ANESTHESIOLOGY

Severe Hypoxemia Prevents Spontaneous and Naloxone-induced Breathing Recovery after Fentanyl Overdose in Awake and Sedated Rats

Philippe Haouzi, M.D., Ph.D., Daniel Guck, D.O., Marissa McCann, B.S., Molly Sternick, B.S., Takashi Sonobe, Ph.D., Nicole Tubbs, B.S.

ANESTHESIOLOGY 2020; 132:1138–50

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Opioid overdose produces a rapid and profound depression of breathing, which, if not corrected, leads to a terminal hypoxic cardiac arrest
- Severe acute hypoxemia produces a rapid inhibition of respiratory neuronal activity through a nonopioid mechanism

What This Article Tells Us That Is New

- The level of hypoxemia reached during fentanyl-induced apnea in unsedated rats affected their ability to "autoresuscitate" and to respond to naloxone
- Fentanyl-induced apnea in urethane-anesthetized rats was not associated with spontaneous recovery when Pao_2 decreased below approximately 16 mmHg during apnea and could not be reversed with naloxone

ABSTRACT

Background: As severe acute hypoxemia produces a rapid inhibition of the respiratory neuronal activity through a nonopioid mechanism, we have investigated in adult rats the effects of hypoxemia after fentanyl overdose-induced apnea on (1) autoresuscitation and (2) the antidotal effects of naloxone.

Methods: In nonsedated rats, the breath-by-breath ventilatory and pulmonary gas exchange response to fentanyl overdose (300 μg · kg^{-1} · min^{-1} iv in 1 min) was determined in an open flow plethysmograph. The effects of inhaling air (nine rats) or a hypoxic mixture (fractional inspired oxygen tension between 7.3 and 11.3%, eight rats) on the ability to recover a spontaneous breathing rhythm and on the effects of naloxone (2 mg · kg^{-1}) were investigated. In addition, arterial blood gases, arterial blood pressure, ventilation, and pulmonary gas exchange were determined in spontaneously breathing tracheostomized urethane-anesthetized rats in response to (1) fentanyl-induced hypoventilation (7 rats), (2) fentanyl-induced apnea (10 rats) in air and hyperoxia, and (3) isolated anoxic exposure (4 rats). Data are expressed as median and range.

Results: In air-breathing nonsedated rats, fentanyl produced an apnea within 14 s (12 to 29 s). A spontaneous rhythmic activity always resumed after 85.4 s (33 to 141 s) consisting of a persistent low tidal volume and slow frequency rhythmic activity that rescued all animals. Naloxone, 10 min later, immediately restored the baseline level of ventilation. At fractional inspired oxygen tension less than 10%, fentanyl-induced apnea was irreversible despite a transient gasping pattern; the administration of naloxone had no effects. In sedated rats, when Pao_2 reached 16 mmHg during fentanyl-induced apnea, no spontaneous recovery of breathing occurred and naloxone had no rescuing effect, *despite circulation being maintained.*

Conclusions: Hypoxia-induced ventilatory depression during fentanyl induced apnea (1) opposes the spontaneous emergence of a respiratory rhythm, which would have rescued the animals otherwise, and (2) prevents the effects of high dose naloxone.

(ANESTHESIOLOGY 2020; 132:1138–50)

Death by opioid overdose is the direct consequence of a rapid and profound depression in breathing,[1] which, if not very rapidly corrected,[2] leads to a terminal hypoxic cardiac arrest.[3] The substratum of this acute breathing depression is to be found in the combination of different factors. The first mechanism is the direct consequence of the long-described presence of opioid receptors in the aggregates of neurons involved in breathing generation and located in the pons and the medulla.[4–6] These key respiratory neuronal networks, essential for breathing generation, see their rhythmic activity depressed or even abolished in the presence of an opioid agent.[5] Second, large doses of opioids produce tetanic muscle contractions involving the chest wall and the abdominal muscles,[7–10] a phenomenon well described with fentanyl.[10] This muscle rigidity is produced by a central mechanism, likely mediated in the locus coeruleus,[11,12] that has been shown to decrease chest wall compliance. Along with these changes in respiratory mechanics, sustained contractions of the expiratory muscles[7]

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org).

Submitted for publication April 15, 2019. Accepted for publication December 20, 2019. Published online first on February 7, 2020. From the Division of Pulmonary and Critical Care Medicine, Department of Medicine, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania.

 Anesthesiology 2020; 132:1138–50. DOI: 10.1097/ALN.0000000000003156

can oppose inspiratory movements.[8] Finally, laryngeal muscle contractions can lead to a glottis closure,[13,14] adding an obstructive component to an otherwise central apnea.

All of these effects can develop within a few minutes or even seconds in victims of opioid overdose, potentially leading to a terminal hypoxemia and death by pulseless electrical activity or ventricular arrhythmia. The two hypotheses tested in the current study were that the severity of hypoxemia developing during fentanyl-induced apnea will dictate the ability of breathing to spontaneously resume as well as the response to naloxone. Our rationale is that brain stem hypoxia has long been shown to inhibit the medullary respiratory activity[15–17] leading to a central depression of breathing[15,18] in small and large mammals.[16,17,19] As hypoxemia-induced ventilatory depression is a nonopioid-mediated mechanism of breathing inhibition, we hypothesized that if Pa_{O_2} is not restored or maintained above critical levels, *via* spontaneous gasping, ventilatory support, or rapid recovery of a regular respiratory rhythm, apnea-induced hypoxemia could prevent (1) breathing resumption and (2) effectiveness of naloxone. To test these two hypotheses, we have first investigated the outcome of fentanyl-induced apnea in a nonsedated rat model in keeping with the level of hypoxemia. As this nonsedated model did not allow for the monitoring of arterial blood gases and arterial blood pressure, these studies were completed in *spontaneously breathing* urethane-anesthetized rats equipped with an arterial line. The combination of these two models has helped us clarify whether hypoxemia-induced ventilatory depression affects the spontaneous recovery as well as the antidotal effects of naloxone after fentanyl overdose.

Materials and Methods

Animal Preparation

Fifty male Sprague-Dawley rats (Charles River, USA), weighing 411 ± 20 g (11 to 13 weeks), were used in these studies. The number of rats used for each part of the study, *i.e.*, pilot experiments as well as the unsedated and anesthetized protocols, is given in the protocol and is summarized in Supplemental Digital Content figure 1 (http://links.lww.com/ALN/C201). Animals were randomly allocated to any part of the protocol by a person who was not involved in the conduct of the experiments or the analysis of data. Experiments were always performed in the morning or early afternoon from 10:00 AM to 1:00 PM, studying typically two to four rats per session for the unsedated model and one animal only for the anesthetized model (see Protocol section). All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*, 8th Edition (National Research Council [US] Institute for Laboratory Animal Research). The Pennsylvania State University Hershey Medical Center Institutional Animal Care and Use Committee approved the study. The rats were housed at the animal resource services at the Pennsylvania State University College of Medicine (Hershey, Pennsylvania), which conforms to the requirements of the U.S. Department of Agriculture and the U.S. Department of Health, Education and Welfare (Washington, D.C.). The Pennsylvania State University College of Medicine is accredited by the American Association for Assessment and Accreditation of Laboratory Animal Care International (Frederick, Maryland), Animal Welfare Assurance Number A3045-01. Rats were provided with food and water *ad libitum*, on a standard 12-h (7:00 AM to 7:00 PM) light/dark schedule, under the direct supervision of veterinarians.

Fentanyl (Fentanyl Citrate Injection, 1,000 mcg Fentanyl/20 ml [50 mcg · ml^{-1}], Hospira, Inc., USA) and naloxone (Naloxone Hydrochloride Injection, USP 4 mg/10 ml [0.4 mg · ml^{-1}], Somerset Therapeutics, LLC, USA) were used in this study; dilutions, when needed, were always done with sterile saline. Two different animal models were used.

Nonsedated (Awake) Rat Model. The day of study, rats were sedated (3.5% isoflurane) for 15 min to place a heparinized custom-designed double lumen venous catheter in the dorsal vein of the tail. At least 2 h were given for full recovery before doing the study. A custom-designed leakproof acrylic cylinder (internal volume 1.4 l) was used for determination of ventilation and pulmonary gas exchange at various fractional inspired oxygen tension (F_{IO_2}). Gas was delivered through the inlet port of the plethysmographic chamber using a precision rotameter with a flow of approximately 2.5 l · min^{-1}.[20] The outlet port was connected through noncompliant tubing and to a Fleisch 01 pneumotachograph connected to a pressure transducer (Sensym, DCLX O1DN; Honeywell, USA) for the determination of the flow of air through the chamber. The fractions of carbon dioxide and oxygen were determined (model No. 17630 infrared, and No. 17620 fuel cell analyzers, respectively; Vacumed, USA) in the gas entering and leaving the chamber for computation of carbon dioxide production ($\dot{V}_{CO_2}$) and oxygen consumption, as previously described.[20] Temperature in the chamber was also continuously monitored *via* a fast responding thermocouple (Thermalert TH5; Physiotemp, USA). Air could be mixed with nitrogen or replaced by oxygen to create any desired level of F_{IO_2}. In addition, the venous catheter was connected through two extension lines to leakproof adapters, allowing the infusion lines to be connected outside the box. The solution of fentanyl (50 μg · ml^{-1}) was infused intravenously using a high precision infusion pump (Fusion 100; Chemyx Inc, USA).

All of the analog output signals, *i.e.*, gas flow, percent carbon dioxide, percent oxygen, and temperature were fed into a digital data acquisition system (PowerLab/chart system, AD Instruments, USA). Signals were sampled at 400 Hz, displayed online, and then stored for additional analysis. To calculate respiratory variables from the raw flow trace, a high-pass filter (greater than 0.5 Hz) was used to subtract the direct current voltage component. The "filtered" signal

was used for the determination of breathing frequency and to estimate minute ventilation($\dot{V}_E$) as previously described.[20] To obtain an estimate of $\dot{V}_E$ from the filtered flow signal, the positive deflections in the plethysmographic trace were integrated over 5-s intervals. The result of this integration was then temperature-corrected based upon the difference between ambient temperatures in the chamber as determined continuously throughout the experiments. Due to the complexity of factors involved in quantitative interpretation of an open-flow plethysmographic signal,[21–23] $\dot{V}_E$ should be seen as a semiquantitative index represented in the same units as are appropriate for direct measurements of ventilation.

Studies in Urethane-anesthetized Rats. Animals were briefly anesthetized by inhalation of 3 to 5% isoflurane for a few minutes before an intraperitoneal injection of urethane (1.8 g/kg) as previously described. Rats were then tracheostomized, and the tracheostomy was connected to a small dead space two-way valve. Inspiratory flow was measured using a pneumotachograph (Series 1100, Hans Rudolph, Inc., USA). A 7-ml mixing chamber was connected to the expiratory port of the valve, where mixed expiratory gas composition was continuously analyzed (Gemini, CWE Inc., USA). A catheter (PE-50; Fischer, USA) was placed into the right carotid artery to continuously monitor arterial blood pressure and for sampling arterial blood. The arterial catheter was connected to a pressure transducer (MLT844 Pressure Transducer, AD Instruments, USA). Two similar catheters were placed into the left and the right jugular veins for injecting fentanyl and naloxone, respectively.

Body temperature was monitored with a rectal probe (Thermalert TH-5, Physitemp, USA) and maintained around 37°C using a heating pad and lamp. At the end of the experiment, rats were euthanized by a lethal injection of high-dose barbiturate IV (Euthasol; Virbac, USA) into the right heart through the jugular catheter.

Arterial blood pressure, mixed expired oxygen and carbon dioxide fractions, and respiratory flow signals were digitized (PowerLab/chart system) for the computation of minute ventilation, oxygen consumption, and $\dot{V}_{CO_2}$. Blood gases and lactate levels were measured using an i-STAT1 blood gas analyzer (ABAXIS, USA). All signals were visualized online. Data were stored for further analysis using LabChart7.

Protocol

Fentanyl-induced Ventilatory Depression and Effects of Naloxone in Awake Rats. Preliminary data were collected in six rats using 50, 100, and 300 $\mu g \cdot kg^{-1}$ of fentanyl over 1 min to determine the maximal dose of fentanyl that would produce an immediate, spontaneously reversible apnea. The selected dose was applied to 17 rats. Nine rats were exposed to air, while eight rats were exposed to a hypoxic mixture ranging from 7.3 to 11.3%. Infusion of fentanyl was maintained for 1 min. The time to apnea and the duration of apnea were determined. For all the animals that recovered a rhythmic activity, naloxone ($2 mg \cdot kg^{-1}$) was administered at 10 min. Whenever an animal was unable to recover a rhythmic activity, naloxone was administered within 1 min after the median recovery time of air-breathing animals.

Fentanyl-induced Ventilatory Depression and Effects of Naloxone in Sedated Rats. Since sedation can alter the response to fentanyl, we first established the dose and rate of fentanyl able to produce an apnea within 10 min in urethane-sedated rats. Our objective was (1) to produce a model that would lead to an apnea after a period of hypoventilation, allowing a low level of Pa_{O_2} to develop at the time of apnea; and (2) to evaluate in this model the effect of hyperoxia.

Seventeen rats were studied in this protocol. Based on the pilot experiments in seven rats (see Results section), the infusion of fentanyl at the rate 2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was maintained until minute ventilation would decrease by half to evaluate the effects of naloxone in nonapneic animals. In the other 10 rats, fentanyl was maintained at the same rate (2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) until an apnea occurred. The inspiratory port of the tracheal valve was connected to a bag containing either air (five rats) or 100% O_2 (five rats); hyperoxia was administered before—and maintained throughout—fentanyl exposure. Arterial blood gases were sampled before fentanyl administration, then within 15 s into the apneic period or during the phase of hypoventilation (for low rate infusion), and just before naloxone administration. Also, arterial blood pressure, which was continuously monitored, was determined at the time of naloxone administration. Whenever naloxone was able to restore eupneic breathing, high-dose fentanyl infusion (25 to 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$, *i.e.*, 10 to 20 times the dose used to produce a depressive effect) was infused again 15 min after naloxone as a proof of its efficacy.

Hypoxia-induced Ventilator Depression. Four rats were equipped with an additional arterial catheter placed in one of the femoral arteries, allowing blood sampling without interrupting the recording of mean arterial blood pressure. The left femoral artery and right carotid artery were also exposed and isolated from the vein, nerve, and surrounding tissues, and a transonic flow probe (MA1PRB, Transonic Systems Inc., USA) was placed around both vessels. The transonic flow probes were connected to a perivascular flowmeter (TS420, Transonic Systems Inc., USA). The inspiratory port of the valve was connected to a bag containing different concentrations of a mixture of air and nitrogen. The animals were exposed to this anoxic mixture until breathing stopped, and an arterial blood gas was sampled within 15 s into apnea. Animals were then mechanically ventilated to prevent a cardiac arrest and to allow breathing to resume. Negative inflections in the tracheal pressure signals were considered as spontaneous breaths.

Data Analysis

As our data are not expected to be normally distributed, they are presented as median and range, and nonparametric

tests were used for statistical analyses. These analyses were conducted using GraphPad Prism 5 (Graphpad Software, USA). Statistical power calculation was conducted before the study. In the unsedated model, anticipating that in air conditions all animals will be responding to naloxone and will display a spontaneous recovery of rhythmic breathing, if 50 to 60% of the animals exposed to a hypoxic mixture are able to recover a spontaneous rhythm or respond to naloxone, a sample of 8 to 11 per group would be required, with a power of 90% and a significance level of 5%. Similarly, assuming that all the sedated animals presenting an apnea after a period of hypoventilation will not respond to naloxone and that hyperoxia may have a rescuing effect in 70 to 80% of animals, four to six animals in each group would be required to identify a statistical difference.

In unsedated animals, our primary outcomes were the onset and duration of apnea (comparison by Mann–Whitney U test). The percentage of animals recovering a regular rhythmic pattern spontaneously and after naloxone was compared between hypoxemic and air-breathing animals using a Fisher's exact test. In addition, minute ventilation ($\dot{V}_E$) and $\dot{V}_E/\dot{V}_{CO_2}$ were compared at baseline, 10 min after fentanyl infusion (before naloxone injection), and 1 min after naloxone administration within each group using a Friedman test. A Wilcoxon matched-pairs signed rank test was used to compare any of these specific time points, if needed. Comparison between groups was done using a Kruskal–Wallis test for multiple comparison and a Mann Whitney U test for two group comparisons. In sedated animals, the percentage of animals recovering a regular rhythmic pattern spontaneously and after naloxone between the control group and the hyperoxic group was our primary outcome (Fisher's exact test). In addition, the ventilatory parameters, blood gas values, and arterial blood pressure were compared using a Friedman and Wilcoxon matched-pairs signed rank test for comparison within each group; a Mann Whitney U test was used for comparisons between groups. $P < 0.05$ was regarded as significant for any of these comparisons that were performed as two-tailed tests.

Results

Studies in Unsedated Animals

Fentanyl Overdose in Air-breathing Unsedated Rats. The selection of the dose of fentanyl was done in a series of pilot experiments (six rats) using a dose of fentanyl of 50, 100, or 300 μg · kg^{-1} administered over 1 min—assuming that a dose of 80 μg · kg^{-1} could produce a lethal apnea.[24] We found that 50 μg · kg^{-1} fentanyl infusion produced a rapid apnea that was followed by a spontaneous recovery of a slow rhythmic pattern. A similar apneic response was produced at the dose of 100 and 300 μg · kg^{-1} that was always followed by a spontaneous recovery, as described below in more detail for the highest dose of fentanyl infusion (300 μg · kg^{-1}) that was chosen for our study.

The response to fentanyl infusion (300 μg · kg^{-1} infused in 1 min) in air was analyzed in nine rats. An example is displayed in figure 1. All of the animals presented a cessation of breathing at 14 s (median, ranging from 12 to 29 s) after the onset of fentanyl infusion (fig. 2) preceded by an abrupt coma. This apnea was associated with the development of generalized muscle rigidity, which affected the axial muscles (tail, abdominal, chest, back, and neck muscles) followed by a tonic extension of the hindlimbs along with jerking and clear expiratory movements. Every animal spontaneously recovered from this central apnea (median 85 s, ranging from 33 to 141 s) after fentanyl injection (fig. 2). This recovery consisted of a slow reappearance of breaths with reduced tidal volume (no gasping pattern was noted), initially irregular but rapidly becoming regular (fig. 1). As displayed in figure 2, where all individual data along with their median are shown, the level of ventilation emerging after the apnea was very low (median 78 *vs.* 314 ml · min^{-1} for baseline; $P = 0.004$, Wilcoxon matched-pairs signed rank test) and remained reduced (one third of the baseline value) for the 10-min period during which breathing was recorded. This hypoventilation was associated with a significant decrease in pulmonary gas exchange ($\dot{V}_{CO_2}$) from 16.1 ml · min^{-1} (median at baseline) to 8.9 ml · min^{-1} (10 min into recovery, $P = 0.004$, Wilcoxon matched-pairs signed rank test). The depression in breathing was, however, out of proportion to the change in metabolism, with a $\dot{V}_E/\dot{V}_{CO_2}$ ratio that was significantly decreased (fig. 2). Administration of naloxone (2 mg · kg^{-1}) increased ventilation immediately (fig. 1) to a median of 513 ml · min^{-1} ($P = 0.004$ when compared to preinjection value, fig. 2). All animals recovered with no clinical neurologic deficits.

Opioid Overdose in Hypoxia. Eight rats were studied in hypoxia (F_{IO_2} ranging from 7.4 to 11.3%). As illustrated in figures 1 and 2, breathing a hypoxic mixture led to an increase in median baseline minute ventilation to 456 ml · min^{-1} ($P = 0.004$, Mann Whitney U test) when compared to air breathing. Yet fentanyl injection still produced a central apnea with the same delay (median 15 s, ranging from 11 to 19 s) as in the air-breathing animals (fig. 2, Mann–Whitney test $P = 0.743$). However, the recovery from apnea was affected as none of the hypoxic rats, except the one rat exposed to 11.3% O_2 (figs. 1 and 2), recovered a spontaneous rhythmic eupneic activity, nor did they respond to naloxone (fig. 2). Overall, only the rat that was exposed to a hypoxic mixture above 10% (one out of eight rats) recovered and was responsive to naloxone *vs.* all (nine out of nine) in air breathing (Fisher's exact test, $P < 0.001$). Of note, in some animals, a pulse pressure signal was still observed during and after naloxone injection on the respiratory flow signal. However, since the pulse pressure signal superimposed on the flow signal was inconsistently observed even in air-breathing animals, the circulatory status at the time of naloxone injection could not be determined in this model. Blood gases and arterial blood pressure

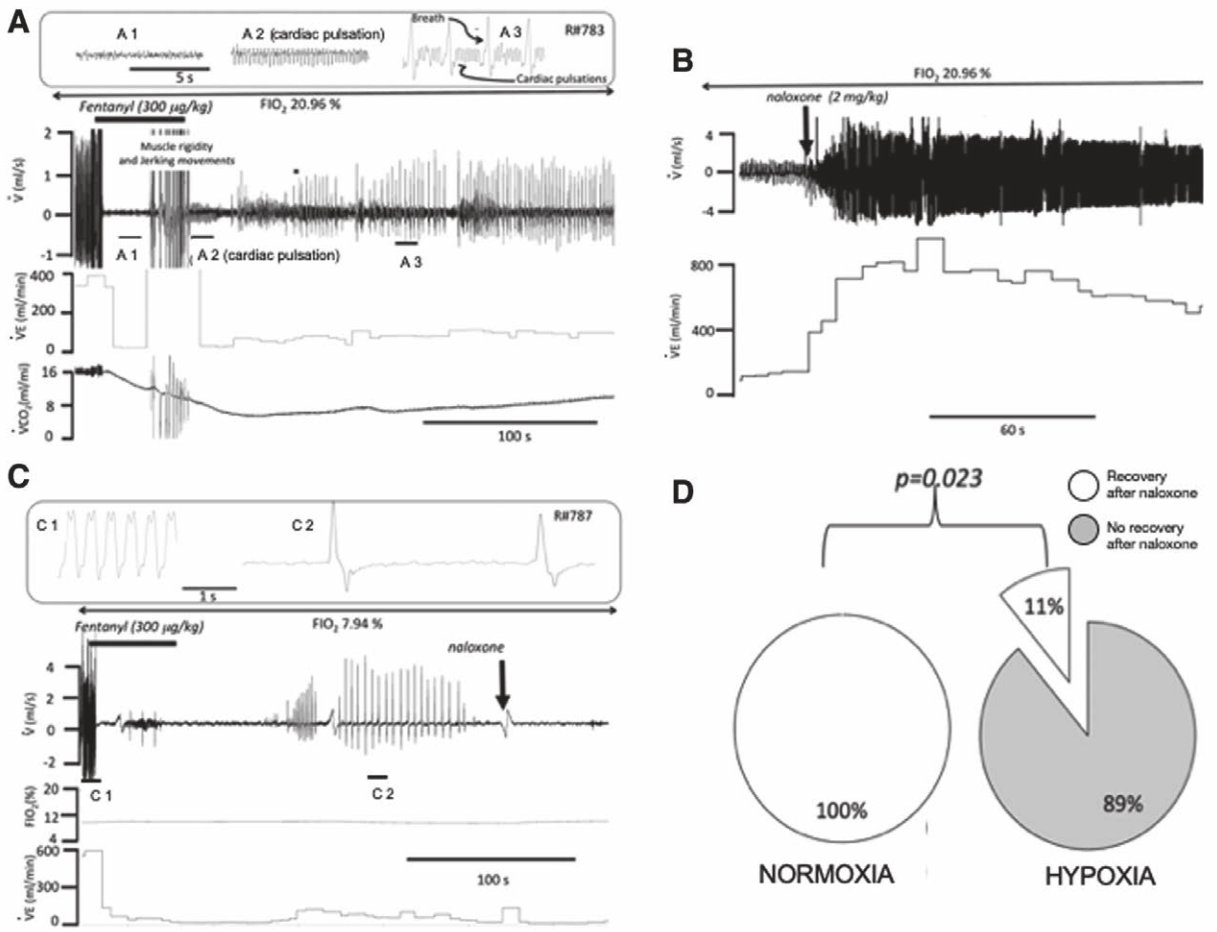

Fig. 1. Effect of hypoxia on fentanyl induced apnea and recovery in unsedated rats. (*A*) Example, in one unsedated rat, of the effects of a bolus injection of fentanyl (300 µg · kg^{-1} · min^{-1} over 1 min) on instantaneous respiratory flow ($\dot{V}$), minute ventilation ($\dot{V}_E$), and carbon dioxide output ($\dot{V}_{CO_2}$). Measurements were performed in an open-flow whole body plethysmograph. A central apnea occurred almost immediately, associated with profound muscle rigidity. *A1* and *A2* display a magnification of the flow signal during the period of apnea. Note the artifact created by arterial pulsation in *A2* that was intermittently observed in only a few rats. Ventilation resumes spontaneously with a very slow frequency and low tidal volume, as shown in *A3*, rescuing the animal. (*B*) Effects of a bolus injection of naloxone (2 mg · kg^{-1}) on the hypoventilation produced by fentanyl overdose (10 min after the administration of fentanyl) in the rat displayed in (*A*). $\dot{V}$ and $\dot{V}_E$ are shown. Note the immediate (within seconds) increase in $\dot{V}_E$ after naloxone. (*C*) Example of the effects of an injection of fentanyl (300 µg · kg^{-1} · min^{-1} over 1 min) on $\dot{V}$, $\dot{V}_E$ and $\dot{V}_{CO_2}$, while breathing 7.8% O_2. In contrast to air breathing animals (*A*), few gasps were observed during apnea, but ventilation never spontaneously resumed. A magnification of the flow signal before fentanyl infusion and during the period of gasping is shown in the inset (*C1* and *C2*). Naloxone was administered less than 1 min after the gasping pattern and had no effect on breathing. (*D*) Incidence of animals responding to naloxone after fentanyl overdose–induced apnea (the *P* value for comparison using a Fisher's exact test is shown). FIO_2, fractional inspired oxygen tension.

were therefore measured in a sedated model, as presented in the following paragraphs.

Studies in Urethane-anesthetized Animals

Selection of the Dose of Fentanyl and Naloxone in Sedated Rats. As mentioned in the Methods section, the objective of this protocol was to allow a period of hypoventilation to precede the apnea and thus hypoxemia to be present at the time the apnea occurs. In a series of pilot experiments, doses ranging from 2.5 to 50 µg · kg^{-1} · min^{-1} were infused in six rats to determine the rate of fentanyl infusion that would produce a complete central apnea within 10 min after a period of hypoventilation. We found that infusing fentanyl at a rate of 2.5 µg · kg^{-1} · min^{-1} for 3 to 5 min was able to depress breathing by half and would stop breathing when infused at the same rate for 10 min. These doses and rates were selected for our protocol to produce either a reversible hypoventilation or an apnea after a period of

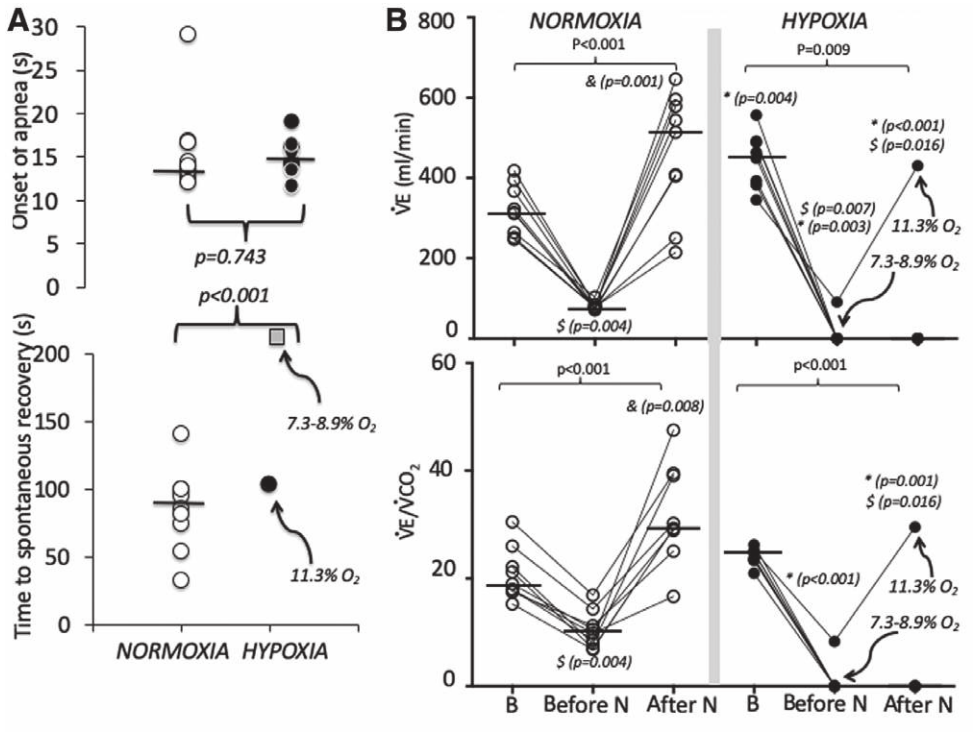

Fig. 2. Effects of hypoxia on the effects of fentanyl induced apnea and recovery in unsedated rats. (*A*) Time to the onset of apnea after fentanyl infusion (*upper*) and time to spontaneous recovery (*right*) in normoxia (*open symbols*) and hypoxia (*black symbols*). Individual data and median are shown. Hypoxemia did not affect the time to apnea but prevented all animals but one (exposed to 11.3%) to spontaneously recover (*P* values are shown for comparison using a Mann–Whitney U test). All rats exposed to fractional inspired oxygen tension less than 10% were unable to recover (*gray square*). (*B*) Minute ventilation ($\dot{V}_E$) and V̇E/carbon dioxide output ($\dot{V}_{CO_2}$) ratio (individual data and median) are displayed in baseline condition (*B*), 30 s before naloxone injection (Before N) and 1 min after naloxone (after N). The *P* values for comparison using a Friedman test are shown with no symbol. In addition, individual comparisons within groups were performed using a Wilcoxon matched-pairs signed rank test ($, comparison to baseline). Comparisons between groups were performed using a Kruskal–Wallis tests or Mann–Whitney U tests (*, comparison between air and hypoxia at the same time point).

hypoventilation. As soon as an apnea occurred, fentanyl infusion was stopped. Also, three doses of naloxone, *i.e.*, 0.4, 1, and 2 mg · kg^{-1}, were investigated. Our aim was to use a dose of naloxone that will be able to *prevent* any depression in minute ventilation after a dose of fentanyl that was 10 to 20 times the dose producing an apnea. Naloxone at the dose of 2 mg · kg^{-1} prevented any new ventilatory depression even when very high doses of fentanyl infusion were used.

Effects of Naloxone on Fentanyl-induced Ventilatory Depression in Sedated Rats. Seven rats received an infusion of fentanyl at the rate of 2.5 μg · kg^{-1} · min^{-1}, which was maintained until minute ventilation decreased by half (fig. 3). This took two periods of 52 to 511 s (median 184 s) of infusion each separated by 15 min to develop (fig. 4). More specifically, after the first injection, ventilation returned toward baseline, while a second fentanyl infusion produced a more sustained breathing inhibition (figs. 3 and 4). This depression in breathing was associated with a decrease in oxygen uptake ($P = 0.003$) and $\dot{V}_{CO_2}$ ($P = 0.001$). As displayed in figure 5, statistically significant hypercapnia and hypoxemia developed, but with no systematic changes in mean arterial blood pressure. A bolus injection of naloxone (2 mg · kg^{-1}) restored ventilation in every instance (figs. 4 and 5). The effects of naloxone were immediate, occurring no more than a few seconds after a bolus administration. This was associated with a recovery of $Paco_2$ and Pao_2 (fig. 5). Whenever a new infusion of fentanyl was initiated at the rate of 50 μg · kg^{-1} · min^{-1} (20 times the rate of fentanyl infusion producing a breathing inhibition before naloxone administration), no further depressive effects on breathing could be observed, as illustrated in figure 3.

Effects of Naloxone on Fentanyl-induced Apnea in Air. Five rats were studied while breathing air during and after an infusion of fentanyl (2.5 μg · kg^{-1} · min^{-1}), which was maintained until an apnea occurred (fig. 4). At this rate, a complete cessation of breathing movements was produced at 9.2 min (time to apnea ranged from 3.4 to 15.1 min). Of note, in two out of five rats, a typical gasping pattern was observed before the animals went apneic. As displayed in figure 4, the

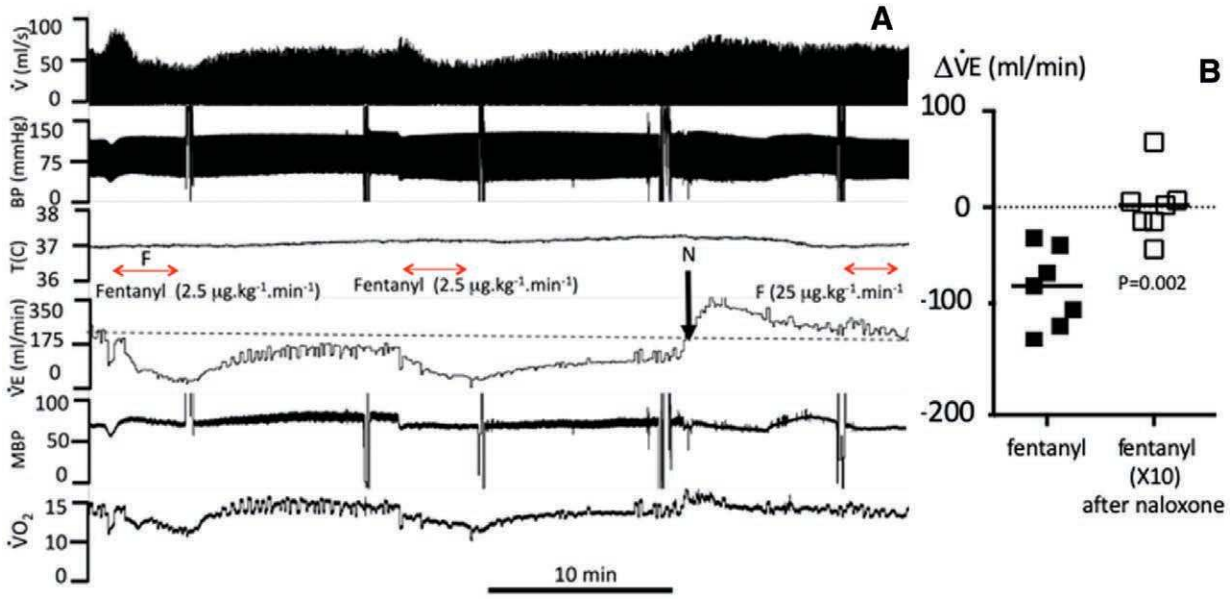

Fig. 3. Effects of low dose fentanyl and naloxone in sedated rats. (*A*) Example of the effects of two infusions of fentanyl (2.5 µg · kg^{-1} · min^{-1}) aimed at decreasing minute ventilation by half and the effect of naloxone (2 mg · kg^{-1}) in one urethane-sedated rat. From *top* to *bottom*, instantaneous inspiratory flow ($\dot{V}$), carotid arterial blood pressure (BP), body temperature (T), minute ventilation ($\dot{V}_E$), mean arterial blood pressure (MBP), and oxygen uptake ($\dot{V}_{O_2}$) are displayed. Note that naloxone antagonizes the depression induced by fentanyl and prevents the ventilatory effect produced by a dose of fentanyl 10 times higher (25 µg · kg^{-1} · min^{-1}). (*B*) Change in minute ventilation (individual data and median) produced by fentanyl (second infusion) before and after naloxone (using a dose of fentanyl 10 to 20 times higher). The *P* value is shown for comparison using a Wilcoxon matched-pairs signed rank test.

cessation of breathing was associated with a statistically significant reduction in mean arterial blood pressure. The most notable changes during the apnea consisted of a reduction in Pa_{O_2} from 79 (range 70 to 95) to 11 (range 6 to 16) mmHg. Naloxone was administered 45 s into apnea, but was unable to rescue any of the animals. Of note, naloxone was administered while a clear pulse pressure was still present on the arterial blood pressure signal (fig. 4). Apnea persisted and led to a cardiac arrest in every animal.

Effects of Naloxone on Fentanyl-induced Apnea in Hyperoxia. Exposing five animals to 100% O_2 during fentanyl infusion (figs. 4 and 5) produced a progressive depression in breathing leading to an apnea that occurred at a time (median 13.1 min, ranging from 4.1 to 16.1 min) that was not different from air breathing. However, all the animals maintained their Pa_{O_2} well above 100 mmHg at the time of apnea with no difference in Pa_{CO_2} between air and hyperoxia (fig. 5). The animals were all rescued within a few seconds after naloxone administration (100% recovery *vs.* 0% in air breathing, $P = 0.008$, Fisher's exact test, two-tailed). Also, arterial blood pressure was maintained around baseline levels at the moment of apnea (fig. 4).

Effects of Isolated Anoxia. In five rats, breathing nitrogen for 15 s led to an increase in minute ventilation, which rapidly decreased before abruptly stopping, after the generation of several large breaths, as shown in figure 6. Pa_{O_2} determined at the onset of apnea revealed that Pa_{O_2} reached 10 (8 to 12) mmHg at the time of apnea. No spontaneous recovery of breathing occurred. Only when mechanical ventilation was initiated did breathing resume; spontaneous breaths were generated within 10 to 60 s of ventilatory support. Spontaneous ventilation remained sustained thereafter.

Discussion

We found that, in unsedated animals, the level of hypoxemia reached during fentanyl-induced apnea dictates the ability of rats to "autoresuscitate" and to respond to naloxone. In addition, fentanyl-induced apnea in urethane-anesthetized animals was not associated with spontaneous recovery when Pa_{O_2} decreased below approximately 16 mmHg during apnea. We could also confirm with this model that the resistance to naloxone is a direct consequence of the effects of hypoxemia (fig. 4).

Limitations of the Study: Doses of Fentanyl, Awake *versus* Sedated Rat Models

The correspondence between the doses of fentanyl used in rats and those responsible for an opioid overdose in an adult human is difficult to establish. According to the recommendations for conversion of doses (from mg · kg^{-1} to mg · m^{-2}),[25] a five times larger coefficient factor should be used to convert

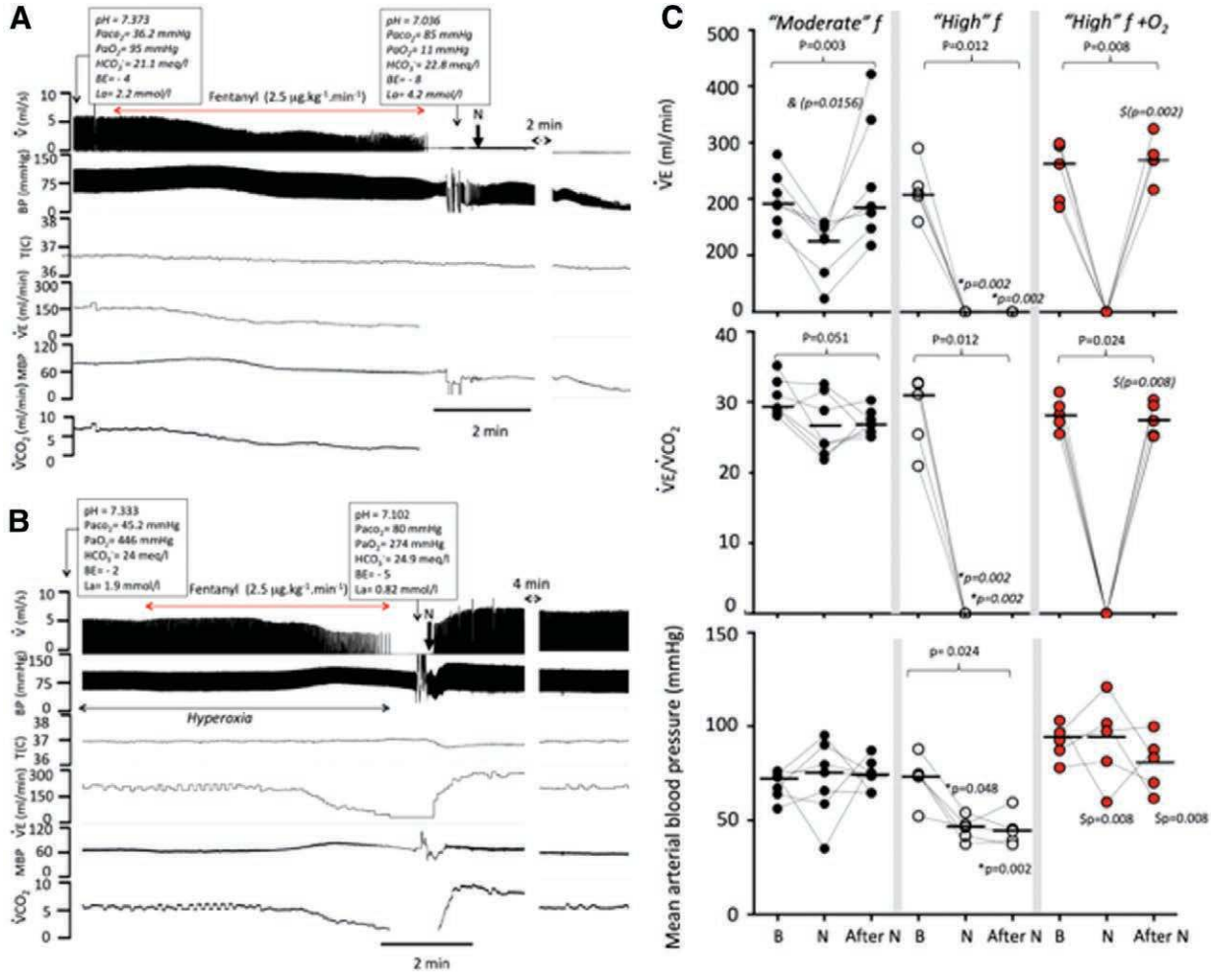

Fig. 4. Effects of oxygen supplement on the recovery from fentanyl-induced breathing depression. (*A*) Example in one urethane-sedated rat of the effects of an infusion of fentanyl (2.5 µg · kg^{-1} · min^{-1}) maintained until an apnea is produced followed by the injection of naloxone (N, 2 mg · kg^{-1}) that was administered while the animal still displayed a significant pulse pressure and sustained mean blood pressure. From top to bottom, instantaneous inspiratory flow ($\dot{V}$), carotid arterial blood pressure (BP), body temperature (T), minute ventilation ($\dot{V}_E$), mean arterial blood pressure (MBP), and carbon dioxide output ($\dot{V}_{CO_2}$) are displayed. The values of arterial pH, Pa_{O_2}, Pa_{CO_2}, bicarbonate (HCO_3^-), base excess (BE), and lactate are also displayed. Note that (1) naloxone could not rescue the apnea produced by fentanyl; (2) a severe hypoxemia was present at the time of naloxone administration; and (3) the evolution was fatal despite a persistent arterial blood pressure signal for several minutes after naloxone injection. (*B*) Example of the effects of an infusion of fentanyl (2.5 µg · kg^{-1} · min^{-1}) aimed at producing an apnea in a urethane-sedated rat exposed to hyperoxia. As in (*A*), naloxone (2 mg · kg^{-1}) was administered within seconds after the onset of the apnea. Same legend as in (*A*). Note that naloxone rescued the apnea produced by fentanyl. (*C*) $\dot{V}_E$, $\dot{V}_E$ / $\dot{V}_{CO_2}$ ratio, and mean blood pressure (individual data and median) are displayed in baseline condition (*B*), 30 s before naloxone injection (before N) and 1 min after naloxone (after N) during moderate fentanyl exposure (infusion maintained until minute ventilation decreased by half), or high level of exposure (infusion maintained until apnea) in air or hyperoxia. The *P* values of all significant differences are shown. The *P* values that are displayed with no symbols were obtained using the Friedman test (comparison within each group). Comparisons between groups were performed using a Kruskal–Wallis test followed by a Mann–Whitney U test for comparison between groups (&, comparison between high and low dose fentanyl in air at the same time point; $, comparison between air and hyperoxia at the same time point; *, comparison between air and hyperoxia at the same time point).

doses from an adult human to a 400-g rodent. Accordingly, 300 µg · kg^{-1} in an adult rat should correspond to a dose of 60 µg · kg^{-1} in a human, while 2.5 µg · kg^{-1} · min^{-1} for 10 min required to lead to apnea in the sedated model would correspond to a total dose of 0.5 µg · kg^{-1} · min^{-1}. Similarly, the dose of naloxone that was used in our study (2 mg · kg^{-1}) corresponds to a dose several-fold higher than the maximal doses recommended in adult humans[2] even after correcting for the difference in body size. It is the view of the authors that the doses of fentanyl used in this study

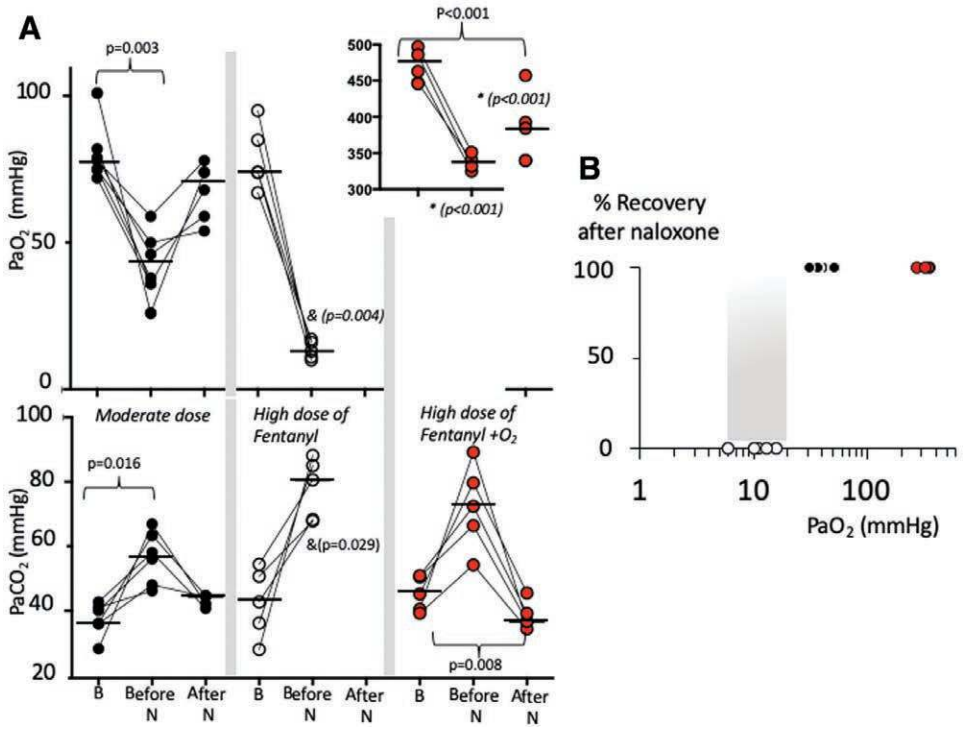

Fig. 5. Effects of fentanyl and naloxone as a function of Pa_{O_2} in sedated rats. (*A*) Pa_{O_2} and Pa_{CO_2} (individual data and median) are displayed in baseline condition (*B*), before naloxone injection (before N) and 1 min after naloxone (after N) during moderate fentanyl exposure (infusion maintained until minute ventilation decreased by half) or high level of exposure (infusion maintained until apnea) in air or hyperoxia. The *P* values of all significant differences are shown. The *P* values that are displayed with no symbols were obtained using Friedman test (comparison within each group). Comparisons between groups were performed using a Kruskal–Wallis test followed by a Mann–Whitney U test for comparison between groups (&, comparison between low and high dose fentanyl in air at the same time point;*, comparison between air and hyperoxia at the same time point). (*B*) Outcome of the effects of naloxone (% of animal rescued by naloxone) as a function of Pa_{O_2} in urethane sedated rats after moderate fentanyl exposure (infusion was maintained until minute ventilation decreased by half, *closed symbols*), or high level of exposure (infusion maintained until apnea) in air (*open symbols*) or hyperoxia (*red symbols*). Note that naloxone rescued all animals that were hypoventilating (moderate level of fentanyl exposure) or those in apnea (high levels of fentanyl exposure) in the hyperoxic condition. All animals that presented an apnea with a Pa_{O_2} less than 16 mmHg, but still with an effective circulation, were resistant to naloxone.

correspond to doses that would lead to a potentially lethal overdose in humans. These doses were also higher than those used by Ren *et al.*,[24] who found that 80 μg · kg^{-1} infused over 1 min was lethal in the group of five rats that he studied. We do not have a clear explanation of why 80 μg · kg^{-1} would always cause a fatal apnea, while in our hands, fentanyl at the dose of 300 μg · kg^{-1} was unable to produce a fatal apnea in most animals. This dose of 300 μg · kg^{-1} was well above the dose required to produce an apnea as established in our pilot experiments. Whether the presence of abdominal contractions during the apnea (fig. 1) when doses of 300 μg · kg^{-1} could have mobilized large lung volumes below the functional residual capacity, could have limited the severity of the hypoxemia during the period of apnea, when compared to the lower dosage,[24] remains to be investigated.

Since the unsedated model was not equipped with an arterial line, the determination of the circulatory status or the severity of hypoxemia at the time of naloxone injection was not possible to establish. The use of the anesthetized model showed that an effective circulation was still present in the hypoxic animals that did not respond to naloxone.

Important differences exist between our unsedated and anesthetized animal models that must be acknowledged.[26] First, in keeping with the difference of doses that depressed breathing in the two models, urethane-anesthetized rats appear to be much more vulnerable to the depressing effects of fentanyl than the awake model. The second difference was the lack of visible muscle rigidity in the urethane-anesthetized rats, while major tonic muscle contractions/contractures involving the back and tail muscles, as well as the abdominal and chest wall muscles, were observed in every awake animal. Similar observations were made by Lui *et al.*[9] in rats anesthetized with thiopental, in which opioid-induced rigidity was absent. In contrast, ketamine anesthesia seemed to preserve fentanyl-induced muscle contractures.[9] The study of the role of muscle rigidity in the ventilatory

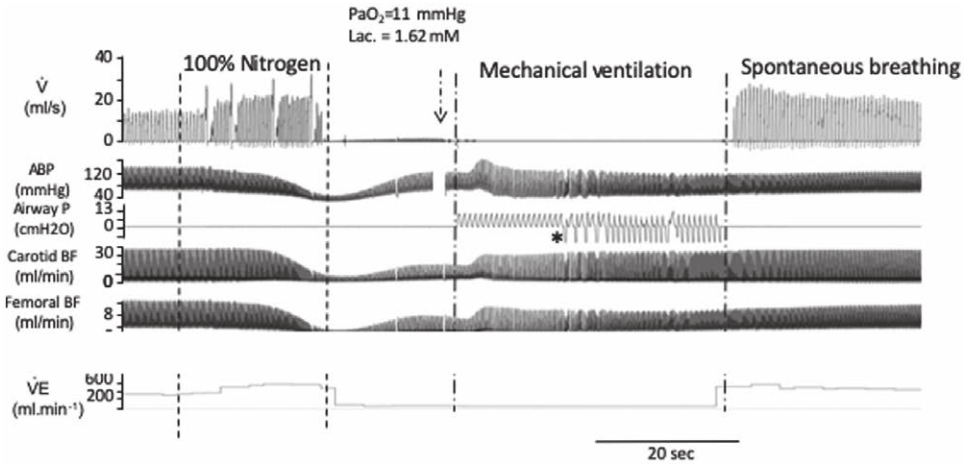

Fig. 6. Example in one rat of the effects of breathing an anoxic mixture. From top to bottom, instantaneous inspiratory flow ($\dot{V}$), carotid arterial blood pressure (ABP), airway pressure (P), carotid and femoral blood flow (BF), and minute ventilation ($\dot{V}_E$) are shown. Anoxic exposure led to a central apnea within 20 s. The off-effects were very rapid after mechanical ventilation, allowing breathing to resume, as shown by the negative inflection of the tracheal pressure signal (*inspiratory effort). Lac., lactate.

response to fentanyl is beyond the scope of the current investigation, but chest wall and abdominal muscle rigidity has been suggested to contribute to the severity of the breathing depression produced by fentanyl.[8] Of note, the dramatic decrease in the $\dot{V}_E/\dot{V}_{CO_2}$ ratio, after fentanyl intoxication in both models, reflected the development of a primary depression in breathing occurring independently of the reduction in metabolism typically produced by hypoxemia in small-sized mammals.[22,27]

Hypoxia-induced Ventilatory Depression during and after Fentanyl-induced Apnea

Hypoxia-induced apnea relies on a complex set of mechanisms, which have long been investigated.[15,18,28–30] Indeed, the stimulation of breathing through the arterial chemoreflex produced by hypoxemia[31–34] is counteracted by the depression of the central pattern generator of breathing when a profound reduction in Pa_{O_2} is present, as illustrated in figure 6. Although breathing inhibition by hypoxia has been extensively studied in the fetal and neonatal period,[35] this effect remains potent in adults during anoxic as well as ischemic insults.[16,19] In adult sedated sheep,[19] we found that hypoxia-induced apnea was produced at Pa_{O_2} averaging 15 ± 1 mmHg, while values of 13 ± 2 mmHg were observed at the onset of the first gasp. Similarly, in adult awake and chloralose-anesthetized dogs,[16] ventilatory depression develops as soon as Pa_{O_2} reaches 19 mmHg with no influence of Pa_{CO_2}. These studies show that in adult large mammals, hypoxic ventilatory depression is produced at Pa_{O_2} values that were in ranges similar to those found in our current study. While fentanyl-suppressed hypercapnia induced breathing stimulation,[36] hypoxia when reaching values less than 16 mmHg significantly added its depressive effects to those produced by fentanyl, but through a nonopioid mechanism of ventilatory inhibition. Indeed, the on-off depressive effects of hypoxia were very rapid (fig. 6). It has been shown that this depressive effect could be mediated *via* changes in ion channel activity–induced neuronal hyperpolarization[30] and modification in neurotransmitter release[35] leading to a disturbance of synaptic interaction within the respiratory neuronal network. These effects are not reversed by naloxone.[28] Yet some of these effects are similar to those produced by opioids,[37–39] at least in their mechanisms of transduction, such as an increase in potassium permeability. This mechanism of action has long been supported by the "antidotal effects" of blockers of potassium channels, like doxapram, or phosphodiesterase-4 inhibitors, like caffeine or aminophylline, that increase cyclic adenosine monophosphate against opioid induced respiratory depression (see review[40]). Interestingly, some of these agents have also been suggested to oppose hypoxia-induced ventilatory depression.[18,28–30,41–43] Regardless of the mechanisms involved, our current data support the view that hypoxia is responsible for a resistance to naloxone after fentanyl-induced apnea when low Pa_{O_2} is present.

Autoresuscitation from Fentanyl-induced Apnea

The mechanisms leading to breathing recovery at such a high dose of fentanyl are not clear. It was prevented when critical low levels of Pa_{O_2} were reached. Whether the slow frequency and low tidal volume respiration observed after an apnea produced by fentanyl are due to a rapid desensitization of opioid receptors in respiratory neurons previously inhibited by fentanyl or a reconfiguration of the central pattern generators using new neuronal circuits "composed" of cells resistant to opioids[1] certainly remains an outstanding question.

The Effects of Circulation

Severe hypoxemia alone produced a decrease in arterial blood pressure associated with a decrease of carotid as well as femoral blood flow in our urethane-sedated rats, as previously reported.[44] Similarly, when fentanyl was infused with a "slow" rate allowing hypoventilation to develop over several minutes before an apnea occurred, arterial blood pressure fell. Yet at the time of naloxone injection, a significant pulse pressure was still present, while mean arterial blood pressure was reduced by only approximately 28 mmHg (fig. 4). A reduction in medullary blood flow can *stimulate* brainstem neurons through a local acidosis. A more severe acute reduction in brainstem blood supply, like during a cardiac arrest, for instance,[19] opposes such a stimulation *via* local brainstem ischemia/anoxia. In the context of a fentanyl overdose, any local hypoxia/ischemia resulting from brainstem hypoperfusion[44] could have amplified the depression in neuronal activity produced by a reduction in Pa_{O_2}. Although we cannot rule out that, in some unsedated animals, a hypoxic depression in cardiac contractility or even a rapidly developing pulseless electrical activity could have prevented the effects of naloxone, the observation that an effective circulation (fig. 4) was still present when naloxone was administered, in the sedated model, suggests that the lack of response to naloxone cannot be explained by its inability to reach the medulla and/or that animals were already in cardiac arrest. Whether high doses of naloxone, higher than 2 mg · kg^{-1}, could have resulted in a different outcome was not investigated.

Clinical Implications

Programs of naloxone distribution aimed at providing naloxone kits to the general population have been developed, since a rapid administration of naloxone (intranasal or intravenous) in a prehospital setting is the only effective treatment able to rescue victims found unconscious and in respiratory failure.[45,46] Our current findings support the view that if no ventilatory support[2] is provided before naloxone administration, this antidote may be ineffective (or less effective). As there is currently no specific antagonist/antidote that could either promote gasping or counteract the acute effects of hypoxia-induced neuronal depression, lay bystanders, who have free access to naloxone, should always provide ventilatory support before administering naloxone[2] in victims of opioid overdose[45,46] with life-threatening breathing depression.

In conclusion, the outcome of fentanyl overdose–induced apnea is dictated by the capacity of a slow but effective spontaneous pattern of breathing to emerge. This new pattern develops in less than 2 min. If Pa_{O_2} reached critically low levels, this rescuing pattern of breathing cannot be produced and is replaced by few gasps that appear unable to produce an autoresuscitation. The response to naloxone was also inhibited, and the outcome was always lethal.

Research Support

Start-up funds were provided by the Department of Medicine, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Haouzi: Division of Pulmonary and Critical Care Medicine, Department of Medicine, Pennsylvania State University, College of Medicine, 500 University Drive, H041, Hershey, Pennsylvania 17033. phaouzi@pennstatehealth.psu.edu.

References

1. Pattinson KT: Opioids and the control of respiration. Br J Anaesth 2008; 100:747–58
2. Boyer EW: Management of opioid analgesic overdose. N Engl J Med 2012; 367:146–55
3. Sakhuja A, Sztajnkrycer M, Vallabhajosyula S, Cheung pasitporn W, Patch R 3rd, Jentzer J: National trends and outcomes of cardiac arrest in opioid overdose. Resuscitation 2017; 121:84–9
4. Montandon G, Horner R: CrossTalk proposal: The preBotzinger complex is essential for the respiratory depression following systemic administration of opioid analgesics. J Physiol 2014; 592:1159–62
5. Mellen NM, Janczewski WA, Bocchiaro CM, Feldman JL: Opioid-induced quantal slowing reveals dual networks for respiratory rhythm generation. Neuron 2003; 37:821–6
6. Lalley PM: Mu-opioid receptor agonist effects on medullary respiratory neurons in the cat: Evidence for involvement in certain types of ventilatory disturbances. Am J Physiol Regul Integr Comp Physiol 2003; 285:R1287–304
7. Drummond GB, Duncan MK: Abdominal pressure during laparoscopy: Effects of fentanyl. Br J Anaesth 2002; 88:384–8
8. Burns G, DeRienz RT, Baker DD, Casavant M, Spiller HA: Could chest wall rigidity be a factor in rapid death from illicit fentanyl abuse? Clin Toxicol (Phila) 2016; 54:420–3
9. Lui PW, Lee TY, Chan SH: Fentanyl-induced muscle rigidity in unanesthetized and ketamine- or thiopental-anesthetized rats. ANESTHESIOLOGY 1989; 70:984–90

10. Streisand JB, Bailey PL, LeMaire L, Ashburn MA, Tarver SD, Varvel J, Stanley TH: Fentanyl-induced rigidity and unconsciousness in human volunteers. Incidence, duration, and plasma concentrations. Anesthesiology 1993; 78:629–34
11. Lai S, Lui P: Inhibition by neuropeptide Y of fentanyl-induced muscular rigidity at the locus coeruleus in rats. Neurosci Lett 2000; 280:203–6
12. Lui PW, Lee TY, Chan SH: Involvement of locus coeruleus and noradrenergic neurotransmission in fentanyl-induced muscular rigidity in the rat. Neurosci Lett 1989; 96:114–9
13. Abrams JT, Horrow JC, Bennett JA, Van Riper DF, Storella RJ: Upper airway closure: A primary source of difficult ventilation with sufentanil induction of anesthesia. Anesth Analg 1996; 83:629–32
14. Bennett JA, Abrams JT, Van Riper DF, Horrow JC: Difficult or impossible ventilation after sufentanil-induced anesthesia is caused primarily by vocal cord closure. Anesthesiology 1997; 87:1070–4
15. Berkenbosch A, DeGoede J: Effects of brain hypoxia on ventilation. Eur Respir J 1988; 1:184–90
16. Guntheroth WG, Kawabori I: Hypoxic apnea and gasping. J Clin Invest 1975; 56:1371–7
17. Lawson EE, Thach BT: Respiratory patterns during progressive asphyxia in newborn rabbits. J Appl Physiol Respir Environ Exerc Physiol 1977; 43:468–74
18. Richter DW, Bischoff A, Anders K, Bellingham M, Windhorst U: Response of the medullary respiratory network of the cat to hypoxia. J Physiol 1991; 443:231–56
19. Haouzi P, Ahmadpour N, Bell HJ, Artman S, Banchs J, Samii S, Gonzalez M, Gleeson K: Breathing patterns during cardiac arrest. J Appl Physiol (1985) 2010; 109:405–11
20. Haouzi P, Notet V, Chenuel B, Chalon B, Sponne I, Ogier V, Bihain B: H2S induced hypometabolism in mice is missing in sedated sheep. Respir Physiol Neurobiol 2008; 160:109–15
21. Bell HJ, Azubike E, Haouzi P: The "other" respiratory effect of opioids: Suppression of spontaneous augmented ("sigh") breaths. J Appl Physiol (1985) 2011; 111:1296–303
22. Haouzi P, Bell HJ, Notet V, Bihain B: Comparison of the metabolic and ventilatory response to hypoxia and H2S in unsedated mice and rats. Respir Physiol Neurobiol 2009; 167:316–22
23. Mortola JP, Frappell PB: Measurements of air ventilation in small vertebrates. Respir Physiol Neurobiol 2013; 186:197–205
24. Ren J, Ding X, Funk GD, Greer JJ: Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. Anesthesiology 2009; 110:1364–70
25. Nair AB, Jacob S: A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016; 7:27–31
26. Moore J, Haouzi P, Van de Louw A, Bell HJ: Hypocapnia-dependent facilitation of augmented breaths: Observations in awake vs. anesthetized rats. Respir Physiol Neurobiol 2012; 180:105–11
27. Frappell P, Lanthier C, Baudinette RV, Mortola JP: Metabolism and ventilation in acute hypoxia: A comparative analysis in small mammalian species. Am J Physiol 1992; 262(6 pt 2):R1040–6
28. Kagawa S, Stafford MJ, Waggener TB, Severinghaus JW: No effect of naloxone on hypoxia-induced ventilatory depression in adults. J Appl Physiol Respir Environ Exerc Physiol 1982; 52:1030–4
29. Nieto-Posadas A, Flores-Martínez E, Lorea-Hernández JJ, Rivera-Angulo AJ, Pérez-Ortega JE, Bargas J, Peña-Ortega F: Change in network connectivity during fictive-gasping generation in hypoxia: Prevention by a metabolic intermediate. Front Physiol 2014; 5:265
30. Trippenbach T, Richter DW, Acker H: Hypoxia and ion activities within the brain stem of newborn rabbits. J Appl Physiol (1985) 1990; 68:2494–503
31. Heymans C: Chemoreceptors and regulation of respiration. Acta Physiol Scand 1951; 22:1–13
32. Dejours P, Girard F, Labrousse Y, Raynaud J: [Oxygen chemoreflex stimulus in ventilation at low altitude in man. II. During muscular exercise]. J Physiol (Paris) 1957; 49:120–4
33. Lahiri S, Roy A, Baby SM, Hoshi T, Semenza GL, Prabhakar NR: Oxygen sensing in the body. Prog Biophys Mol Biol 2006; 91:249–86
34. Fitzgerald RS, Lahiri S: Reflex Responses to Chemoreceptor Stimulation, Comprehensive Physiology. Hoboken, John Wiley & Sons, Inc., 2011
35. Bissonnette JM: Mechanisms regulating hypoxic respiratory depression during fetal and postnatal life. Am J Physiol Regul Integr Comp Physiol 2000; 278:R1391–400
36. Berkenbosch A, Teppema LJ, Olievier CN, Dahan A: Influences of morphine on the ventilatory response to isocapnic hypoxia. Anesthesiology 1997; 86:1342–9
37. Dahan A, Aarts L, Smith TW: Incidence, reversal, and prevention of opioid-induced respiratory depression. Anesthesiology 2010; 112:226–38
38. Montandon G, Ren J, Victoria NC, Liu H, Wickman K, Greer JJ, Horner RL: G-protein-gated inwardly rectifying potassium channels modulate respiratory depression by opioids. Anesthesiology 2016; 124:641–50
39. Roozekrans M, van der Schrier R, Okkerse P, Hay J, McLeod JF, Dahan A: Two studies on reversal of opioid-induced respiratory depression by BK-channel blocker GAL021 in human volunteers. Anesthesiology 2014; 121:459–68
40. Dahan A, van der Schrier R, Smith T, Aarts L, van Velzen M, Niesters M: Averting opioid-induced respiratory depression without affecting analgesia. Anesthesiology 2018; 128:1027–37
41. Bruce RD, Darnall RA, Althaus JS: Aminophylline reduces hypoxic ventilatory depression without

increasing catecholamines. Pediatr Pulmonol 1986; 2:218–24
42. Darnall RA Jr: Aminophylline reduces hypoxic ventilatory depression: Possible role of adenosine. Pediatr Res 1985; 19:706–10
43. Hilaire G, Voituron N, Menuet C, Ichiyama RM, Subramanian HH, Dutschmann M: The role of serotonin in respiratory function and dysfunction. Respir Physiol Neurobiol 2010; 174:76–88
44. Van Beek JH, Berkenbosch A, De Goede J, Olievier CN: Response of vertebral and carotid blood flow to isocapnic changes in end-tidal oxygen tension. Respir Physiol 1986; 63:65–77
45. Straus MM, Ghitza UE, Tai B: Preventing deaths from rising opioid overdose in the US - The promise of naloxone antidote in community-based naloxone take-home programs. Subst Abuse Rehabil 2013; 2013
46. Lewis CR, Vo HT, Fishman M: Intranasal naloxone and related strategies for opioid overdose intervention by nonmedical personnel: A review. Subst Abuse Rehabil 2017; 8:79–95