in mediating this respiratory depressive effect (37). By contrast, other rhythmonic preinspiratory neurons residing outside of the pre-Bötzinger complex (RTN/pFRG) appear to be opioid resistant, and may actually limit the depressive effects of opioids on breathing (23, 36). These respiratory effects on rhythmonic neurons may occur via presynaptic rather than direct effects on rhythmonic respiratory neurons (3, 4). While it has not been specifically studied, opioids may influence the generation of ABs via the modulation of the activity of one or more of these neuronal populations.

Pre-Bötzinger complex neurons in vitro are also capable of generating fictive AB discharge patterns in the absence of peripheral inputs (30). The synaptic mechanisms involved in the central generation of ABs have some unique characteristics compared with those governing the generation of normal eupneic breaths (28, 29). Further, a specific set of inspiratory neurons in this region of the ventral respiratory group appear to be active only during fictive AB discharges (62). These unique “sigh-only” medullary neurons provide a potential target for opioids to exert their influence over the production of ABs.

A second interesting possibility is that morphine administration affects the discharge and the central encoding of pulmonary vagal afferents, and thereby suppressed ABs. After all, vagal afferents are known to be very important in triggering and/or controlling the incidence of ABs (5, 20, 34), and opioid-related respiratory changes are at least partially mediated by their effect on receptors located on pulmonary vagal afferents (24, 69, 70).

However, if morphine had indeed suppressed or eliminated ABs by desensitizing pulmonary vagal afferents, then we would have expected to see a decrease in fB and an increase in estVt during breathing in both room air and in hypoxia. The apparent lack of effect morphine on breathing pattern during room air exposures in our data argue against their having been a desensitization of pulmonary vagal afferents; If there were, breathing pattern in both conditions should have been affected. Nevertheless, because the P value for an interaction effect in the estVt data approached but did not reach significance, we cannot say for certain that the change in breathing pattern was isolated to the condition of hypoxia. Rather our results only allow us to say that morphine tended to increase estVt independently of O2 status.

**Study limitations.** Effective morphine dose for pain management in naive rats (i.e., a clinically relevant dose in acute administration) can vary between 2 and 10 mg/kg (1, 25). We selected the dose of 5 mg/kg because it is well within the relevant dose range in these animals, and in preliminary experiments this dose was also sufficient to cause obvious signs of opioid-induced sedation (i.e., impaired righting reflex, and lack of escape response to general handling). Importantly however, this dose did not significantly depress breathing. The morphine dose we used also did not decrease the hypoxic ventilatory response, although there was a trend toward a change in breathing pattern. It is possible that morphine doses in the lower region of the recommended dose range would have a less potent effect on AB generation. However, to test the effect of morphine on AB production at lower doses where obvious sedation does not occur, more complicated assessments of the relative degree of analgesia will need to be carefully performed to confirm systemic analgesic effectiveness (14).

Our results indicate that acute administration of morphine in naive rats causes a powerful elimination of ABs, an effect we can say persists over at least 2 h of treatment. It is well-established that other side effects of opioid treatment such as respiratory depression, respiratory instability during sleep, sedation, nausea, dry mouth, and constipation remain a prevalent concern over extended or long-term chronic use (19, 48, 52, 71). Nevertheless, based solely upon our present work it remains uncertain if morphine continues to suppress or eliminate ABs if animals are provided administration of morphine over several hours, or even days to weeks.

In the present study we monitored animals noninvasively using a technique known as whole body open-flow plethysmography. The main advantages of this technique are that fact that it is noninvasive so animals can be studied repeatedly across several days of intervention, and several hours of continuous monitoring at a given time are easily performed. However, plethysmography provides indirect measurements, and as with any form of this monitoring approach important technical limitations on the data should be realized and this has been the topic of extensive study and discussion elsewhere (31, 38, 49, 59). Our animal monitoring provided an indirect index of tidal volume and minute ventilation which we have referred to as estVt and estV˙E, respectively. Our breathing frequency data is equivalent to direct measurement since technical limitations of indirect monitoring apply to the analysis of the amplitude of the signal, not the cycle length. Our count of ABs was likewise robust since these were assessed based upon a ratio vs. the normal eupneic breaths recorded in the same background condition, and not the absolute value of the amplitude signal. Nevertheless, we have empirically validated our plethysmographic determinations of estVt and estV˙E across the physiological range against direct respiratory flow measurements made in the same animals, and we reliably obtain values of estVt and estV˙E that are also very consistent with the accepted range of values reported across a wide survey of literature on rat physiology published over the last several decades (12, 16, 18, 27, 32, 39, 41, 45, 61, 66).

**Implications of results.** Our data suggest that patients receiving opioid therapy for pain management or sedation are unlikely to generate spontaneous deep breaths, and even if they do they will be generated far less frequently than normal. This leaves patients more susceptible to developing atelectasis and related pulmonary complications. This is especially a concern for patients in critical care or postsurgical settings (10). Interestingly, patients receiving opioid treatment systemically are known to be more likely to develop pulmonary complications compared with patients receiving regional opioid-based analgesia (2, 51, 57, 67). Based upon our findings, this difference may be related to the fact that systemic opioid administration suppresses AB production.

We found that a stimulation of the carotid bodies via hypocapnic hypoxia triggered AB production, despite their total disappearance in room air breathing. This finding offers an interesting therapeutic approach toward preventing pulmonary complications that are common in vulnerable ICU and postsurgical patients who may be receiving opioid therapy, complications that may be secondary to or exacerbated by an elimination of spontaneous sigh breaths. Opioid treatment
could be combined with a respiratory stimulant that acts via the peripheral chemoreceptors. Such a pharmacological approach to preventing the opioid-induced elimination of ABs could be coadministered and may be an effective approach to reducing the incidence or severity of pulmonary complications.

Summary. We found that acute opioid administration eliminates augmented breaths in normal room air breathing. However, by stimulating the peripheral respiratory chemoreflex pathways they can be “triggered,” despite the systemic presence of the opioid. There are important implications for this respiratory side effect: the elimination or suppression of augmented breaths may predispose vulnerable subjects to pulmonary complications related to the progressive development of atelectasis. Our results also show that the opioid-induced elimination of ABs cannot be attributed to an effect of general sedation, and that endogenous opioids do not appear to be involved in regulating the production of ABs.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s). .

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