

Two Studies on Reversal of Opioid-induced Respiratory Depression by BK-channel Blocker GAL021 in Human Volunteers

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ABSTRACT

Background: Opioid-induced respiratory depression is potentially lethal. GAL021 is a calcium-activated potassium (BK_{Ca}) channel blocker that causes reversal of opioid-induced respiratory depression in animals due to a stimulatory effect on ventilation at the carotid bodies. To assess in humans whether GAL021 stimulates breathing in established opioid-induced respiratory depression and to evaluate its safety, a proof-of-concept double-blind randomized controlled crossover study on isohypercapnic ventilation (study 1) and subsequent double-blind exploratory study on poikilocapnic ventilation and nonrespiratory end points (study 2) was performed.

Methods: In study 1, intravenous low- and high-dose GAL021 and placebo were administered on top of low- and high-dose alfentanil-induced respiratory depression in 12 healthy male volunteers on two separate occasions. In study 2, the effect of GAL021/placebo on poikilocapnic ventilation, analgesia, and sedation were explored in eight male volunteers. Data are mean difference between GAL021 and placebo (95% CI).

Results: Study 1: Under isohypercapnic conditions, a separation between GAL021 and placebo on minute ventilation was observed by 6.1 (3.6 to 8.6) l/min ($P < 0.01$) and 3.6 (1.5 to 5.7) l/min ($P < 0.01$) at low-dose alfentanil plus high-dose GAL021 and high-dose-alfentanil plus high-dose GAL021, respectively. Study 2: Similar observations were made on poikilocapnic ventilation and arterial pCO₂. GAL021 had no effect on alfentanil-induced sedation, antinociception and no safety issues or hemodynamic effects became apparent.

Conclusion: GAL021 produces respiratory stimulatory effects during opioid-induced respiratory depression with containment of opioid-analgesia and without any further increase of sedation. Further studies are needed to confirm these preliminary data. ([ANESTHESIOLOGY 2014; 121:459-68](#))

OPIOIDS are the cornerstone of treatment of moderate to severe, acute, and chronic pain. Opioids, however, come with serious side effects, of which respiratory depression is potentially lethal.¹ In the perioperative setting, the estimated incidence of opioid-induced respiratory depression (OIRD) is 0.5 to 2%.¹ In chronic pain patients, the incidence of OIRD is unknown.² Recent publications stress the fact that the number of fatalities from legally prescribed opioids for the treatment of chronic pain is high.^{2,3} This is predominantly attributed to an increased awareness of clinicians to diagnose and treat chronic pain and the apparent ease at which legally prescribed opioids change hands.³

Taken the presented data, both in perioperative medicine and in the treatment of chronic pain the elimination of opioid-induced respiratory complications is important. Not only will it reduce morbidity and mortality, but it will possibly result in improved pain treatment with less suffering from inadequate pain relief, which often occurs due to the fear of OIRD.

What We Already Know about This Topic

- The opioid receptor antagonist naloxone reverses both opioid-induced respiratory depression and analgesia
- GAL021, which blocks calcium-activated potassium channels in the carotid bodies, increases ventilatory drive and antagonizes opioid-induced respiratory depression in animals

What This Article Tells Us That Is New

- In a double-blind, randomized, placebo-controlled crossover study, GAL021 stimulated ventilation in male volunteers with alfentanil-induced respiratory depression at a clamped and elevated end-tidal carbon dioxide partial pressure, increasing both tidal volume and respiratory rate
- GAL021 also stimulated poikilocapnic ventilation during alfentanil administration, without affecting sedation, antinociception, hemodynamics, or safety parameters

Current clinical practice is to treat OIRD with the opioid antagonist naloxone, which, however, reverses OIRD as well as analgesia, and comes with other sometimes deleterious

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side effects.^{1,4} A potent respiratory stimulant that effectively counteracts OIRD without any interaction with the opioid receptor system is lacking.¹ Various experimental drugs that enhance respiration are currently under investigation, including serotonin-agonists, ampakines, phosphodiesterase inhibitors, and potassium-channel blockers.^{1,5} In the current study, we investigated the efficacy of a new agent, GAL021 (fig. 1), which inhibits calcium-activated potassium channels at the carotid bodies (*i.e.*, large conductance Ca^{2+} /voltage-activated K^{+} -channels, BK_{Ca} -channels, formerly known as Maxi-K-channels).⁶ In rodents and monkeys, GAL021 dose-dependently increased ventilatory drive and antagonizes opioid (morphine/fentanyl)- and nonopioid (midazolam, isoflurane/propofol)-induced respiratory depression.⁷⁻⁹

We performed two studies to assess the effect of GAL021 on respiratory and nonrespiratory end points. The first study was a randomized controlled trial that was designed as a *first-in-class* study to confirm the effects of GAL021 on established (opioid-induced) respiratory depression under isohypercapnic conditions. The main aim of the study was to assess whether the results confirm the mechanism of action of GAL021 in humans under conditions of a depressed ventilatory control system. To further explore the properties of GAL021 we performed an exploratory or learn-study to assess the effects of GAL021 on ventilation under nonclamped conditions and on nonrespiratory variables (hemodynamics, antinociception, sedation, and adverse events). Our main hypothesis is that GAL021, given on top of established OIRD, is able to stimulate breathing without major effects on nonrespiratory end points.

Materials and Methods

Both studies (the proof-of-concept study [study 1] and learn-study [study 2]) had a randomized, double-blind, placebo-controlled crossover design. The protocol was performed after approval was obtained from the Medical Ethics Committee of the Biomedical Research Ethics Review Foundation (BEBO, Assen, The Netherlands) and the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands) and was registered at www.trial-register.nl under number NTR3718. The studies were conducted from October 31, 2012 to February 11, 2013. An *a priori* power analysis was performed for study 1 and yielded 12 subjects to detect a respiratory effect of GAL021 greater than placebo (see Sample Size). After completion of study 1, the effect of GAL021 *versus* placebo was studied in study 2 on respiratory and nonrespiratory variables, now against a background of poikilocapnia (*i.e.*, the subjects breathed

room air). Study 2 was designed to study (1) the effect of GAL021 on alfentanil-induced respiratory depression under “real life” (*i.e.*, noncarbon dioxide clamp) conditions, and (2) to get an impression of the effect of GAL021 on nonrespiratory variables, including hemodynamics, pain responses, and sedation. In this learn-study, the number of subjects was set at eight, considering the magnitude of effects observed in study 1. The protocol allowed expansion of study 2 to a maximum of 36 subjects in case further exploration was required. In Studies 1 and 2 adverse events were recorded.

Subjects

Healthy men, aged 18 to 45 yr and body mass index 18 to 30 kg/m^2 , were recruited through an advertisement on a dedicated Web site. All subjects gave written and oral informed consent. The subjects underwent a full medical screening, including medical history taking, a physical examination, blood chemistry and hematology, and an electrocardiogram to assess eligibility. Participants were healthy with no history of major medical or psychiatric disease, alcohol abuse, daily consumption of caffeine greater than six servings, smoking in the last year, and any other investigational drug administered within 3 months before inclusion. Finally, participants had to fast for at least 6 h before the administration of study drug.

Study Design

Upon arrival in the laboratory all subjects received two intravenous access lines, one for administration of alfentanil and another for administration of GAL021 (Galleon Pharmaceuticals Corp., Horsham, PA) or placebo (NaCl 0.9%). An arterial line was placed in the radial artery of the non-dominant arm for alfentanil blood sampling in Studies 1 and 2 (appendix), and measurement of blood pressure, cardiac output, and arterial pCO_2 in study 2. For safety monitoring, the electrocardiogram, blood pressure, heart rate, and oxygen saturation were measured continuously.

Drugs. GAL021 was prepared as a sterile product ready for dilution (colorless, pH 3.1). GAL021 and placebo (normal saline) were diluted in lactated Ringer’s solution (final volume $\approx 250 \text{ ml}$) and administered intravenously by an infusion pump.

Alfentanil and GAL021 Infusions in Studies 1 and 2.

A stepped drug infusion regimen was applied as depicted in figure 2. First, alfentanil was administered intravenously: a loading infusion of $1.33 \mu\text{g kg}^{-1} \text{ min}^{-1}$ for 6 min, followed by a subsequent maintenance infusion of $0.3 \mu\text{g kg}^{-1} \text{ min}^{-1}$ given over 104 min, in order to achieve a 25 to 30% decrease in minute ventilation (ALF-low). If this level of respiratory depression was not reached during the first infusion, a second dose of $1.33 \mu\text{g kg}^{-1} \text{ min}^{-1}$ was administered and the maintenance infusion was increased to $0.6 \mu\text{g kg}^{-1} \text{ min}^{-1}$; in case of an overshoot in respiratory depression during the loading infusion, the maintenance infusion was halved. After 30 min of steady-state ventilation, a concurrent intravenous infusion of GAL021 or placebo was started (GAL-low): a

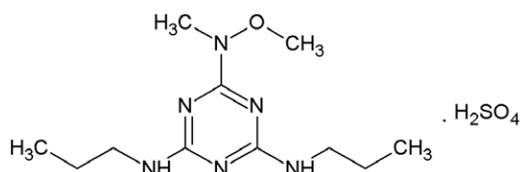


Fig. 1. The chemical structure of GAL021 dihydrogensulfate.

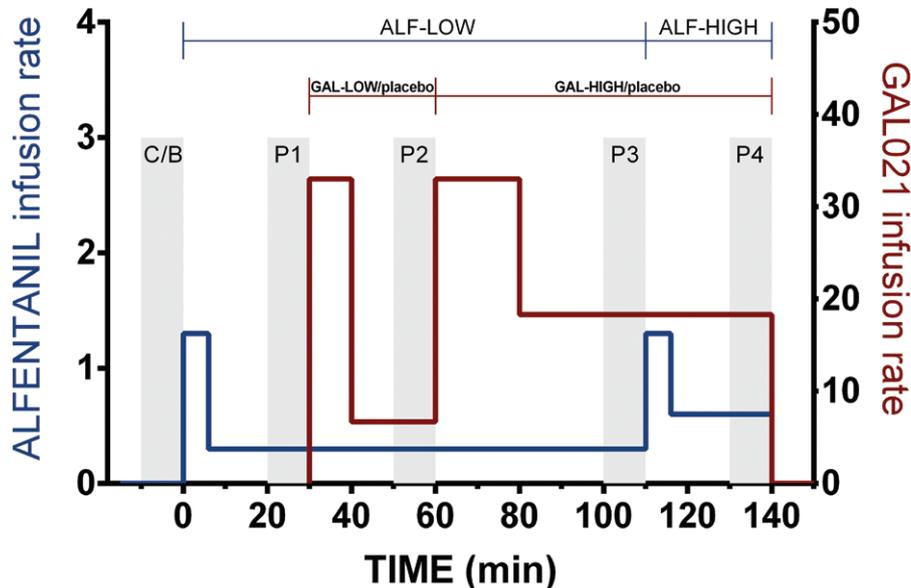


Fig. 2. Schematic representation of the design of studies 1 and 2. The blue line is the infusion rate of alfentanil. In study 1, the infusion could be adapted depending on magnitude of the ventilatory (a doubling of loading and maintenance dose in case the target of respiratory depression, 25–30%, was not attained; a reduction of the maintenance dose by 50% in case of an overshoot in respiratory depression). The red line is the infusion rate of GAL021 or placebo. In study 1, respiration was measured under isohypercapnic conditions; in study 2 poikilocapnic ventilatory and nonventilatory variables were obtained. B represents baseline (no drug, no added inspired carbon dioxide), C represents the carbon dioxide clamp before any drug infusion, P1 represents low-dose alfentanil (ALF) infusion before GAL021 or placebo infusion (ALF-low), P2 the combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low plus GAL021-low), P3 the combination of low-dose alfentanil with high-dose GAL021 or placebo (ALF-low plus GAL021-high), and P4 the combination of high-dose alfentanil with high-dose GAL021 or placebo (ALF-high plus GAL021-high). Alfentanil and GAL021 infusion rate are in $\mu\text{g kg}^{-1} \text{h}^{-1}$.

loading infusion of $33.3 \mu\text{g kg}^{-1} \text{min}^{-1}$ for 10 min, followed by a maintenance infusion of $6.67 \mu\text{g kg}^{-1} \text{min}^{-1}$ for 20 min (total infusion time of GAL-low 30 min). Next, the GAL021 infusion was increased (GAL-high) with a loading infusion of $33.3 \mu\text{g kg}^{-1} \text{min}^{-1}$ for 20 min, followed by a maintenance infusion of $18.3 \mu\text{g kg}^{-1} \text{min}^{-1}$ for 60 min (total infusion time of GAL-high 80 min). During the final 30 min of the GAL-high infusion, the infusion rate of alfentanil was increased (ALF-high) with a repeat loading as given in ALF-low (in case no adjustments were made this was $1.33 \mu\text{g kg}^{-1} \text{min}^{-1}$ for 6 min), followed by a maintenance dose twice that of ALF-low (in case no adjustments were made the maintenance infusion was $0.6 \mu\text{g kg}^{-1} \text{min}^{-1}$ given over 24 min). The target reduction in ventilation at ALF-high was 50 to 60%. Hereafter, both alfentanil and GAL021/placebo infusions were ended.

Inhaled Gas Concentrations and Ventilation Measurements.

During (breath-to-breath) ventilation measurement the subjects breathed through a facemask connected to a pneumotachograph system (#4813; Hans Rudolph, Shawnee, KS). The signal from the pneumotachograph was integrated to yield a volume signal. The inspired and expired oxygen and carbon dioxide partial pressures (pO_2 and pCO_2) were measured at the mouth with a capnograph (Datex Capnomac, Helsinki, Finland).

In study 1, ventilation was measured at the background of isohypercapnia. To that end, varying concentrations of inhaled oxygen, carbon dioxide, and nitrogen were delivered to the

subjects via three computer-controlled mass flow controllers (Bronkhorst, Veenendaal, The Netherlands) ensuring the strict control of the end-tidal pO_2 and pCO_2 independent of the ventilatory response (see studies by Dahan *et al.*^{10,11} for an elaborate explanation of the dynamic end-tidal forcing technique). The elevated end-tidal pCO_2 was such that the target predrug clamped minute ventilation was between $20 \pm 2 \text{ l/min}$. The inspired oxygen concentration was also manipulated to keep the end-tidal pO_2 in the normoxic range (110 mmHg) throughout the study. In study 2, the subjects breathed room air without any additional inspired carbon dioxide.

Study Episodes. For analyses purposes five time points are defined in study 1 and four in study 2 (see also fig. 2):

Study 1: Period C is defined as the 10-min period before any drug infusion, but with carbon dioxide clamp, P1 is the 10-min period during low-dose alfentanil infusion before any GAL021 or placebo infusion (ALF-low), P2 is the 10-min period where low-dose alfentanil is combined with low-dose GAL021 or placebo infusion (ALF-low plus GAL-low), P3 is the 10-min period where low-dose alfentanil is combined with a high-dose GAL021 or placebo infusion (ALF-low plus GAL-high) and P4 is the 10-min period where high-dose alfentanil is combined high-dose GAL021 or placebo (ALF-high plus GAL-high).

Study 2: Period B is the 10-min period before any drug infusion. P1 to P4 are defined as in study 1. No carbon dioxide clamp was applied in study 2.

Design of Study 1. When ventilation had reached a steady state at the elevated end-tidal $p\text{CO}_2$, alfentanil infusion was started (see Alfentanil and GAL021 Infusions). For analyses purposes, 10-min averages of inspired minute ventilation, tidal volume, respiratory rate, end-tidal $p\text{CO}_2$, and oxygen saturation were obtained at periods C, P1 to P4 (fig. 2). Each subject participated twice in study 1, once receiving alfentanil and GAL021, once receiving alfentanil and placebo. The washout-period between sessions was at least 1 week.

Design of Study 2. Eight subjects who previously participated in study 1 were included in study 2. Selection of the subjects was based on their availability and unrelated to the respiratory responses in study 1. Subjects in study 2 were tested twice, once receiving alfentanil and GAL021, once receiving alfentanil and placebo, with at least 1 week between sessions. In this study, the infusion schemes of alfentanil and GAL021/placebo were similar to that of study 1. The subjects breathed room air throughout the study. The following procedures were performed to collect data at regular intervals (at B, P1 to P4; fig. 2):

1. Ventilation was measured for 5 to 10 min (while breathing room air) using the facemask/pneumotachograph system.
2. Hereafter a blood sample was obtained for blood gas analysis. Here we report on the arterial $p\text{CO}_2$. The sample was analyzed with an I-Stat 1 system (Abbott Point of Care, Abbott Park, IL) using CG8+ cartridges.
3. Next, alfentanil-induced antinociception was measured using an electrical pain model. Two electrodes were placed on the skin over the shinbone of the right leg.¹² An electrical stimulus train was generated by a computer-interfaced current stimulator (Leiden University Medical Center, Leiden, The Netherlands). After starting the stimulator, the current increased from 0 by 0.5 mA/s and the subject indicated, by pressing a button on the control panel, when pain was first observed (pain detection threshold) and by pressing another button when he could not tolerate the pain any further (pain tolerance). This ended the stimulus train. If a muscle response was triggered during this procedure, the electrodes were relocated until no further response was observed. This procedure was practiced at the beginning of the experimental session. Four baseline values were obtained before any drug infusion. These values were averaged and served as predrug control values. Here we present the pain threshold data.
4. Just before respiratory measurements, the subjects were queried about the magnitude of sedation by means of a visual rating scale from 1 to 100 mm, where 1 mm equals no sedation and 100 mm equals maximum sedation.¹²
5. Throughout part 2 of the study, the mean arterial pressure and cardiac output were measured using the FloTrac/Vigileo system (Edwards Lifesciences Corp., Irvine, CA) connected to the arterial line. Minute averages were obtained from the device.
6. Heart rate (Datex Cardiocap) and oxygen saturation (Masimo SET pulse oximeter, Irvine, CA) were collected throughout the study.

Randomization and Allocation

This was a double-blind study. Randomization was performed by a study-independent statistician according to a computer-generated nonrestricted randomization schedule and shared with the local pharmacy. Subjects were allocated in a 1:1 ratio. The pharmacy prepared the study drugs and dispensed them into identical syringes marked solely with the subject and visit number. The drugs were delivered to the research team on the morning of the experiment. The pharmacy further delivered alfentanil syringes in a solution of 0.5 mg/ml. Unblinding of the study was performed after data closure.

Sample Size

Sample size determination was performed for minute ventilation at P3 and P4 and was based on data from a previous study that showed that changes in minute ventilation (over a 10-min assessment period) had an intrasubject variance ranging from 6 to 9%.¹³ Sample sizes of 8 and 12 yielded respectively 80 and 90% power to observe a statistically significant within-cohort difference ($\alpha = 0.05$, one-sided). The sample size was set at 12 subjects for study 1. In case of discontinuation, the subject was replaced by another and both experimental sessions were performed. The sample size of part 2 of the study was set arbitrarily at eight.

Statistical Analysis

The evaluable population consisted of all subjects who completed both crossover periods in both studies. Data are presented as mean (95% CI) and point estimates of the difference between treatments (95% CI). To get an indication of the ability of GAL021 to increase ventilation relative to placebo, the data of study 1 were analyzed with a mixed model analysis of covariance with treatment segment, and treatment \times segment as fixed factors and subject, subject \times treatment, and subject \times segment as random factors and the value at segment P1 (ALF-low) as covariate. Analysis was performed for segments P2, P3, and P4 separately, with P value less than 0.01 considered significant (SAS; SAS Institute Inc., Cary, NC).

Results

Study 1

Three subjects withdrew consent after completing one single experimental session for reasons of discomfort. Data of these three were not included in the analysis; three new subjects were enrolled and completed the study. Twelve subjects completed both experimental sessions. Median (range) age of the subjects was 21.5 (19 to 31) yr, median weight 72.3 (62.9 to 84.3) kg, and body mass index 22.3 (20.2 to 26.5) kg/m^2 . All subjects completed the study without major side effects (see Adverse Events).

On average, carbon dioxide was clamped at 48.8 (SD = 0.2) mmHg and 49.2 (SD = 0.04) mmHg in placebo and GAL021 experiments, respectively. Ventilation levels reached at period

C were 20.8 (19.4 to 22.3) l/min (placebo) and 19.8 (19.3 to 20.4) l/min (GAL021; table 1). Three subjects required an additional loading infusion of alfentanil because of a limited effect of the initial loading infusion on ventilation as specified in the protocol. Six subjects received a reduced maintenance infusion because of an initial overshoot in ventilatory depression. The effects of alfentanil, GAL021, and placebo on ventilation, tidal volume, respiratory rate, and oxygen saturation are given in table 1. Most importantly, a separation between

GAL021 and placebo on minute ventilation was observed at P3 (ALF-low plus GAL-high) and P4 (ALF-high plus GAL-high) by 6.1 (3.6 to 8.6) l/min and 3.6 (1.5 to 5.7) l/min, respectively (both $P < 0.01$ vs. placebo; fig. 3A). The effects on minute ventilation were due to effects on tidal volume (at P3; fig. 3B) and respiratory rate (at P3 and P4; fig. 3C). No effect of either alfentanil or GAL021/placebo was observed on oxygen saturation (table 1). A scatter plot of the individual ventilation data is given in figure 4.

Table 1. Effect of GAL021 on Respiratory Variables Obtained under Clamped End-tidal pCO₂ Conditions (Part 1 of the Study)

	Clamp	P1	P2	P3	P4
Ventilation (l/min)					
Placebo	20.82 (19.4–22.3)	12.63 (11.3–14.0)	12.05 (10.7–13.4)	10.77 (9.4–12.2)	9.32 (8.2–10.5)
GAL021	19.84 (19.3–20.4)	13.31 (12.2–14.4)	14.00 (12.1–15.9)	16.86 (14.1–19.6)	12.94 (10.8–15.1)
Mean difference	-0.98 (-2.2 to 0.2)	0.68 (-0.6 to 2.0)	1.95 (0.6–3.3)	6.09 (3.6–8.6)*	3.62 (1.5–5.7)*
Tidal volume (ml)					
Placebo	1,196 (1,116–1,275)	823 (760–886)	818 (766–869)	811 (728–894)	812 (719–906)
GAL021	1,174 (1,095–1,252)	851 (792–910)	901 (822–980)	1,032 (927–1,137)	905 (796–1,014)
Mean difference	-22 (-83 to 39)	28 (-26 to 82)	83 (21–146)	221 (124–318)*	93 (-13 to 198)
Respiratory rate (min⁻¹)					
Placebo	18.2 (16.8–19.6)	15.4 (14.0–16.8)	14.8 (13.4–16.3)	13.6 (12.6–14.5)	12.6 (11.2–13.9)
GAL021	17.7 (16.4–19.0)	16.0 (14.5–17.5)	15.8 (14.0–17.6)	16.5 (14.4–18.7)	14.9 (13.1–16.8)
Mean difference	-0.5 (-1.3 to 0.2)	0.6 (-0.3 to 1.5)	1.0 (0.0–1.9)	3.0 (1.2–4.8)*	2.4 (0.7–4.0)*
End-tidal pCO₂ (mmHg)					
Placebo	48.8 (47.4–50.2)	48.9 (47.6–50.3)	48.9 (47.6–50.3)	49.0 (47.7–50.2)	48.6 (47.3–49.9)
GAL021	49.2 (47.9–50.3)	49.2 (48.0–50.3)	49.2 (47.9–50.4)	49.3 (48.2–50.4)	49.2 (48.0–50.4)
Mean difference	0.4 (-0.8 to 1.6)	0.2 (-1.0 to 1.4)	0.3 (-0.9 to 1.4)	0.3 (-0.8 to 1.5)	0.6 (-0.4 to 1.5)
Oxygen saturation (%)					
Placebo	98.6 (97.9–99.3)	97.3 (96.6–98)	97.4 (96.6–98.1)	97.6 (96.4–98.7)	97.3 (96.3–98.3)
GAL021	98.9 (98.4–99.5)	97.5 (96.7–98.3)	97.7 (96.8–98.5)	98.1 (97.3–98.9)	97.0 (96.2–97.9)
Mean difference	0.3 (-0.1 to 0.8)	0.3 (-0.3 to 0.9)	0.3 (-0.3 to 0.9)	0.5 (-0.2 to 1.2)	-0.3 (-0.9 to 0.4)

Values are mean (95% CI); mean difference = GAL021 - Placebo.

* $P < 0.01$ vs. placebo.

ALF = alfentanil; B = baseline (no drug, no added inspired CO₂); C = CO₂ clamp before any drug infusion (only in part 1 of the study); P1 = low-dose alfentanil infusion before any GAL021 or placebo infusion (ALF-low); P2 = combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low plus GAL021-low); P3 = the combination of low-dose alfentanil with high-dose GAL021 or placebo (ALF-low plus GAL021-high); P4 = the combination of high-dose alfentanil with high-dose GAL021 or placebo (ALF-high plus GAL021-high).

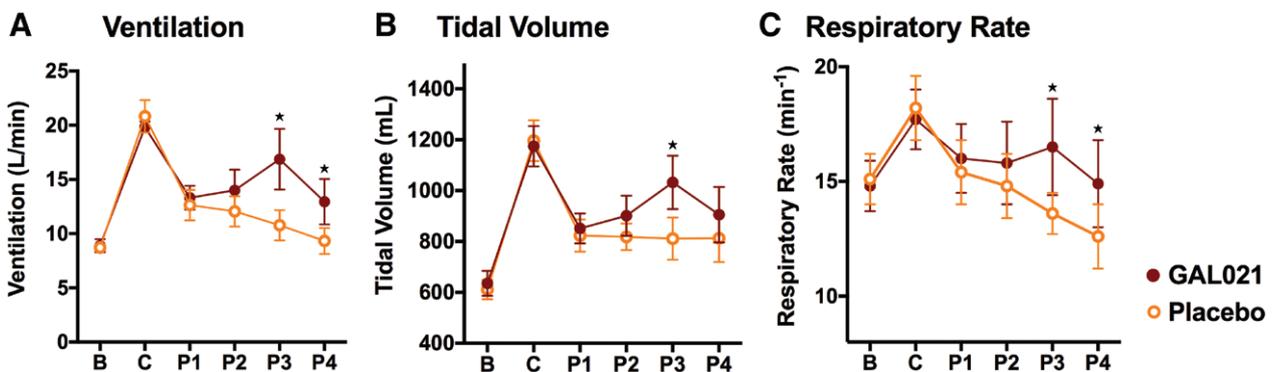


Fig. 3. Results of study 1: Effect of GAL021 versus placebo on minute ventilation (A), tidal volume (B), and respiratory rate (C). B represents baseline (no drug, no added inspired carbon dioxide), C represents the carbon dioxide clamp before any drug infusion, P1 represents low-dose alfentanil (ALF) infusion before any GAL021 or placebo infusion (carbon dioxide-clamp plus ALF-low), P2 the combination of low-dose alfentanil and low-dose GAL021 or placebo (carbon dioxide-clamp plus ALF-low plus GAL021-low), P3 the combination of low-dose alfentanil with high-dose GAL021 or placebo (carbon dioxide-clamp plus ALF-low plus GAL021-high), and P4 the combination of high-dose alfentanil with high-dose GAL021 or placebo (carbon dioxide-clamp plus ALF-high plus GAL021-high). Values are mean \pm 95% CI. * $P < 0.01$ versus placebo.

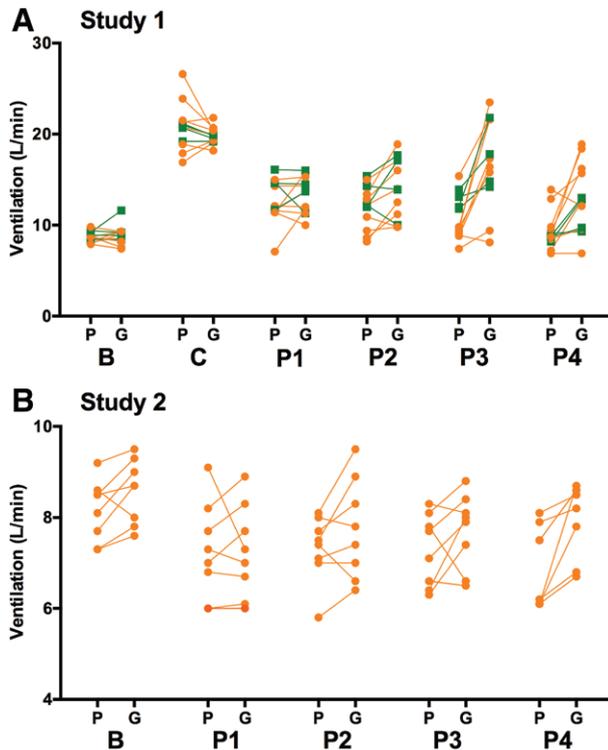


Fig. 4. (A) Scatter plot of the effect of treatment on ventilation of study 1 at baseline (B), clamped ventilation (C) and time points P1 (carbon dioxide-clamp plus alfentanil (ALF)-low), P2 (carbon dioxide-clamp plus ALF-low plus GAL021-low), P3 (carbon dioxide-clamp plus ALF-low plus GAL021-high), and P4 (carbon dioxide-clamp plus ALF-high plus GAL021-high). The subjects that participated in studies 1 and 2 are indicated in orange. (B) Scatter plot of the effect of treatment on ventilation of study 2 at baseline (B) and time points P1 (ALF-low), P2 (ALF-low plus GAL021-low), P3 (ALF-low plus GAL021-high), and P4 (ALF-high plus GAL021-high). The subjects that participated in studies 1 and 2 are indicated in orange.

Study 2

Eight subjects of study 1 participated in study 2 and completed both experimental sessions. All subjects completed the study without major side effects (see Adverse Events). Examination of (poikilocapnic) ventilation and arterial $p\text{CO}_2$ (table 2 and fig. 5) shows separation between GAL021 and placebo at P3 and P4 with mean differences in effect estimates of 0.6 (0.1 to 1) l/min and -3.4 (-6.2 to -0.6) mmHg at P1 and 1.0 (0.5 to 1.6) l/min and -1.5 (-3.1 to -0.1) mmHg at P4, respectively. No treatment differences were observed for plasma alfentanil concentrations, blood pressure, cardiac output, pain threshold, and sedation (table 2). A scatter plot of the individual ventilation data is given in figure 4.

Adverse Events

Both alfentanil and GAL021 were well tolerated by the subjects and no interventions were required. Adverse events occurring in studies 1 and 2 are given in table 3. Apart from evident opioid-related side effects such as pruritus and nausea, specific differences were observed between studies 1 and

2 (feeling warm/sweating, nausea, headache) and between treatments (pain at infusion site).

Discussion

Respiratory Depression

Opioid-induced respiratory depression remains an important concern taken its possible morbidity and potential fatal consequences. Indeed both in the acute and chronic opioid-use settings numerous (near)-fatalities have been reported.¹⁻³ Current clinical practice to reverse OIRD is by intravenous or intramuscular injection of the opioid receptor antagonist naloxone. While naloxone is an effective reversal agent it comes with disadvantages, including reversal of analgesia, sympathicoexcitation, and risk of re-arousal if not continuously administered (due to its short half-life of 15 to 30 min).⁴ Furthermore, some opioids are difficult to reverse related to their high affinity for the opioid receptor (e.g., buprenorphine).¹⁴ Hence, there is a need for an agent that selectively stimulates breathing without any effect on other physiological systems. Such an agent should stimulate respiration without any interaction with the opioid receptor system. Various agents have been evaluated to that end, including modulators of potassium channels, serotonin receptor agonists, agents that enhance glutamatergic transmission, and phosphodiesterase inhibitors.^{1,5} Here we present data on a novel agent, GAL021. Previous animal data demonstrated that GAL021 induces potent ventilatory stimulation during OIRD, without affecting analgesia. In conscious rats and nonhuman primates (*Cynomolgus* monkeys) an infusion of GAL021 reversed morphine-induced respiratory depression producing a rapid and dose-dependent diminution of the evoked respiratory depression for the duration of the infusion.⁷⁻⁹ Termination of GAL021 infusion led to the return of OIRD. Importantly, GAL021 did not diminish morphine-induced analgesia in rats as tested by the tail-flick assay.⁷

Our proof-of-concept study (study 1) assessed the ability of GAL021 to increase ventilation during established alfentanil-induced respiratory depression in a group of healthy male volunteers. This study was performed at clamped and elevated end-tidal carbon dioxide levels. This was done to quantify ventilatory changes without the confounding stimulatory or inhibitory effects of changes in arterial $p\text{CO}_2$. Application of this technique allows the assessment of the true pharmacological effect of a drug on the ventilatory control system. The observations made in study 1 on carbon dioxide-clamped ventilation are in close agreement with observations made in study 2 on poikilocapnic ventilation and arterial $p\text{CO}_2$ (fig. 5, B and C, table 2). Since these two variables are interconnected (an increase in arterial $p\text{CO}_2$ stimulates breathing and hyperventilation reduces arterial $p\text{CO}_2$), their evaluation is best done jointly. Combining these variables show that under “real-life” conditions, a separation between GAL021 and placebo on ventilation, during alfentanil-induced respiratory depression, is observed. The

Table 2. Effect of GAL021 on Poikilocapnic Ventilation, Arterial pCO₂ and Nonrespiratory Variables (Study 2)

	Baseline	P1	P2	P3	P4
Ventilation (l/min)					
Placebo	8.15 (7.7–8.6)	7.28 (6.6–8.0)	7.32 (6.8–7.8)	7.28 (6.7–7.8)	6.95 (6.3–7.6)
GAL021	8.58 (8.1–9.1)	7.23 (6.5–7.9)	7.74 (7.0–8.5)	7.84 (7.4–8.3)	7.98 (7.4–8.6)
Mean difference	0.43 (0.1–0.8)	–0.04 (–0.6 to 0.5)	0.42 (–0.1 to 0.9)	0.56 (0.1–1.0)	1.03 (0.5–1.6)
Arterial pCO₂ (mmHg)					
Placebo	43.4 (42.0–44.8)	47.4 (44.9–49.9)	47.7 (46.2–49.3)	48.6 (46.1–51.0)	51.7 (48.1–55.3)
GAL021	42.6 (40.6–44.5)	46.4 (42.3–50.5)	46.7 (43.3–50.0)	45.3 (41.7–48.9)	48.0 (43.1–52.9)
Mean difference	–0.8 (–2.4 to 1.7)	–0.9 (–4.3 to 2.5)	–1.2 (–3.1 to 0.7)	–3.4 (–6.2 to –0.6)	–3.6 (–5.7 to –1.6)
Arterial pO₂ (mmHg)					
Placebo	95.1 (92.1–98.1)	95.8 (91.7–99.8)	93.1 (90.3–96.0)	88.1 (82.3–93.9)	94.0 (87.1–100.9)
GAL021	94.7 (90.8–98.5)	93.8 (86.6–101.0)	94.6 (83.0–106.2)	96.0 (88.2–103.8)	94.2 (84.3–104.1)
Mean difference	–0.4 (–8.1 to 7.3)	–2.0 (–6.6 to 2.6)	1.5 (–3.7 to 6.7)	7.9 (1.9–13.9)	0.2 (–3.9 to 4.3)
Cardiac output (l/min)					
Placebo	7.7 (6.5–9.0)	6.1 (5.2–7.0)	6.0 (4.9–7.0)	6.3 (4.5–8.0)	6.3 (4.7–7.8)
GAL021	7.2 (5.6–8.8)	6.0 (4.6–7.4)	6.2 (4.4–8.1)	6.6 (5.2–8.1)	6.8 (5.3–8.4)
Mean difference	0.5 (–0.1 to 1.1)	–0.1 (–0.9 to 0.8)	0.2 (–1.1 to 0.6)	0.3 (–0.5 to 1.2)	0.5 (–0.5 to 1.0)
Mean arterial pressure (mmHg)					
Placebo	95 (89–101)	87 (83–91)	86 (83–90)	87 (83–92)	86 (82–90)
GAL021	95 (89–101)	86 (78–95)	87 (79–96)	85 (80–90)	83 (76–91)
Mean difference	0 (–3 to 3)	–1 (–5 to 3)	1 (–3 to 4)	–2 (–5 to 1)	–3 (–6 to 0)
Heart rate (min^{–1})					
Placebo	63 (56–70)	54 (50–58)	53 (48–58)	54 (49–59)	54 (49–58)
GAL021	61 (49–73)	53 (46–60)	53 (47–60)	57 (50–63)	61 (58–64)
Mean difference	3 (–5 to 12)	1 (–5 to 7)	0 (–6 to 6)	3 (–3 to 9)	7 (2–13)
Pain threshold (% of baseline)					
Placebo	100	113 (101–125)	118 (100–136)	123 (107–139)	140 (114–165)
GAL021	100	124 (98–150)	131 (108–154)	142 (108–175)	160 (120–201)
Mean difference		11 (–20 to 42)	13 (–14 to 40)	19 (–18 to 55)	21 (–33 to 75)
Sedation VAS (mm)					
Placebo	13 (–5 to 32)	66 (46–86)	49 (20–77)	48 (24–71)	63 (42–85)
GAL021	11 (–6 to 22)	62 (44–80)	47 (25–69)	56 (32–80)	71 (49–92)
Mean difference	–2 (–5 to 0)	–4 (–13 to 6)	–2 (–21 to 18)	8 (–2 to 18)	7 (–9.0 to 24)

Values are mean (95% CI). Mean difference is GAL021 – Placebo.

ALF = alfentanil; B = baseline (no drug, no added inspired CO₂); P1 = low-dose alfentanil infusion before any GAL021 or placebo infusion (ALF-low); P2 = combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low plus GAL021-low); P3 = the combination of low-dose alfentanil with high-dose GAL021 or placebo (ALF-low plus GAL021-high); P4 = the combination of high-dose alfentanil with high-dose GAL021 or placebo (ALF-high plus GAL021-high); VAS = visual analogue scale.

data from study 1 indicate that ventilation increases due to changes in tidal volume and respiratory rate.

Rat studies suggest that GAL021 acts mainly through an effect on the carotid bodies. GAL021 dose-dependently increases carotid sinus activity, while its effects on ventilation were diminished upon carotid body denervation.¹⁵ The carotid bodies, located at the bifurcation of the common carotid artery, contain the peripheral chemoreceptors, which are responsible for about 30% of the tonic ventilatory drive and respond to hypoxia with a brisk hyperventilatory response.¹⁶ Type 1 carotid body cells (which are sensitive to hypoxia) express various potassium channels (including BK_{Ca}-channels). Upon BK_{Ca}-channels blockade, type 1 cells release neurotransmitters that activate the sinus nerve, and consequently increase respiratory drive.^{17,18} In *Slo^{–/–}* mice that lack various subunits of the BK_{Ca}-channel, the effects of GAL021 were severely diminished (but not abolished).¹⁹ Jointly these data suggest that GAL021 acts

through blockade of the BK_{Ca}-channel of the type 1 carotid body cells but additional mechanisms at the carotid bodies or other sites are not excluded.

Hemodynamics

BK_{Ca}-channels are expressed in vascular smooth muscles and may play a role in regulating cerebral and systemic vascular tone.²⁰ We previously observed that doxapram produces a sharp increase in cardiac output before any effect on ventilation was apparent.²¹ Apart from its effects at TASK-channels, doxapram, like GAL021, interacts with the BK_{Ca}-channel,²² and this channel may be the site of action of the cardiostimulatory effects of doxapram.²⁰ In the current study, however, GAL021 was without effects on mean arterial pressure, heart rate, and cardiac output. This indicates a differential effect of GAL021 and doxapram on the cardiovascular system and suggests that the BK_{Ca}-channel is not the site of action of the cardiovascular stimulatory effects of doxapram.

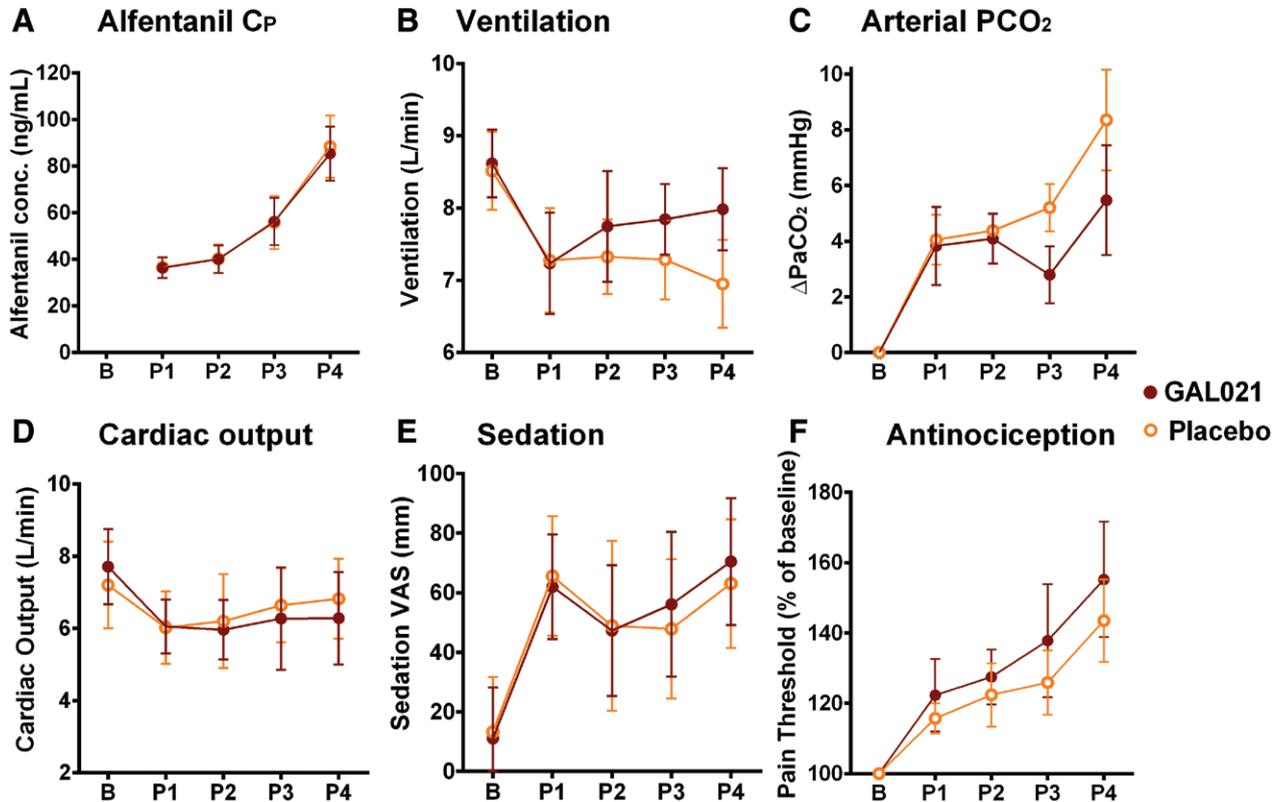


Fig. 5. Results of study 2: (A) Plasma alfentanil concentrations (CP), (B) poikilocapnia ventilation, (C) arterial pCO₂, (D) cardiac output, (E) sedation, and (F) antinociception. B represents baseline (no drug), P1 represents low-dose alfentanil (ALF) infusion before any GAL021 or placebo infusion (ALF-low), P2 the combination of low-dose alfentanil and low-dose GAL021 or placebo (ALF-low plus GAL021-low), P3 the combination of low-dose alfentanil with high-dose GAL021 or placebo (ALF-low plus GAL021-high), and P4 the combination of high-dose alfentanil with high-dose GAL021 or placebo (ALF-high plus GAL021-high). Values are mean \pm 95% CI.

Table 3. Adverse Events Observed in Studies 1 and 2

	Pruritus	Feeling Warm/ Sweating	Lightheadedness	Nausea	Headache	Pain at Infusion Site	Limb Weakness	Hallucinations	Total
	Incidence in study 1–Incidence in study 2								
Placebo	4–3	1–1	3–1	6–1	7–1	0–0	1–0	0–0	22–7
GAL021	3–4	6–3	0–0	3–2	4–0	6–2	2–0	1–0	25–11

The first number represents the number of subjects that experience the adverse event in study 1, the second in study 2.

Analgesia and Sedation

Since potassium channels are involved in nociceptive processing,²³ it is important to assess the effect of GAL021 on analgesia and pain responses. Both in animal studies and in the current study opioid-analgesia was not affected by GAL021.⁷ However, our study was not powered to detect possible effects from GAL021 on analgesia. Although the average effect of analgesia during GAL021 infusion was higher than during placebo infusion (difference +10 to 20%; fig. 5F), further studies using multiple pain models are required to assess the true effect of GAL021 on opioid-analgesia. Similarly, we found no effect of GAL021 on opioid-induced sedation. This is important as some other respiratory stimulants do increase sedation.²⁴ Like analgesia,

the absence of effects of GAL021 on sedation needs additional investigation.

Safety

An important part of our studies was the assessment of the safety of GAL021. In a previous study, the safety of GAL021 has been addressed in 30 healthy volunteers without the presence of another drug.¹³ Apart from the observation of a burning sensation at the GAL021 infusion site; adverse events were similar between GAL021- and placebo-treated subjects. This is in agreement with our observation. The observation of injection site pain could be attributed to the low pH of the GAL021 infusate (pH \approx 3.5). We additionally observed perspiration and hot flushes in 6 (of 12)

subjects receiving GAL021, especially during hypercapnia (table 3). This suggests a sympathetic effect of GAL021 under hypercapnic conditions. Interestingly, some adverse events occurred in study 1 but not study 2, such as nausea and headache. We attribute this to the hypercapnic conditions of study 1.

Doxapram and Ampakines

Apart from GAL021, various nonopioid respiratory stimulants are clinically available or under investigation (reviewed in studies by Dahan *et al.*¹ and Boom *et al.*⁵). One of the first agents that was developed to induce respiratory stimulation is doxapram, available since 1962.²⁵ Doxapram inhibits background potassium channels (TASK1, TASK3, TASK1/TASK3 heterodimer) as well as BK_{Ca}-channels expressed on type 1 carotid body cells.^{22,26} A recent study in perioperative patients showed that a 1 mg/kg bolus dose of doxapram produces modest respiratory stimulation following total intravenous anesthesia.²⁷ We recently tested the effect of a continuous infusion of doxapram (total dose 2.7 mg/kg given over 94 min) on alfentanil-induced respiratory depression (plasma concentration 60 to 100 ng/ml), using a study design similar to the current study.²¹ We observed no effect on ventilation under both isohypercapnic (*i.e.*, at a clamped and elevated end-tidal pCO₂) or poikilocapnic conditions at two thirds of the maximum recommended dose. Possibly higher doxapram dosages are required to induce reversal but its side effects profile (which includes panic attacks, sympathicoexcitation [causing hypertension secondary to elevations of cardiac output], sweating, nausea, convulsions) precluded higher infusion rates than used by us. Newly developed TASK-3 antagonists showed an improved efficacy profile compared with doxapram in the rat.²⁶ The effect in humans has not been tested as yet.

Alternative respiratory stimulants, which include serotonin and dopamine receptor ligands, while effective in animals, are without significant effect in humans.^{1,5} An exception is the ampakine CX717.²⁴ A recent study showed that oral CX717 increased the slope of the nonsteady-state ventilatory response to hypercapnia during alfentanil-induced respiratory depression, without affecting analgesia in healthy male volunteers (albeit at the expense of enhanced sedation). A caveat of that study is the use of a nonsteady-state approach (rather than a steady-state approach as used in this study) in measuring the ventilatory response to hypercapnia, prohibiting the exact quantification of OIRD (see studies by Berkenbosch *et al.*²⁸ and Bourke *et al.*²⁹ for a discussion on this topic). Ampakines act through activation of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors.^{1,5} Glutamatergic transmission through AMPA receptors within the brainstem respiratory centers (most importantly the preBöttinger complex) plays a crucial role in respiratory rhythmogenesis; AMPA receptor activation leads predominantly to an increase in respiratory frequency and less to the desired increase in tidal volume.

Conclusions

Our studies demonstrated the stimulatory effects of the BK_{Ca}-channel blocker GAL021 on carbon dioxide-clamped ventilation during the condition of established OIRD. In an exploratory study, GAL021 also stimulated poikilocapnic ventilation during alfentanil administration, while it had no impact on sedation, antinociception, hemodynamic, or safety parameters. While our data suggest that GAL021 is an attractive alternative to other respiratory stimulants taken its observed efficacy and favorable side effect profile, the current studies are not definitive. Our studies may be used to power future studies, which should address the ability of GAL021 to reverse OIRD at higher opioid concentrations and respiratory depression induced by other agents (*e.g.*, anesthetics and sedatives) and drug combinations (in clinical studies).³⁰ Furthermore, its effect on nonrespiratory systems should be explored further.

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Competing Interests

James McLeod is employee of Galleon Pharmaceuticals Corp., Horsham, Pennsylvania, the manufacturer of GAL021. All other authors report no conflict of interest. The Leiden University Medical Center respiratory laboratory is currently funded by some other parties involved in opioid-induced respiratory depression and reversal of respiratory depression: Mundipharma (Cambridge, United Kingdom) and Revive Therapeutics (Vaughan, Ontario, Canada).

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Appendix: Alfentanil Assay

Plasma was separated within 15 min of blood collection and centrifuged for 10 min at 3,500 revolutions per min. Plasma samples were immediately stored at –20°C until analysis. For the construction of the calibration curve human control plasma was spiked with adequate volumes of alfentanil dissolved in 50:50 methanol/water to give a concentration range of 0–5,000 ng/ml. For the analysis, 50 μ l plasma samples, standards, blanks, and quality controls were transferred into 1 ml tubes. Proteins were precipitated with 150 μ l acetonitrile, which contained 1,000 ng/ml propranolol (internal standard). The tubes were vortexed for 30 s and centrifuged for 5 min at 3,000 revolutions per min. The supernatant was transferred to a 96-well plate for analysis. Aliquots (10 μ l) were injected by a Shimadzu SIL-5000 auto sampler (Columbia, MD) on an Atlantis T3 column (4.6 \times 50 mm, 5 μ m particle size; Milford, MA), which was used for separation of the analytes from the plasma components. The analytes were eluted using gradient elution with a mobile phase consisting of

aqueous 0.1% formic acid (mobile phase A) and 0.1 % formic acid in acetonitrile (mobile phase B) at a flow rate of 1 ml/min by using the following gradients: 5% mobile phase B for the first 0.3 min, linear gradient from 5 to 100% for 1 min, back to 5% mobile phase 5 in 0.1 min and hold at 5% mobile phase B until the total analysis time of 3 min. The column was coupled to an API Qtrap 4000 mass spectrometer (ABI Sciex Qtrap, Toronto, Canada) for detection. The ions were produced in the positive mode by electro-spray ionization supplied by a turbo spray ion source at 4,500 V and detected in multiple reaction-monitoring mode. Transitions were 417.2 \rightarrow 268.5 for alfentanil and 260.0 \rightarrow 116.0 for propranolol. Retention times were 1.66 and 1.65 min respectively for alfentanil and propranolol.

Correlation coefficients of the calibration curves were above 0.998. The calibration curve was fitted quadratic with a weighting factor of 1/x. The lower limit of quantification was 2 ng/ml. Variations in accuracy and intra- and interday precision were <15% over the calibration range of 2–5,000 ng/ml.