Carbon dioxide responses at extreme conditions: opioid effects and tolerability

Schrier, R.M. van der

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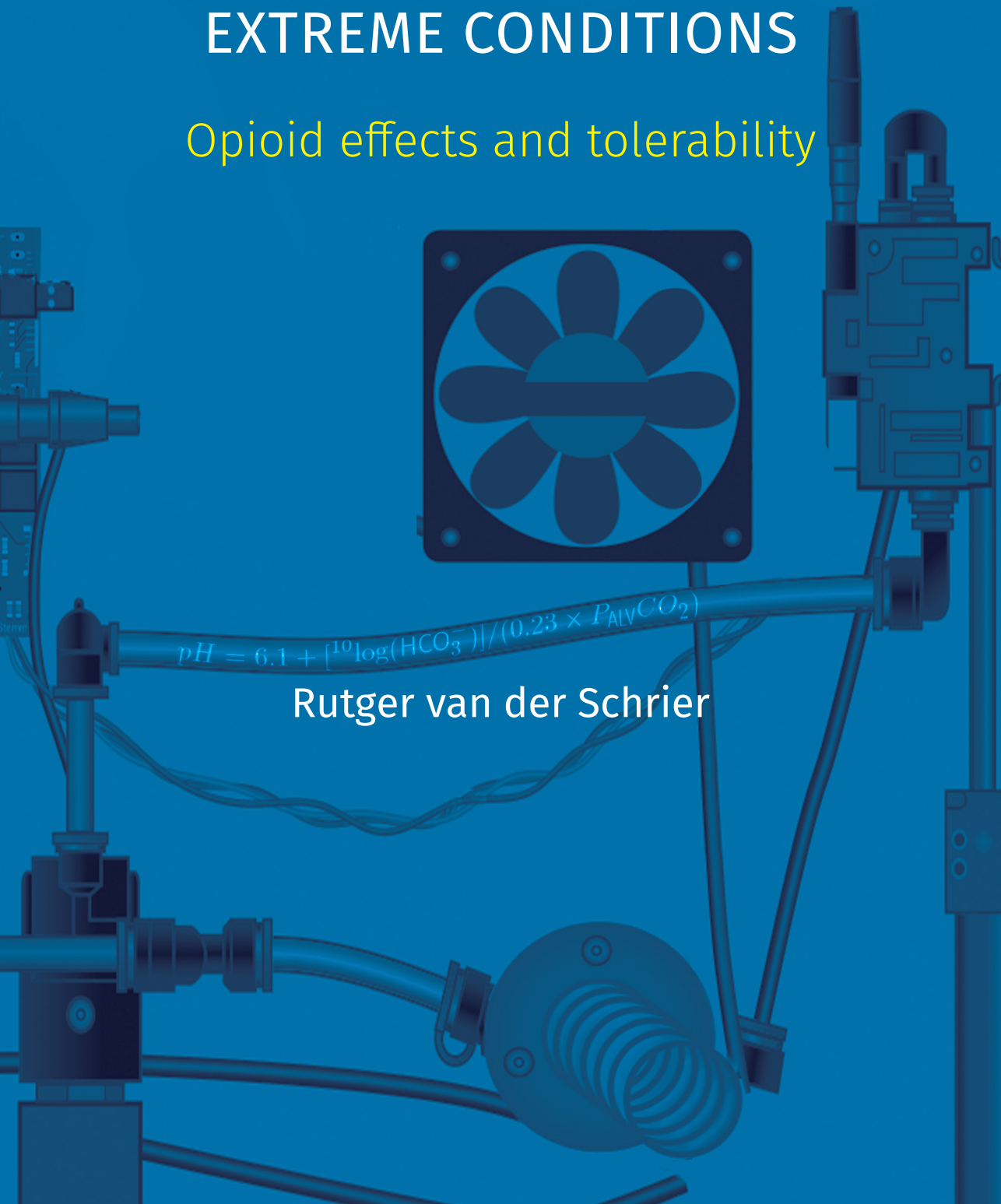
Opioid effects and tolerability



Rutger van der Schrier

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Chapter 1

Introduction

Introduction

The realm of the anesthesiologists has expanded significantly over the last decades. While the anesthesiologist initially provided anesthesia care to the patient during all types of surgery, his or her scope of practice has enlarged to include preoperative screening of patients with often a decisive role in the ultimate management of the patient, the treatment of acute and chronic pain patients, dedicated involvement in the intensive care unit, emergency room and resuscitation teams and involvement in out-of-hospital urgency care. The American surgeon Atul Gawande recounts the patients' life before and after the advent of anesthesia.[1] As he explains "…. anesthesia allowed …. more complex, invasive and precise [patient care]". Today, it is safe to assert that anesthesiologists are vital medical professionals inside most if not all medical centers.

It is important to realize that the scientific foundations of anesthesiology include anatomy, physiology and pharmacology in health and disease. No other medical specialist has a deeper understanding of these subjects than the anesthesiologist. Hence it is not surprising that there are many distinguished experts in these particular domains that were or are anesthesiologists. One such expert was the anesthesiologist (and physicist) John Severinghaus (1922-2021), who is regarded a pioneer not just in anesthesia but also in respiratory physiology. His research on the control of breathing still stands at the basis of our current knowledge of how the acid base system impacts breathing and how opioid drugs impede breathing.[2] Other anesthesiologists who changed the practice of medicine include Virginia Apgar (1909-1974; she contributed to the field of neonatology),[3] Henry Beecher (1904-1976; he contributed to the field of medical ethics and described and explored the mechanisms of placebo analgesia)[4] and Harold Griffith (1894-1985; he established the first postoperative recovery room and was first to use muscle relaxants),[5] to name a few of our colleagues who played crucial roles in developments in and outside of anesthesia.

Importantly, while we have come a long way since the dawn of anesthesia, many outstanding questions related to anesthesia, pharmacology and physiology and their complex interaction remain unanswered. Three such questions are:

- *What is the evolutionary advantage of the ability of anesthetics to induce a state of reversable loss of consciousness in mammals?*
- *What is the evolutionary advantage of opioid-induced respiratory depression, an observation made in most animals?*

and

- *Why are humans less well able to cope with inhaled carbon dioxide compared to other mammals such as the rat (rattus) and naked-mole rat (heterocephalus glaber)?*

These "philosophical" questions are seldom if ever posed, possibly because they are difficult to resolve; they revolve around the pivotal question:

- *What are the mechanisms of action of opioids and anesthetics for wanted and unwanted effects?*

Note that also carbon dioxide and ethanol are anesthetics. While we seem to have a crude idea of their mechanisms of action, the essentials are still poorly defined and differences among various drugs of the same class are not well understood. One such question, for example, is what role the various intracellular transduction pathways play in the various effects that opioids exert, such as analgesia, hyperalgesia, tolerance, gastrointestinal impairement (nausea/vomiting/constipation), sedation, euphoria and dysphoria, dizziness, lightheadedness, confusion and delirium, insomnia, pruritis, vasodilation and orthostatic hypotension, muscle rigidity (wooden chest syndrome), reward, addiction and moderate to potentially fatal respiratory depression.

The above designates that the most important tools of the anesthesiologist and pain specialist, anesthetics but particularly opioids, are associated with serious adverse effects. The most devastating adverse effect is depression of respiration, which is the topic of this thesis. It is currently well known, even to non-medical trained individuals, that opioids do cause life-threatening respiratory depression (opioid-induced respiratory depression or OIRD) and many deaths arise from exogenously consumed opioids for medical purposes or hedonistic pleasure.[6] These exogenous opioids interact with opioid receptors that are part of the endogenous opioid system. This system consists of four opioid receptors (mu-, kappa-, delta - and the atypical nociception-receptor), ubiquitously present in the central nervous system and their endogenous ligands (β-endorphins, enkephalins, dynorphins, and nociceptin/orphanin FQ). The endogenous opioid system is involved in nociception and endogenous pain modulation but has also other roles as was discovered when studying opioid-receptor knockout mice and *in* and *ex vivo* animal preparations.[7–10] One observation was that by acting at the mu-opioid receptor within the brainstem and pontine respiratory networks (especially at the preBötzinger complex and the parabrachial Kolliker-Fuse nucleus), exogenous opioids affect respiratory rhythmogenesis.[8–10] Consequently, exogenously administered high-dose opioids, such as morphine, oxycodone, oxymorphone, methadone or fentanyl, initiate a sequence of events, starting with irregular breathing, followed by cyclic breathing, and eventually apnea (Figure 1), that will eventually lead to complete cardiorespiratory collapse from hypoxia and cardiac dysrhythmia and arrest, if left untreated.[11–13]
And even when treated appropriately with the opioid reversal agent naloxone, cardiorespiratory collapse is difficult to reverse when potent opioids (e.g. carfentanil or highdose fentanyl) are (ab)used or multiple drugs are consumed together, such as opioids and ethanol, antidepressants, benzodiazepines or alpha-2-agonists, etc.[14] The current opioid epidemic in the United States of America, with more than 100,000 deaths per year, exemplifies the devastating effects of opioids on the ventilatory control system.[15]

Most of these individuals died from OIRD; and OIRD is often linked to opioid abuse and

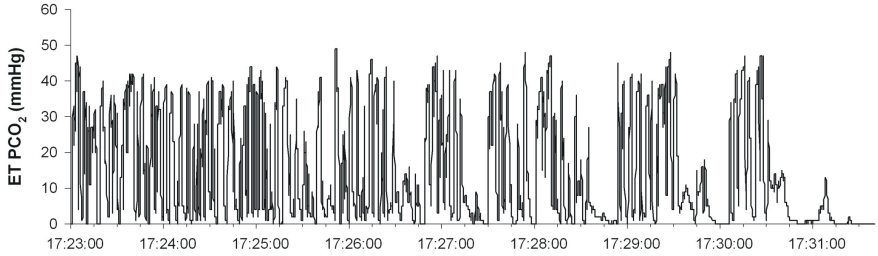

Figure 1: Capnogram of a patient overdosed on morphine. A period of irregular breathing is followed by cyclic breathing, gasping and apnea. Data from Frank Overdyk, with permission.

addiction, other devastating opioid adverse effects. Opioids are at their worst when they are consumed for hedonistic pleasures or in case of addiction. Overdoses then easily occur during compulsive use and loss of control or after a period of abstinence following detoxification or incarceration. Prescription opioids obtained from medical care givers for acute or chronic pain and used inside or outside of the hospital are also associated with OIRD and death.[11–13] This is often related to absence of monitoring, patient (genetic) make-up, underlying morbidity, polypharmacy, diversion or abuse.

In this thesis I address several more mundane questions, most importantly:

- *How do commonly used drugs interact with opioids on ventilatory control?*
- *How can we prevent opioid-induced respiratory depression?*

and finally:

- *How does carbon dioxide interact with the respiratory physiology of mammals?*

These three questions relate to the safety of individuals exposed to opioids or carbon dioxide (CO_2). The rationale for the study of opioids is given above. The rationale for studying CO_2 is manifold: (i) CO_2 is used as a physiological challenge of the ventilatory control system and consequently used to quantify the effect of opioids (as is demonstrated in Chapters 2 and 3); (ii) CO_2 was used in the operating room as respiratory stimulant at the end of surgery by the anesthesiologist to accelerate the recovery from anesthesia; (iii) at high-dose it was used as anesthetic before the availability of modern inhalational anesthetics; (iv) it is still used as agent to euthanize laboratory animals; (v) and possibly most important in current day society, with the increased need for CO_2 capture, storage and transportation of gas to underground or undersea facilities, incidents causing the release of CO_2 may occur and will then cause clouds with high CO_2 levels within populated areas that may be inhaled by bystanders (see Figure 2) with unknown physical and psychological consequences. Because of all of these items, a systematic study of CO_2 is mandated with respect to tolerability and toxicity.

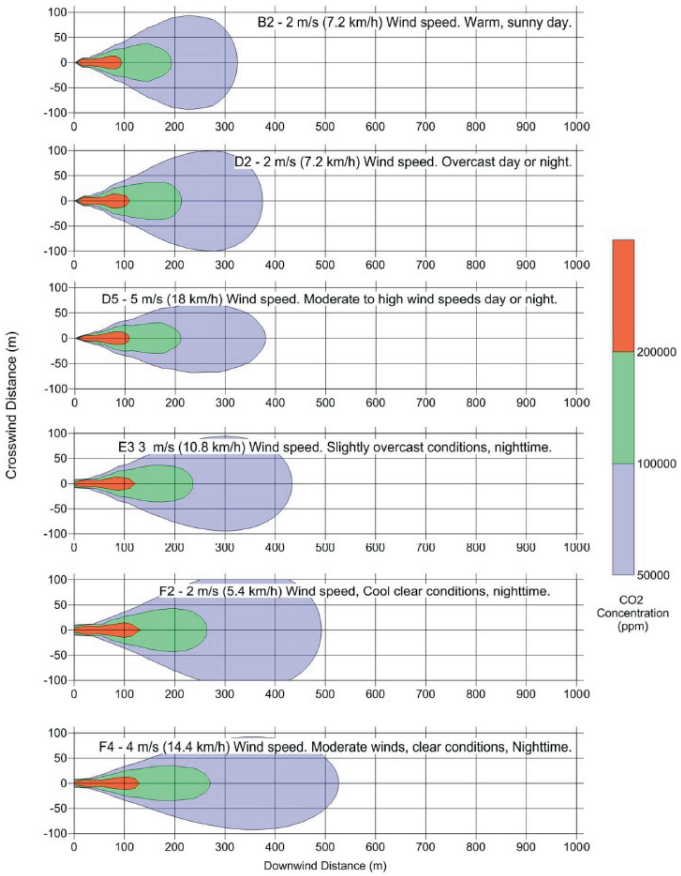

Figure 2: Predicted 1-min average ground-level CO_2-concentrations in parts per million (ppm) at various weather conditions following the release of CO_2 from a ruptured pipeline. Data from Shell Global Solutions International BV, The Hague, with permission.

Summarizing, in this thesis, **Chapter 2** reports on a study on the interactive effects of ethanol and oxycodone on respiratory depression in young and elderly volunteers. **Chapter 3** discusses the effect of the antidepressant paroxetine and antipsychotic quetiapine combined with oxycodone on ventilation in young volunteers. In both studies, the ventilatory control system was studied via the measurement of the hypercapnic ventilatory response. **Chapter 4** gives an update of the large number of reversal agents available or being developed to treat or prevent OIRD. Finally, in **Chapter 5** the tolerability and toxicology of CO_2 as observed in rats and men is discussed. Additionally, a translational model is presented that links the acidbase data between the two species, allowing extrapolation of the animal data to humans.

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Chapter 2

Influence of ethanol on oxycodone-induced respiratory depression

A dose-escalating study in young and elderly individuals

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Introduction

Opioids produce potentially life-threatening respiratory depression through their action at μ-opioid receptors present in brainstem respiratory centers.[1,2] Numerous studies have shown that exogenous opioids decrease the ventilatory responses to hypercapnia and hypoxia, cause irregular breathing and at high dose cause the complete cessation of rhythmic respiratory activity.[1,2] Recent studies indicate that a large number of visits to the emergency department are because of abuse or misuse of legally prescribed opioids (e.g., the use of higher doses than prescribed, the use of prescription opioids in sometimes opioid-naive individuals who are not pain patients) that involved concomitant ethanol consumption.[3,4] Additionally, data from the forensic literature indicate that the combined use of opioids and ethanol is a frequent observation in postmortem blood analysis.[5,6] These data suggest a possibly contributing role of ethanol in the deleterious respiratory effects of (prescription) opioids, possibly due to the enhancement of sedation.[7]

Ethanol has a predominant depressant effect on the central nervous system due to enhancement of γ-aminobutyric acid–mediated and reduction of glutamatergic neuronal transmission.[8,9] Since these receptor systems play an important role in the generation of respiratory activity,[10,11] it is expected that the combination of opioids and ethanol potentiates respiratory depression. Somewhat surprisingly, just few studies addressed the effect of ethanol and its combination with opioids on breathing. Setnik et al.[12] observed a decrease in end-tidal carbon dioxide concentration when 0.7 g/l ethanol was administered on top of 80 mg oral morphine. This suggests a stimulatory effect of ethanol on opioid-induced respiratory depression (OIRD). Studies on the effect of just ethanol are equivocal with respect to its effect on breathing with studies showing a small stimulatory effect,[13–16] no effect,[17] or a depressant effect.[18,19] We relate these divergent outcomes to differences in doses, study methods, and populations tested.

In the current proof-of-concept study, we examined the effect of ethanol (0, 0.5, and 1 g/l) on OIRD induced by 20 mg oral oxycodone. We studied healthy and opioid-naive young adult and elderly volunteers. The main endpoint of our study was ventilation at an end-tidal partial pressure of carbon dioxide of 55 mmHg derived from the steady-state ventilatory response to hypercapnia. Our study is a first to study relatively high opioid and ethanol doses in opioid-naive elderly healthy individuals. Since elderly individuals display an increased sensitivity to opioids and ethanol,[20–22] we used a dose-escalating, single-blind design, allowing us to continuously evaluate the safety of participants and make an informed decision on whether to continue to a next cohort in case of specific issues such as low oxygen saturation or long-term apneas. The relatively high opioid dose was chosen to emphasize the magnitude of OIRD, but our approach is not academic as various studies show the presence of relatively high opioid doses on first prescriptions.[23,24] We hypothesize that ethanol enhances OIRD and that particularly the elderly population is at higher risk for respiratory depression when oxycodone is combined with ethanol. In this study, ethanol was measured in the exhaled air.

Methods

Subjects

This three-way crossover dose-escalating study (within each age cohort) was performed from October 2013 to December 2014. The protocol was approved by the local Institutional Review Board (IRB; Commissie Medische Ethiek, Leiden, The Netherlands) and the Central Committee on Research Involving Human Subjects (CCMO) in The Hague, The Netherlands. Participants were recruited by advertisement in the local newspaper and flyers posted within the facilities of Leiden University (Leiden, The Netherlands). All subjects gave written informed consent before enrollment in the study. After receiving informed consent, the subjects gave their medical history and received a physical examination; then blood chemistry (renal and liver functions) results were obtained. An independent physician who was not part of the research team performed the screening. The study was registered at trialregister.nl under number NTR4123.

Inclusion criteria were the absence of any medical (e.g., current pulmonary, cardiac, renal, or metabolic), neurologic, or psychiatric illness; age 20 to 30 yr (young participants) or 65 yr or older (elderly participants); and a body mass index (BMI) of 18 to 35 kg/m2. The BMI range was relatively large to obtain a representative sample of individuals in the population. Exclusion criteria were abnormalities on physical examination or blood chemistry, a high risk of obstructive sleep apnea as determined by the STOP-BANG questionnaire (score greater than 4),25 pregnancy/lactation, weekly ethanol intake of more than 3 units/day or more than 21 units/week, illicit drug use in 3 months before enrollment or a positive urine dip-stick drug test during screening or on the morning of the study (Alere Toxicology Plc., Oxfordshire, United Kingdom; the stick tests for cocaine, amphetamine, cannabinoids, phencyclidine, methadone, benzodiazepines, tricyclic antidepressants, and barbiturates), current use of any medication, and a Mini Mental State Examination less than 28. The latter exclusion criterion was to prevent that subjects with some form of cognitive impairment participated.

Intervention

Subjects received oral oxycodone on three occasions with at least 2 weeks between study days. On all three visits, the subjects received an intravenous ethanol infusion that was initiated before the 20 mg oxycodone immediate release tablet (Mundipharma Pharmaceuticals BV, The Netherlands) intake but after baseline respiratory measurements. Treatments were not assigned randomly. A dose escalation of the ethanol dose was performed from placebo on visit 1 to breath ethanol concentrations of 0.5 g/l on visit 2 and 1.0 g/l on visit 3, as requested by the IRB. Participants and the research nurses were not informed on the order of treatment.

A clamping method was used to achieve the target pseudosteady-state breath ethanol concentration.[25] The clamping method is based on the fact that after ethanol elimination is fully saturated, the ethanol elimination is constant and independent of concentration. As a result,

a change in infusion rate will result in a proportional change in steady-state blood ethanol concentration within 30 min. Each subjects received an intravenous infusion of 10% ethanol w/v in 5% glucose diluted in 0.9% NaCl. Dilution was necessary to prevent infusion site pain from the initially high infusion rates of ethanol. The initial infusion rate was based on the estimated body water as derived from weight, age, and sex. After 10 min, the infusion rates were adapted according to the measured breath ethanol concentration (Alco Sensor IV meter; Honac Nederland BV, The Netherlands). Once the target steady-state measured breath ethanol concentration was reached and after a set of respiratory measurements, the oxycodone tablet was ingested with 100 ml noncarbonated water.

The expired breath ethanol concentration of 0.5 g/l corresponds to 1 unit/h ethanol in women and 3 units/h in men, while 1 g/l corresponds to 3 units/h in women and 5 units/h in men.

Apparatus

Steps in end-tidal pressure of carbon dioxide ($PETCO_2$) were applied using the dynamic-end-tidal forcing technique. The dynamic-end-tidal forcing technique enables changes in $PETCO_2$ while keeping the end-tidal oxygen concentration constant. The technique is described in detail elsewhere.[26] In brief, subjects breathed through a mask over mouth and nose that was attached to a pneumotachograph/pressure transducer system (#4813; Hans Rudolph Inc., USA) and to a set of mass flow controllers (Bronkhorst High Tech, The Netherlands) for the delivery of oxygen, carbon dioxide, and nitrogen. The mass flow controllers were controlled by a computer running the custom-made RESREG/ACQ software (Leiden University Medical Center, The Netherlands). The software allows for the steering of the end-tidal gas concentrations (by varying the inspired concentration) and the acquisition of respiratory variables. The inspired and expired oxygen and carbon dioxide partial pressures were measured at the mouth using a capnograph (Datex Capnomac, Finland). Heart rate and arterial oxygen saturation measured by pulse oximetry (SpO_2; Masimo Corporation, USA) were measured continuously.

Measurements

Respiratory measurements without inhalation of any inspired carbon dioxide and hypercapnic ventilatory response (HCVR) curves were obtained at four separate periods: before any drug administration, after the measured steady state breath ethanol concentration was reached, and 60 and 120 min after oxycodone administration. Baseline variables were minute ventilation, respiratory rate, tidal volume, SpO_2. Additionally, the number of apneic events was measured in which an apneic event was defined as the absence of inspiratory flow (as measured by the pneumotachograph) for at least 10s measured in the 6 min before carbon dioxide inhalation. To obtain the HCVR, four steps in the end-tidal partial pressure of carbon dioxide were applied with step sizes of 4.5 mmHg (0.6 kPa), 9 mmHg (1.2 kPa), 13.5 mmHg (1.8 kPa), and 18.0 mmHg (2.4 kPa) above resting $PETCO_2$. Each $PETCO_2$ step lasted 6 to 8 min, assuming at least 2 min of

steady-state ventilation. The order of the steps was arbitrarily set. Throughout each HCVR, the end-tidal oxygen partial pressure was maintained at a normoxic level of 105 mmHg (14 kPa). Only in case of desaturations, supplemental inspired oxygen fraction was given (0.5 to 1.0).

Information on subjectively experienced sedation was obtained at baseline and after drug administration. We used a 100-mm visual analogue scale that represents the subjective feeling of alertness/drowsiness and that ranges from 0 (fully alert) to 100 (unable to keep the eyes open).[27]

Safety

Stopping rules were applied when the subject indicated that he or she wanted to discontinue the experiment or in case of an adverse event. Adverse events were defined by loss of respiratory activity for 90 s or longer, despite active stimulation of the subject, end-tidal partial pressure of carbon dioxide greater than 67.5 mmHg, SpO_2 less than 85% for at least 2 min, or any other situation or condition that may have interfered with the health of the participant. Additionally, the investigators were allowed to stimulate the subjects to breathe or give supplemental oxygen in case they felt this was needed to prevent any adverse event. In case of adverse events, continuation into the next cohort was determined in close cooperation with our department's Data Safety and Monitoring Board (Leiden, The Netherlands).

Data and Statistical Analysis

The placebo ethanol infusion before oxycodone intake was defined as the control condition. The study was powered to detect a reduction of the slope of the HCVR by 0.4 L · min-1 · mmHg-1 (SD 0.4) at the combination of oxycodone and a breath ethanol concentration of 1 g/l versus control. Assuming similar variances of the slope before and after treatment, a sample size of 12 per age group would lead to a power greater than 90% to detect these effects at $P < 0.05$. The sample size analysis was performed in SigmaPlot v12.0 (Systat Software Inc., USA). In the event of discontinuation or withdrawal of informed consent, the data were discarded and the participant was replaced. Independent study monitoring was performed. Data analysis was launched after the monitor filed the final report, ensuring that all Good Clinical Practice requirements were met.

The slope of the HCVR was estimated in R (The R Foundation for Statistical Computing, www.r-project.org, accessed January 1, 2017). To that end, data analysis was automated: (1) from the raw data, the medians of the 1-min breath-to-breath minute ventilation were calculated; (2) all measurements obtained without carbon dioxide stimulation (baseline ventilation) and measurements during the final 2 min of each hypercapnic step, representing steady-state hypercapnic ventilation, were selected for further analysis; (3) The HCVR data (VI vs. $PETCO_2$) were fitted to obtain the slope of the HCVR (fig. 1).

To get an indication on the effect of treatment on the variability of breathing, the percentage coefficient of variation (%CV) was calculated for tidal volume and the duration of one

breath (inspiratory time plus expiratory time) during the 6 min before carbon dioxide stimulation, without taking the apneic events into account.

Three time points were included in the statistical analysis: control, ethanol infusion (before oxycodone administration), and ethanol combined with oxycodone. For the latter time point, we had two measurements (60 and 120 min after oxycodone intake). We included the data point that displayed the greatest degree of depression of ventilation in the analysis. We did so to take into account the large intersubject variability in oxycodone pharmacokinetics.[28]

Statistical analysis was performed on respiratory variables without carbon dioxide inhalation (ventilation, respiratory rate, tidal volume, $PETCO_2$, SpO_2, number of apneas, and %CV) and data obtained during carbon dioxide inhalation (HCVR slope and primary endpoint VE55). VE55 is the ventilation at 55 mmHg, which was extrapolated from the HCVR, and takes both the slope and position of the HCVR into account, hence gives a better reflection of the respiratory effect of the intervention.

Data analysis was on the total population, and subgroup analysis was performed (young vs. elderly participants) as a priori specified. The data were analyzed using a population averaged model with infusion (expired breath ethanol concentration 0, 0.5, and 1 g/l), visit and infusion × visit as fixed factors, subject as a repeated statement and baseline values as covariate. For pairwise comparisons, a Bonferroni correction was applied. A linear mixed model with a Poisson distribution and log link was used to test whether significant differences in the number of apneas were observed among treatment levels. Data analysis was performed using SPSS v23.0 for Windows (IBM Corporation, USA); $P < 0.05$ were considered significant. Data are presented as mean (95% CI) or median (range), unless otherwise stated.

Results

Participants and Adverse Events

Forty (13 young and 27 participants in the older age group) subjects were screened, of whom 14 (1 young and 13 elderly) subjects did not participate for logistic reasons (other commitments on the day of the study) or because they did meet exclusion criteria (BMI greater than 35 kg/m2, mini-mental state examination less than 28). Twenty-six subjects were treated, of whom one young and one elderly female subjects terminated the study early due to discomfort. Twenty-four participants (12 young and 12 elderly), all Caucasians, completed all three sessions; their characteristics are given in table 1. Mean (range) age of the young participants was 24 (21 to 28) yr and that of the participants in the elderly group was 70 (66 to 77) yr. Mean measured breath ethanol concentrations over time are given in figure 1. An example of the effect of 1 g/l ethanol combined with oxycodone on the HCVR obtained in one elderly participant is given in figure 2. All young subjects completed the study without any serious adverse effects. Two elderly subjects required supportive care because of SpO_2 levels of less than 80%.

Table 1: Participant characteristics

	Young Participants	Elderly Participants
Sex distribution (M/F)	6/6	6/6
Age, yr	24 ± 2 (21–28)	70 ± 4 (66–77)
Weight, kg	75 ± 11 (59–89)	70 ± 10 (61–81)
Height, cm	181 ± 8 (170–193)	169 ± 8 (162–186)
BMI, kg/m^2	22.9 ± 2.3 (18.6–26.5)	24.4 ± 2.7 (21.7–31.6)
Weekly ethanol intake (all)	10 ± 4 (4–15)	9 ± 7 (2–21)
Weekly ethanol intake, men	11 ± 4 (10–15)	9 ± 7 (2–21)
Weekly ethanol intake, women	10 ± 4 (4–8)	10 ± 8 (2–21)

Values are mean ± SD (range). One unit of ethanol intake equals a consumption of 250 ml of 5% alcohol.
BMI = body mass index; F = female; M = male.

They were stimulated to take several deep breaths upon which their oxygen saturation increased to values greater than 90% within 2 min. Hence, their actual decline in oxygen saturation is partly masked by these per-protocol interventions. No other adverse events occurred in the elderly population.

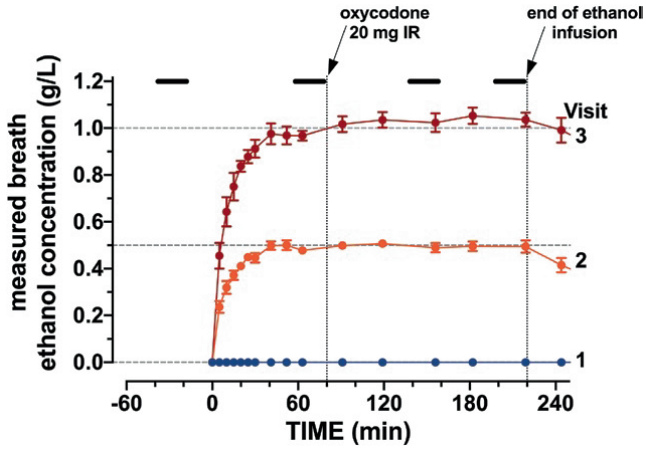

Figure 1: Measured breath ethanol concentration profiles (mean and 95% CIs) obtained during infusion of 0, 0.5, and 1.0 g/l. At t (time) = 0 min, the infusion of ethanol started; at t = 80 min, a 20 mg oxycodone immediate release (IR) tablet was ingested. Respiratory measurements are indicated by the black bars.

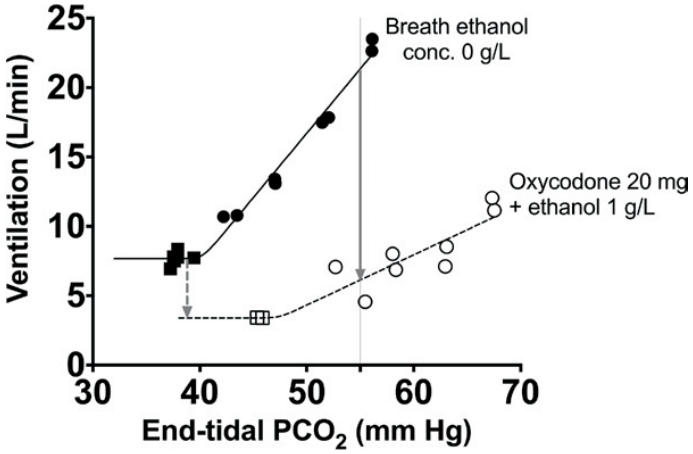

Figure 2: Ventilatory response to hypercapnia of an elderly participant under placebo conditions (breath ethanol concentration of 0 g/l infusion) and during coadministration of ethanol (breath concentration of 1 g/l) and 20 mg oxycodone immediate release. Squares denote resting data points without added inspired carbon dioxide; circles denote data obtained at added inspired carbon dioxide. All data points are 1-min median values obtained at steady-state ventilation. The line through the data is the data fit. The horizontal part of the data fit represents the estimation of resting ventilation, the linear ramp is the hypercapnic ventilatory response curve. The continuous vertical gray line depicts VE55 or the extrapolated minute ventilation at an end-tidal partial pressure of carbon dioxide (PCO_2) of 55 mmHg; the continuous gray arrow is the decline in VE55 due to oxycodone and 1 g/l ethanol. The broken gray arrow is the decline in resting ventilation due to oxycodone and 1 g/l ethanol.

Sedation

Ethanol had no effect on the sedation visual analogue scale (max. score 21 mm on the 100-mm scale; $P > 0.05$ vs. control); however, the intake of oxycodone did result in significant sedation (sedation range 45 to 47 mm among treatments; $P < 0.001$ vs. control), which was independent of the measured breath ethanol concentration (table 2).

Apnea

Apneic events were observed in 11 (of the 12) young participants (total number of events 68), with more than two events occurring in at least one measurement window in four subjects. In this subpopulation, we counted 42 apneic events of which 8 occurred during the combination of oxycodone and ethanol 1 g/l (table 3; fig. 3). In the elderly group, 11 (of 12) participants had at least one apneic episode (total number of events 120). In nine participants, there were more than two events in at least one of the measurement windows. In this subpopulation, there were 118 apneas, with 54 occurring when oxycodone was combined with ethanol 1 g/l. Comparing the two age groups showed a greater number of apneic events in the older subjects with respect

Table 2: Influence of Ethanol at Target Breath Concentrations of 0, 0.5, and 1 g/l, 20 mg oxycodone, and Their Combination on Respiratory Variables and Sedation Score

Alcohol Concentration	Baseline	Ethanol Infusion	Oxycodone 20 mg + Ethanol Infusion
Number of apneic events lasting >10 s			
0 g/l	0 (0–5)	0 (0-5)	1 (0–3) *
0.5 g/l	0 (0–4)	0 (0–5)	1 (0–8) †
1.0 g/l	0 (0–5)	0 (0–3)	1 (0–11) †
Ventilation, l/min			
0 g/l	8.0 (7.4–8.5)	8.0 (7.5–8.5)	5.8 (5.4–6.2) ‡
0.5 g/l	8.2 (7.5–8.9)	7.5 (6.9–8.1)	5.1 (4.4–5.7)
1.0 g/l	8.1 (7.6–8.6)	8.0 (7.5–8.5)	4.7 (4.1–5.4) †
Tidal volume, ml			
0 g/l	734 (641–827)	684 (611–757)	529 (469–590) ‡
0.5 g/l	722 (619–825)	638 (573–702)	504 (455–552)
1.0 g/l	691 (602–780)	642 (562–722)	468 (408–527)
Respiratory rate, min-1			
0 g/l	12 (10–13)	13 (11–14)	11 (10–11) *
0.5 g/l	12 (11–13)	12 (11.3–14)	10 (9–11)
1.0 g/l	13 (11–14)	13 (11–14)	9 (8–10)
End-tidal PCO_2, mmHg			
0 g/l	37 (35–38)	37 (35–38)	41 (39–44) ‡
0.5 g/l	36 (35–38)	37 (35–38)	44 (42–45) †
1.0 g/l	37 (35–38)	36 (34–38)	44 (42–46) †
Oxygen saturation, %			
0 g/l	98 (98–99)	98 (97–99)	94 (92–95) ‡
0.5 g/l	98 (97–99)	96 (96–97)‡	90 (87–92) †
1.0 g/l	98 (97–98)	97 (96–98)‡	89 (86–93) †
Slope of the HCVR, l * min-1 * mmHg-1			
0 g/l	2.0 (1.7–2.3)	2.0 (1.6–2.4)	1.4 (1.2–1.6) *
0.5 g/l	2.1 (1.7–2.5)	2.0 (1.6–2.4)	1.4 (1.2–1.6)
1.0 g/l	2.0 (1.7–2.4)	1.8 (1.5–2.0)	1.3 (1.0–1.5)
Ve55, l/min			
0 g/l	33.2 (28.1–38.2)	33.4 (27.9–39.0)	18.6 (15.6–21.6) ‡
0.5 g/l	33.9 (28.4–39.4)	33.5 (28.3–38.7)	16.3 (13.3–19.3) †
1.0 g/l	34.6 (29.5–39.8)	33.4 (29.4–37.4)	15.7 (12.7–18.6) †
Sedation score, mm			
0 g/l	8 (4–12)	12 (7–17)	47 (38–56) ‡
0.5 g/l	5 (3–7)	12 (8–17)	45 (36–54)
1.0 g/l	4 (3–6)	16 (12–21)	46 (36–55)

Values are mean (95% CI), except for number of apneas, which are median (range); VE55 is the extrapolated ventilation at an end-tidal partial pressure of carbon dioxide of 55 mmHg and is derived from the estimated slope of the hypercapnic ventilatory response slope.
*P < 0.01 vs. control (0 g/l ethanol infusion without oxycodone). †P < 0.01 vs. oxycodone and 0 g/l concomitant ethanol infusion. ‡P < 0.001 vs. control. HCVR = the hypercapnic ventilatory response; PCO_2 pressure of carbon dioxide.

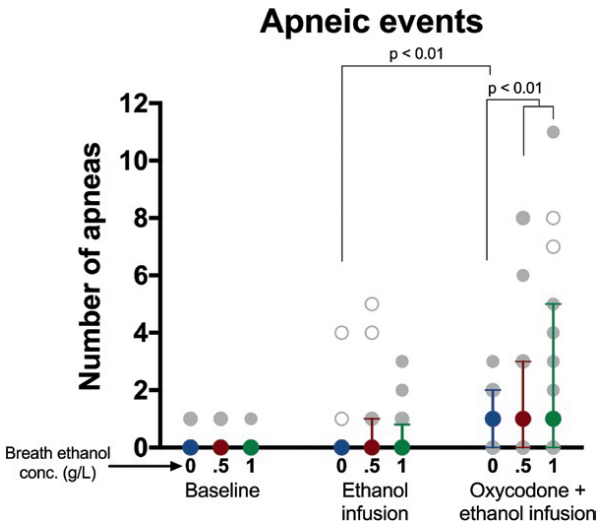

Figure 3: Effect of 0, 0.5, 1 g/l ethanol (breath concentration), 20 mg oxycodone immediate release tablet, and their combination on apneic events. Open circles are the data from the young subject population, closed gray circles from the elderly population. Blue data points are median values ± interquartile range (IQR) at a breath ethanol concentration of 0 g/l; red data points are median values ± IQR at a breath ethanol concentration of 0.5 g/l; green data points are median values ± IQR at a breath ethanol concentration of 1 g/l.

to total events and those occurring during oxycodone plus ethanol 1 g/l ($P = 0.02$, Fisher exact test). In all subjects, apneic events were preceded by irregular breathing (increase in %CV, see the Variability subsection below) and reduced respiratory rates.

Oxygen Saturation, Ventilation, and End-tidal Pco_2

Ethanol infusion alone had just a modest effect on SpO_2 (reduction by 2 and 1% at 0.5 and 1.0 g/l ethanol, respectively; table 2; fig. 4) without affecting any of the other variables. Oxycodone had a significant depressant effect on ventilation. Combining oxycodone with ethanol produced a significant additional increase in respiratory depression, which was reflected by the further decrease in ventilation, with the lowest ventilation observed during the combined treatment of 1 g/l ethanol and oxycodone (mean ventilation 4.7 [4.1 to 5.2] l/min; table 2; fig. 4). At this treatment level, a significant age effect was detected with greater depression of ventilation in the elderly group (young, 5.4 [4.6 to 6.2] l/min vs. elderly 4.1 [3.2 to 5.0] l/min; $P = 0.04$). Additional signs of respiratory depression from the ethanol oxycodone combination were the increase in $PETCO_2$ and reduction in SpO_2, with similar effects at the two ethanol levels (mean increase in $PETCO_2$ 5, 8, and 8 mmHg and decrease in SpO_2 by 5, 8, and 8% at breath ethanol concentrations of 0, 0.5, and 1 g/l, respectively; table 2; fig. 4).

Hypercapnic Ventilatory Response

Ethanol had no effect on the slope of the HCVR curve (table 2). Oxycodone did reduce the slope by 30 to 40% with no further effect from concomitant ethanol administration. The VE55 or the ventilation at an extrapolated endtidal carbon dioxide concentration of 55 mmHg takes both the HCVR slope and its position into account, and hence gives a better reflection of the respiratory effect of the intervention. Oxycodone but not ethanol affected VE55 significantly with a 44% reduction relative to baseline values 60 to 120 min after oxycodone intake ($P < 0.001$ vs. control). Adding ethanol further reduced VE55 to 52% (0.5 g/l) and 55% (1.0 g/l) of baseline values ($P < 0.01$ vs. control; fig. 4).

Variability

The variability data are given in fig. 5, A and B, which show that for tidal volume the mean %CV increased significantly to 55% (47 to 63%) at the combination of oxycodone and 1 g/l ethanol ($P < 0.01$ vs. oxycodone and oxycodone plus ethanol 0.5 g/l). Similar observations were made for breath time (the sum of inspiratory time and expiratory time) with the highest variability observed during the combination of oxycodone and 1 g/l ethanol (49% [41 to 57%], $P < 0.05$ vs. oxycodone).

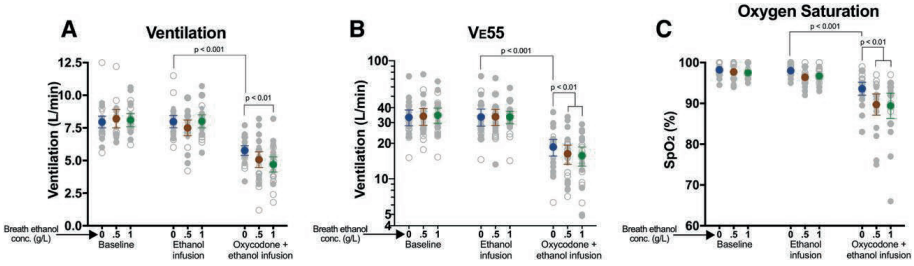

Figure 4: Effect of 0, 0.5, 1 g/l ethanol (measured breath concentration), 20 mg oxycodone immediate release tablet, and their combination on minute ventilation (A), VE55 (B), and oxygen saturation (C). Open circles are the data from the young subject population, closed gray circles from the elderly population. Blue data points are mean values ± SD at a breath ethanol concentration of 0 g/l; red data points are mean values ± SD at a breath ethanol concentration of 0.5 g/l; green data points are mean values ± SD at a breath ethanol concentration of 1 g/l. SpO2 = oxygen saturation measured by pulse oximetry.

Age Effect

Apart from number of apneic events and ventilation, no age effects were observed for any of the respiratory variables.

Table 3: Number of Apneic Events in the Young Participants and Participants in the Elderly Group at the Three Breath Alcohol Concentrations Observed in Visits 1–3

	Baseline			Ethanol Infusion			Oxycodone Plus Ethanol Infusion		
Target breath alcohol concentration, g/l	0	0.5	1.0	0	0.5	1.0	0	0.5	1.0
Young participants (21–28 yr)									
Participant 1	0	1	2	1	4	1	0	1	0
Participant 2	0	0	0	0	0	1	0	0	0
Participant 3	0	0	2	0	0	0	0	0	0
Participant 4	1	4	1	0	0	0	0	0	0
Participant 5	0	1	0	1	0	0	0	0	0
Participant 6	0	0	0	0	0	1	0	0	0
Participant 7	0	0	2	0	1	1	0	2	1
Participant 8	0	0	0	0	1	8	0	1	7
Participant 9	0	0	1	0	5	3	0	0	1
Participant 10	0	0	1	0	0	1	0	0	0
Participant 11	0	0	0	0	1	0	0	0	0
Participant 12	0	0	0	0	0	0	0	0	1
Range	0-1	0-4	0-2	0-1	0-4	0-8	0-0	0-2	0-7
No. of subjects with >2 apneic events	0	1	0	0	2	2	0	0	1
Participants in the elderly group (66–77 yr)									
Participant 13	0	0	0	0	0	0	0	0	0
Participant 14	0	0	1	0	1	1	1	1	5
Participant 15	0	0	3	0	0	3	0	0	2
Participant 16	0	0	1	0	0	0	0	0	1
Participant 17	1	0	2	0	1	3	0	0	11
Participant 18	1	0	1	0	1	6	0	2	3
Participant 19	0	0	0	0	0	1	0	0	0
Participant 20	0	0	1	0	0	1	0	0	5
Participant 21	0	0	2	0	0	3	0	0	2
Participant 22	0	0	3	0	0	1	0	3	4
Participant 23	0	0	1	1	1	8	0	0	11
Participant 24	0	0	1	1	0	8	0	0	11
Range	0-1	0-0	0-3	0-1	0-1	0-8	0-1	0-3	0-11
No. of subjects with >2 apneic events	0	0	2	0	0	6	0	1	7

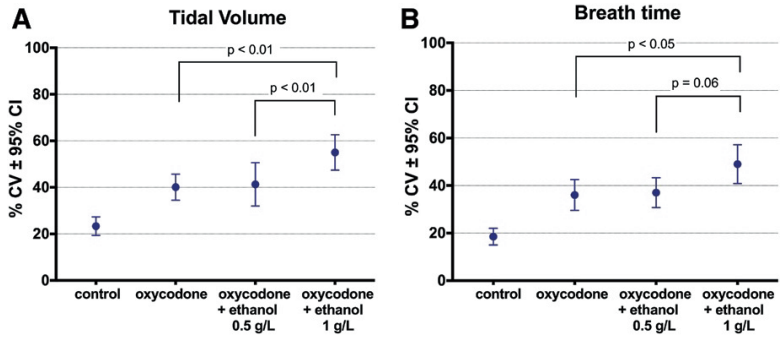

Figure 5: Variability of tidal volume (A) and breath time (= inspiratory + expiratory time; B) during exposure to 20 mg oxycodone immediate release and the combination of 20 mg oxycodone and 0.5 and 1.0 g/l ethanol. Values are mean ± 95% CI. CV = coefficient of variation.

Discussion

We performed a proof-of concept trial on the coadministration of immediate release 20 mg oxycodone and two levels of ethanol (breath concentrations of 0.5 and 1.0 g/l) on the control of breathing in a mixed population of young and elderly opioid-naive participants. We observed that 20 mg oxycodone significantly reduced minute ventilation, the slope of the HCVR, and VE55. Baseline ventilation and VE55 were further impaired by the concomitant administration of ethanol, independent of dose. Especially, but not exclusively, in the elderly population, the ethanol oxycodone combination produced repeated apneic events (table 3) resulting in frequent episodes of oxygen desaturation.

Except for some small drops in SpO_2, we did not observe major effects of 0.5 and 1.0 g/l ethanol on ventilatory control. In contrast, when combined with oxycodone, ethanol enhanced the OIRD. Although our study was not mechanistic in nature, it is important to discuss possible underlying mechanisms. The lack of major effects on ventilatory control from ethanol (despite the occurrence of mild sedation) is in close agreement with previous observations, showing either no effect of ethanol on the control of breathing or some small stimulatory effects.[13–17] This may be related to a persistent rise in brain network activity, observed at low ethanol concentrations.[29] The interactive effect on OIRD is likely due to an effect of ethanol on oxycodone pharmacokinetics or pharmacodynamics: (1) possibly ethanol increased the oxycodone concentration in plasma as is observed for other opioids although this is reported mostly after oral ethanol ingestion[30]; (2) ethanol may have increased the level of sedation induced by the opioid.[7] Although this did not seem the case in our study, as the level of self-reported sedation was not further increased by ethanol (table 2), the reliability of self-assessment may be diminished at higher ethanol levels with a false overoptimistic perception of functional ability

commonly observed in young and older ethanol users.[20,31] Animal studies do indicate the enhancement of sedation as cause of ethanol–opioid interaction on OIRD[7]; (3) ethanol may have interacted with oxycodone at the level of the μ-opioid receptor.[32] Possible scenarios are the rapid sensitization of μ-opioid receptors by ethanol and/or ethanol-induced increase in density of opioid receptors in areas of the brain involved in the control of breathing; (4) finally, the negative effect of ethanol and oxycodone on ventilatory control may be due to the combination of ethanol-induced enhanced γ-aminobutyric acid–mediated and inhibited glutamatergic neurotransmission and opioid-induced opioidergic neuronal inhibition within the respiratory networks of the brainstem.[1,2,8,9] All of these mechanisms remain speculative at present, and further studies are required to understand the complex interaction of opioids and ethanol on the control of breathing in young and elderly humans. We relate the observation that the elderly population shows greater respiratory depression than our younger participants to the higher sensitivity of the elderly to opioids and ethanol, to their lesser resilience and physiologic reserve, and possibly to greater oxycodone plasma concentrations.[20–22]

We studied a single dose of oxycodone in opioid-naive individuals and therefore cannot predict the effect of ethanol on OIRD in chronic opioid users and abusers from our data. However, recent animal work indicates that tolerance to opioid analgesia is not associated with tolerance to the respiratory effects of opioids.[33] Moreover, ethanol interacts with synaptic transmission in opioid pathways and inhibits or reverses the development of tolerance to OIRD due to sensitization of μ-opioid receptors expressed on neurons in the respiratory centers of the brainstem.[32] Additionally, forensic studies show that ethanol is associated in a high percentage of opioid mortality cases.[5,6] We may assume that many of these fatalities were not naive to opioid consumption.[34] All together, these findings suggest that our experimental clinical study may be applicable to individuals who regularly consume opioids for medical or hedonistic reasons.

In common with previous observations in rodents and humans,[35,36] we observed an increase in respiratory variability with oxycodone (fig. 5). Ethanol further increased the variability in tidal volume and breath time (the sum of inspiratory and expiratory times) with the greatest variability observed at the combination of oxycodone and 1 g/l ethanol. Importantly, the increase in respiratory variability preceded apneas in all volunteers independent of age. This indicates that variability may be an important predictor of the severity of OIRD and possibly also of imminent respiratory adverse events.

Study Limitations

The design of our study was dose escalating and single blind as requested by our IRB. The committee was most concerned with the safety of the elderly participants. We observed frequent apneic episodes in nine (i.e., more than two events) elderly volunteers and episodes of low SpO_2 below 85% in two of these subjects. Due to our rapid intervention with supplemental oxygen and verbal stimulation, the drops in SpO_2 were short-lived. Therefore, it was decided, after consultation with the data safety monitoring board, to keep the subjects in the study

and/or have them proceed to the next cohort. This chain of decision-making was the reason for nonrandomization. How much the absence of randomization and double blinding affected the outcome of our study remains unknown. We may assume that our safety interventions caused underestimation of the true respiratory depressant effects of oxycodone and ethanol treatment, especially in the elderly population.

We did not measure oxycodone concentrations in plasma. The pharmacokinetics of oral oxycodone is highly variable.[28] To take into account some of this variability, we measured the respiratory effects at multiple time points after oxycodone ingestion and report peak effect, which we associate with the peak concentration of the opioid at its target site. Further pharmacokinetic–pharmacodynamic modeling studies will enable the full characterization of the interactive effects of ethanol and oxycodone on the ventilatory control system. Our current study suggests an additive interaction of ethanol on OIRD; however, our data were obtained over a rather restrictive concentration range for obvious safety reasons. At higher ethanol and opioid concentrations, greater respiratory effects will occur due to a further increase in central nervous system depression (from both ethanol and opioids), loss of upper airway patency and further depression of respiratory centers in the brainstem. Given the safety issues observed, we do not believe that studies outside the concentration ranges tested by us in the current study, in especially elderly volunteers, are desirable.

In conclusion, our studies in healthy volunteers provide evidence that combining prescription opioids with ethanol leads to significant and clinically relevant levels of respiratory depression and increases the risk of toxicity through a pharmacologic interaction, an effect that is more pronounced in elderly individuals.

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Chapter 3

Effect of Paroxetine or Quetiapine Combined With Oxycodone vs Oxycodone Alone on Ventilation During Hypercapnia

A Randomized Clinical Trial

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Introduction

Ventilation in humans is tightly controlled by feedback mechanisms involving carbon dioxide.[1,2] When chemical receptors in the brain and the carotid body sense increased carbon dioxide, ventilation increases to remove carbon dioxide from the body.[1,2] Opioids decrease this ventilatory response to hypercapnia (Figure 1),[2–5] which can lead to severe respiratory depression and death.[6] Some other drugs, such as benzodiazepines, have minimal effects on ventilation on their own at standard doses, but can exacerbate opioid induced respiratory depression.[7]

In 2016, the US Food and Drug Administration (FDA) required that drug labeling for benzodiazepines and opioids include boxed warnings about increased potential for respiratory depression with their simultaneous use.[7] Following this labeling change, the FDA took proactive steps to review whether other drugs that might be used in place of benzodiazepines (as prescribed or off-label) may exacerbate opioid induced respiratory depression and conducted in vivo rat studies with 14 drugs from diverse pharmacological classes.[8] The selective serotonin reuptake inhibitor (SSRI) paroxetine and the atypical antipsychotic quetiapine exacerbated oxycodone induced respiratory depression.[8] To further investigate these findings, this clinical trial involving healthy participants assessed whether paroxetine-oxycodone or quetiapine-oxycodone combinations decreased the ventilatory response to hypercapnia compared with oxycodone alone.

Methods

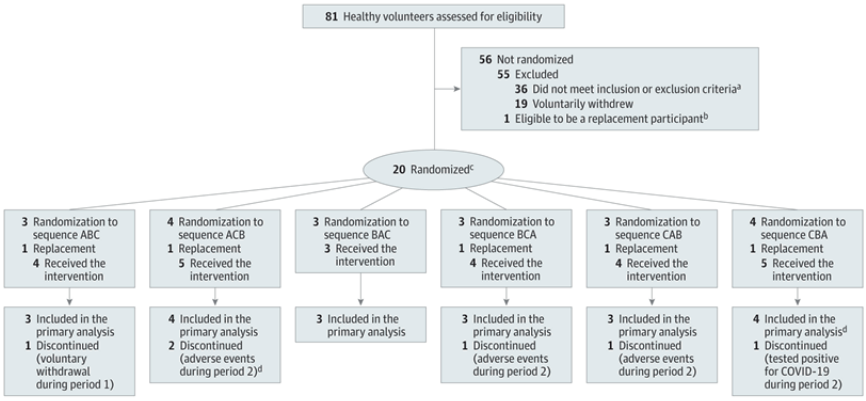
Study Setting and Dates

A randomized, double-blind, 3-way crossover trial involving healthy participants at a clinical pharmacology unit (Spaulding Clinical Research, West Bend, Wisconsin) from January to May 2021 evaluated the effects of paroxetine or quetiapine combined with oxycodone, compared with oxycodone alone, on the ventilatory response to hypercapnia (Figure 1). The Advarra Institutional Review Board approved this study (https://www.advarra.com). All participants provided written informed consent.

Participants and Randomization

Participants were recruited by standard approaches for healthy volunteer clinical pharmacology studies (ie, online advertising and emails or texts to individuals in the site's database). Self-identified race and ethnicity were collected in an open ended format by clinical staff as recommended by the FDA's guidance document Collection of Race and Ethnicity Data in Clinical Trials.[9] Key inclusion criteria were ages 18 to 50 years, nonsmoking, and negative test results for alcohol or illicit drugs. Participants were excluded if they had a history of sleep disorders, panic disorder, panic attacks, generalized anxiety disorder, hypoventilation syndrome, or sleep apnea; used opioid or psychotropic drug within 60 days of the study start; had a Mallampati

A Flow of participants in the study



B Study drug interventions

Treatment group	Study drugs[e]				
	Day 1	Day 2	Day 3	Day 4	Day 5
A	Placebo + oxycodone 10 mg	Placebo	Placebo	Placebo	Placebo + oxycodone 10 mg
B	Paroxetine 40 mg + oxycodone 10 mg	Paroxetine 40 mg	Paroxetine 40 mg	Paroxetine 40 mg	Paroxetine 40 mg + oxycodone 10 mg
C	Quetiapine 50 mg 2/d + oxycodone 10 mg	Quetiapine 100 mg 2/d	Quetiapine 150 mg 2/d	Quetiapine 200 mg 2/d	Quetiapine 200 mg + oxycodone 10 mg

C Illustration of the ventilatory response to hypercapnia at baseline[f]

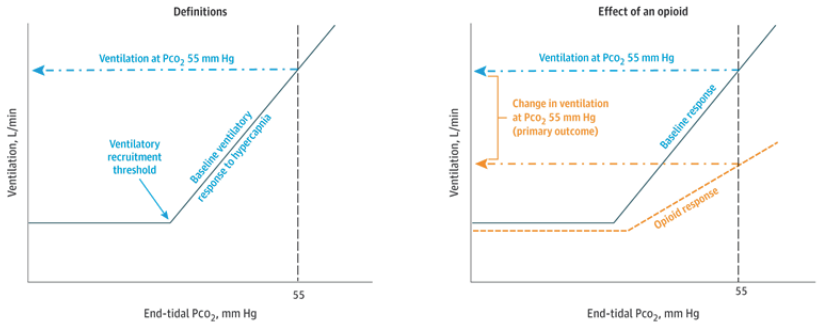


Figure 1: Flow of Participants in Study, Interventions and Overall Study Design:
a. Ten participants had a Mallampati score greater than 2, (predicts difficult tracheal intubation); 5, deemed unlikely to comply with protocol; 5, tested positive for alcohol or illicit drugs; 7, abnormal medical history, laboratory results, or physical examination findings
b. Participant was not needed as a replacement.
c. Five participants replaced the 6 who did not complete all treatment periods. The study design planned for 5 replacements.
d. One participant was included in the primary analysis for only day 1, after which the participant discontinued.
e. See the Methods section for timing of study drug administration. Participants received 4mg of ondansetron 30 minutes before each dose of oxycodone on days 1 and 5 only to prevent nausea and vomiting.
f. Ventilation increases at an approximately linear rate after carbon dioxide (PCO_2) is higher than the ventilatory recruitment threshold (VRT). The opioid causes small decreases in ventilation below the VRT, shifts the VRT to the right, and decreases the rate of rise in ventilation as PCO_2 increases further.

score (predicts difficult tracheal intubation) greater than 2; or could not tolerate the ventilatory assessment procedure during screening.

Participants were randomized to 1 of 6 treatment sequences (Figure 1) using a random number generator in R statistical software. Randomization was conducted in block sizes of 6 for the first 18 participants, and the remaining 2 participants were randomly assigned in 2 of the 6 treatment sequences. Replacement participants were assigned to the treatment sequence of the participant they replaced.

Study Procedures and Interventions

Participants checked in to the clinic the day before the study started, received study drugs on days 1 through 5 (oxycodone on days 1 and 5, and paroxetine or quetiapine [or matched placebos] on days 1 through 5), and checked out on day 6. (See Figure 1 for study drug dosing details.) This was repeated twice with 7 days ofwashout between periods. Study drugs were administered to align the time of maximum concentration for all drugs at the 5-hour time point (paroxetine at 0 hours, oxycodone at 3 hours, quetiapine at 3 and 14 hours). Each period included 16 ventilatory assessments (0 [predose], 4, 5, 6, 8, and 24 hours on days 1 and 5 and 0, 4, 5, and 6 hours on day 4) and 26 blood samples (0 [predose], 3, 4, 5, 6, 8, 9, 12, and 24 hours on days 1 and 5 and 0, 3, 4, 5, 6, 8, 9, and 12 hours on day 4). Plasma concentrations of paroxetine, quetiapine, oxycodone, and selected metabolites were measured by validated liquid chromatography and tandem mass spectrometry.

Participant safety was monitored with clinical laboratory tests, vital signs, electrocardiograms, and physical examinations. Continuous pulse oximetry and telemetry were performed on days when oxycodone was administered,and naloxone was available for participants with severe respiratory depression. Criteria for discontinuation of the study drugs included apnea defined as discontinuation of rhythmic breathing for more than 90 seconds, end-tidal carbon dioxide higher than 67.5 mmHg, or oxygen saturation less than 85% lasting more than 2 minutes.

Ventilatory Assessments

During each assessment, participants sat in an upright position with a fitted mask attached to a pneumotachometer and went through preparatory steps of relaxed breathing (5 minutes of room air then 3 minutes of 100% oxygen), hyperventilation to decrease end-tidal carbon dioxide (1-2 minutes 100% oxygen), followed by rebreathing.[10,11] Upon switching the circuit to the rebreathing bag (7% carbon dioxide, 93% oxygen),participants were instructed to take 3 deep breaths and then breathe normally. This causes approximate equilibration of carbon dioxide in mixed venous blood, arterial blood, brain, and lung with the rebreathing bag.[1,11] Subsequently, carbon dioxide increases at an approximately linear rate as exhaled carbon dioxide is rebreathed through the closed circuit, which increases ventilation above a certain carbon dioxide threshold (Figure 1).[1,12] The procedure continued until end-tidal carbon dioxide was approximately 55 mm Hg. Rebreathing data were reviewed by 2 independent assessors blinded to

study treatment and time of assessments to evaluate completeness of data for study outcomes.

Outcomes and Sample Size Calculation

The primary end point was the minute ventilation when endtidal carbon dioxide was 55 mmHg (Figure 1), which has been used in prior drug-induced respiratory depression studies.[4,5] The primary outcome comparisonswere performed between paroxetine or quetiapine combined with oxycodone vs placebo combined with oxycodone, assessed separately on days 1 and 5. Day 1 was included because quetiapine can cause more sedation after 1 dose than after 5 days of dosing,[13] and it was not known if a similar pattern would be observed with ventilation. Comparisons between paroxetine or quetiapine alone vs placebo on day 4 were secondary outcomes.

Additional secondary outcomes included the maximum plasma concentration and area under the curve (AUC) for plasma concentration vs time of oxycodone when combined with paroxetine or quetiapine compared with oxycodone with placebo. Multiple exploratory outcomes were assessed as specified in the protocol and statistical analysis plan, including pharmacokinetic parameters for paroxetine and quetiapine, additional respiratory measurements including during relaxed room-air breathing, sedation assessments, and pharmacokinetic-pharmacodynamic (concentration-response) modeling. Although reporting summary statistics for exploratory outcomes was prespecified, comparisons between study treatments for the exploratory outcomes were a post hoc assessment. In addition, study drug maximum plasma concentration and AUC were compared based on cytochrome-P450 2D6 (CYP2D6) metabolizer phenotype status as a post hoc assessment.

Sample size requirements were calculated based on 2 primary outcomes (day 1 and day 5) and adjusted for multiplicity (α = .025). The assessments with paroxetine or quetiapine were considered as separate experiments. A sample size of 20 participants was determined to have 90% power at a 1-sided significance level to detect a 4-L/min decrease in the primary end point (ventilation at 55 mmHg end-tidal carbon dioxide) assuming a standard deviation of 5 L/min, based on prior opioid ventilatory studies.[4,5] A 4-L/min decrease was the estimated approximate effect size from 10mg of oxycodone and would indicate that paroxetine or quetiapine was further decreasing hypercapnic ventilation by a similar amount.[4,5] The protocol allowed for enrollment of up to 5 replacement participants to account for discontinuations.

Statistical Analysis

All participants who completed paired rebreathing assessments with placebo plus oxycodone and at least 1 of the other 2 study treatments (paroxetine plus oxycodone or quetiapine plus oxycodone) for day 1 or day 5 were included in the primary analysis without imputation of missing data. Study treatments were compared using a linear mixed-effects model with baseline ventilation at an end-tidal carbon dioxide of 55 mmHg as a continuous variable; treatment, sequence,and period as categorical variables; and participant as a random effect. A similar analysis was performed on day 4 as a secondary outcome. For pharmacokinetic analyses, all

concentrations less than the lower limit of quantitation were considered 0. Maximum oxycodone concentration and AUC were log-transformed and the values between study treatments were compared using a linearmixed-effects model on days 1 and 5 with treatment as a categorical variable and participant as a random effect. Pharmacokinetic-pharmacodynamic modeling included drug concentration as a continuous variable and random effects by participant on the intercept and concentration variable. Demographics are reported with standard descriptive statistics.

A 1-sided P value was used to assess the primary outcomes because the study aim was to evaluate whether the study drugs decreased ventilation, and a value $< .025$ was considered significant based on Bonferroni correction for 2 primary outcomes. A 1-sided P value $< .025$ was also considered significant for the secondary ventilation outcome, and these outcomes are reported with 1-sided upper 97.5% CIs. For secondary and exploratory outcomes assessing pharmacokinetics, a difference in exposurewas concluded if the 2-sided 90% CI of the geometric mean ratio [GMR] excluded 1, which is standard in pharmacokinetic studies.[14] Post hoc comparisons are reported with 2-sided 95% CIs and a difference was reported if the CIs excluded 0. Secondary and exploratory CIs are not adjusted for multiplicity, and all analyses except for primary outcomes should be interpreted as exploratory because of the potential for type I error due to multiple comparisons. Statistical analyses were performed in R (version 4.1.2; The R Project for Statistical Computing).

Results

Study Participants

Twenty-five participants (20 originally randomized and 5 replacement participants; Figure 1) were enrolled (median age, 35 years [IQR, 30 to 40 years]; 11 female [44%]). Table 1 contains additional participant characteristics, including resting respiratory measurements and CYP3A4 and CYP2D6 metabolizer phenotypes. Nineteen participants completed the trial and 1 additional participant completed through day 1 of period 2 and had placebo plus oxycodone data available (Figure 1). Primary outcomes data were available for 20 participants on day 1 and 19 participants on day 5.

Primary Outcomes

The mean ventilation at 55 mm Hg end-tidal carbon dioxide with the paroxetine plus oxycodone combination on day 1 was 29.2 L/min (95% CI, 25.7 to 32.7); with quetiapine plus oxycodone, 33.0 L/min(95% CI, 30.0 to 36.0); with placebo plus oxycodone, 34.1 L/min (95% CI, 31.1 to 37.2). The day 5 values were 25.1 L/min (95% CI, 21.2 to 29.0) with paroxetine plus oxycodone; 34.7 L/min (95% CI, 30.9 to 38.5) with quetiapine plus oxycodone, and 35.3 L/min (95% CI, 31.4 to 39.2) with placebo plus oxycodone (Table 2).

Compared with placebo plus oxycodone, paroxetine plus oxycodone significantly decreased

Table 1: Study Participant Demographics and Baseline Characteristics

Characteristics	No. (%) (N=25)
Sex	
Male	14 (56)
Female	11 (44)
Race, No. (%)[a]	
Asian	2 (8)
Black or African American	12 (48)
White	11 (44)
Hispanic or Latino ethnicity	5 (20)
Body weight, median (IQR), kg	68 (61-81)
BMI, median (IQR)	24.8 (22.0-26.1)
Resting respiratory measurements, median (IQR)	n = 24
Minute ventilation, L/min	7.8 (7.3-9.0)
Respiratory rate, breaths/min	15 (12-17)
Tidal volume, L	0.61 (0.54-0.68)
End-tidal carbon dioxide, mmHG	37.1 (35.6-39.0)
Oxygen saturation, %	97.1 (95.9-98.0)
CYP3A4 metabolizer phenotype	
Extensive metabolizers	25(100)
CYP2D6 metabolizers phenotype	
Extensive metabolizers	19 (76)
Intermidiate metabolizers	6 (24)

Abbreviations: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; CYP, cytochrome P450.
[a]Self-identified race and ethnicity were reported by participants in an open-ended format.

ventilation on day 1 (mean difference [MD], -4.9 L/min [1-sided 97.5% CI, $-\infty$ to -0.6]; $P = .01$) and on day 5 (MD, -10.2 L/min [1-sided 97.5% CI, $-\infty$ to -6.3]; $P < .001$), while quetiapine plus oxycodone did not significantly decrease ventilation on day 1 (MD, -1.2 L/min [1-sided 97.5% CI, $-\infty$ to 2.8]; $P < .28$) or day 5 (MD, -0.6 L/min [1-sided 97.5% CI, $-\infty$ to 3.2]; $P < .37$).

Secondary Outcomes

Figure 2 show pharmacodynamic data across days 1, 4, and 5. On day 4, the oxycodone administered on day 1 had washed out, allowing for a comparison between effects of paroxetine and quetiapine alone and placebo. Mean ventilation at 55 mmHg end-tidal carbon dioxide on day 4 was 32.4 L/min (95%CI, 28.2 to 36.5) with paroxetine alone, 42.8 L/min (95% CI, 38.7 to 46.8) with quetiapine alone, and 41.7 L/min (95% CI, 37.7 to 45.6) with placebo (Table 2). Compared with placebo, paroxetine alone significantly decreased ventilation (MD, -9.3 L/min [1-sided 97.5% CI, $-\infty$ to -3.9]; $P < .001$), whereas quetiapine alone did not significantly decrease ventilation (MD, 1.1 L/min [1-sided 97.5% CI, $-\infty$ to 6.4]; $P = .67$). Paroxetine did not significantly increase oxycodone maximum plasma concentration (GMR, 1.06 [90% CI, 0.96 to 1.17]) or AUC (GMR, 1.03

(90% CI, 0.91 to 1.17) on day 1 but did significantly increase oxycodone maximum plasma concentration (GMR, 1.30 [90% CI, 1.19 to 1.43]) and AUC (GMR, 1.10 [90% CI, 1.02 to 1.19]) on day 5 (Table 2). Quetiapine did not significantly increase oxycodone AUC on day 1 (GMR, 1.06 [90% CI, 0.98 to 1.15]) but did significantly increase oxycodone maximum plasma concentrations on days 1 (GMR, 1.25 [90% CI, 1.14 to 1.37]) and 5 (GMR, 1.39 [90% CI, 1.22 to 1.57]) and AUC on day 5 (GMR, 1.27 [90% CI, 1.19 to 1.36]).

Exploratory Outcomes

Figure 3 displays the oxycodone-alone concentration response model and the day 5 primary end point observed data for the drug combinations. Multidrug concentration response analysis showed that increasing concentrations of paroxetine and oxycodone were each associated with decreased hypercapnic ventilation (paroxetine slope, -0.13 L/min per ng/mL [95% CI,-0.17 to -0.09]; oxycodone slope, -0.24 L/min per ng/mL [95% CI, -0.35 to -0.12]), whereas an increasing concentration of quetiapine or its metabolite norquetiapine was not associated with decreasedhypercapnic ventilation (quetiapine slope, 0.015 L/min per ng/mL [95% CI, 0.007 to 0.022]; norquetiapine slope, -0.015 L/min per ng/mL [95% CI, -0.038 to 0.001]; oxycodone slope, -0.25 L/min per ng/mL [95% CI, -0.34 to -0.16]).

Post Hoc Assessments

Compared with placebo plus oxycodone at the 5-hour time point on day 5, paroxetine plus oxycodone increased resting end-tidal carbon dioxide (41.4 vs 37.4 mmHg; MD, 4.0 mmHg [95% CI, 2.4 to 5.6 mm Hg]), decreased resting oxygen saturation (95.5% vs 96.6%; MD, -1.1% [95% CI, -2.1% to -0.1%], and decreased the slope of the hypercapnic ventilatory response curve (1.00 vs 1.44 L/min per mmHg; MD, -0.44 L/min per mmHg [95% CI, -0.85 to -0.03]); quetiapine plus oxycodone increased resting end-tidal carbon dioxide (40.4 vs 37.4 mmHg; MD, 3.0 mmHg [95% CI, 1.4 to 4.6]), decreased resting oxygen saturation (95.2% vs 96.6%; MD, -1.4% [95% CI, -2.4% to -0.4%]), and increased participant-reported sedation (40 vs 25mm; MD, 15mm [95% CI, 3 to 28]).

Adverse Events

No serious adverse events occurred. Twenty-two participants (88%) experienced 1 or more adverse events. The most common adverse eventswere nausea (64%), dizziness (52%), headache (48%), somnolence (32%), and fatigue (32%).

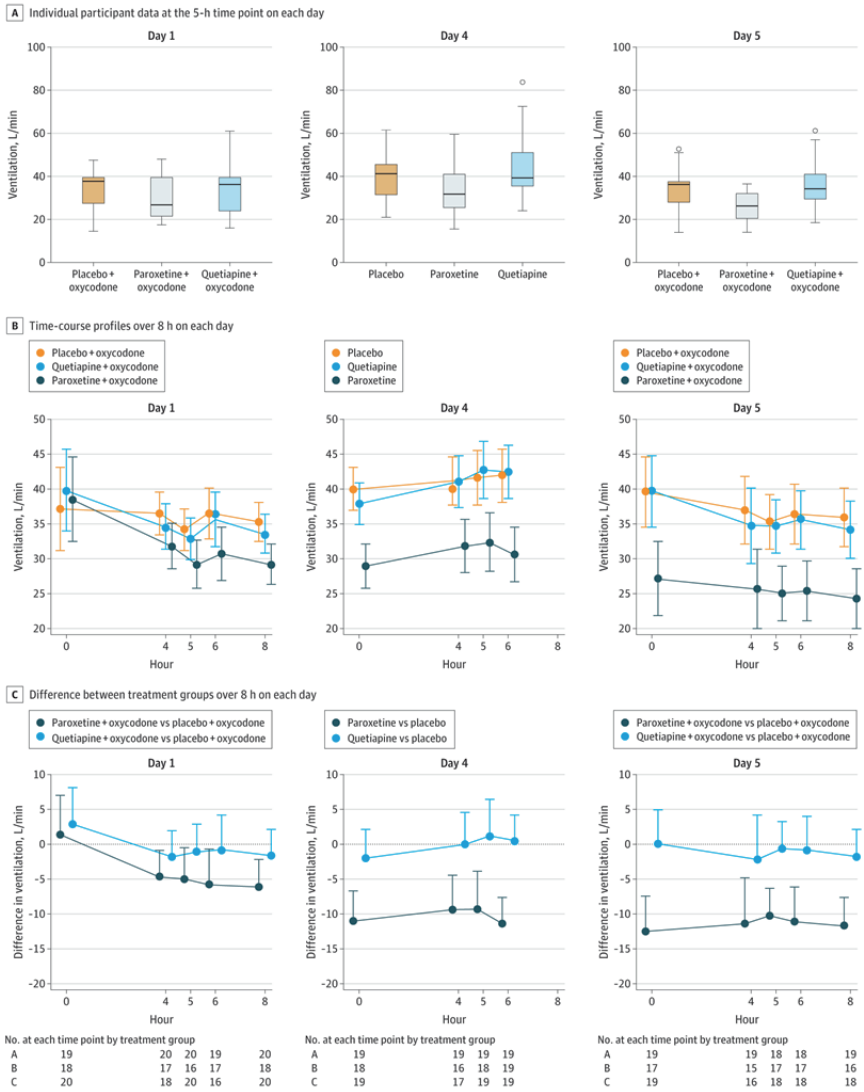


Figure 2: Minute Ventilation at End-Tidal Carbon Dioxide of 55 mmHg
A: Bars indicate medians; box borders, IQRs; and circles, outside the range. Whiskers extending from box borders to the last observation within 1.5 × the IQR.
B: For dosing administration, see the Figure 1. Data points indicate model-estimated means and whiskers 2-sided 95%CIs.
C: The primary outcome comparisons at 5 hours are on days 1 and 5; secondary outcomes, day 4, the secondary outcome comparison. Data points indicate the model-estimated mean difference; whiskers, the upper 1-sided 97.5%CIs.

Table 2: Primary and Secondary Outcomes

	No. of participants	Mean (2-sided 95% CI)		Mean difference (1-sided 97.5% CI)	P value[a]
Primary Outcomes					
Ventilation at 55 mmHG end-tidal PCO2, L/min		Paroxetine + oxycodone	Placebo + oxycodone		
Day 1	20	29.2 (25.7 to 32.7)	34.1 (31.1 to 37.2)	-4.9 ($-\infty$ to -0.6)	0.01
Day 5	19	25.1 (21.2 to 29.0)	35.3 (31.4 to 39.2)	-10.2 ($-\infty$ to -6.3)	< 0.001
		Quetiapine + oxycodone	Placebo + oxycodone		
Day 1	20	33.0 (30.0 to 36.0)	34.1 (31.1 to 37.2)	-1.2 ($-\infty$ to 2.8)	0.28
Day 5	19	34.7 (30.9 to 38.5)	35.3 (31.4 to 39.2)	-0.6 ($-\infty$ to 3.2)	0.37
Secondary outcomes					
Ventilation at 55 mmHg end-tidal PCO2, L/min		Paroxetine	Placebo		
Day 4	19	32.4 (28.2 to 36.5)	41.7 (37.7 to 45.6)	-9.3 ($-\infty$ to -3.9)	< 0.001
		Quetiapine	Placebo		
Day 4	19	42.8 (38.7 to 46.8)	41.7 (37.7 to 45.6)	1.1 ($-\infty$ to 6.4)	0.67
		GM (CV%)		GMR (2-sided 90% CI)	Pvalue[b]
Oxycodone maximum plasma concentration, ng/mL		Paroxetine + oxycodone	Oxycodone + placebo		
Day 1	19	19.1 (25)	18.0 (30)	1.06 (0.96 to 1.17)	0.33
Day 5	19	23.4 (26)	18.0 (26)	1.30 (1.19 to 1.43)	< 0.001
		Quetiapine + oxycodone	Oxycodone		
Day 1	20	22.9 (28)	18.3 (30)	1.25 (1.14 to 1.37)	0.64
Day 5	19	24.9 (26)	18.0 (26)	1.39 (1.22 to 1.57)	< 0.001
Oxycodone AUC, ng/mL x h		Paroxetine + oxycodone	Oxycodone		
Day 1	20	107 (29)	104 (20)	1.03 (0.91 to 1.17)	0.64
Day 5	19	112 (30)	102 (24)	1.10 (1.02 to 1.19)	0.05
		Quetiapine + oxycodone	Oxycodone		
Day 1	20	113 (25)	107 (25)	1.06 (0.98 to 1.15)	0.24
Day 5	19	129 (23)	102 (24)	1.27 (1.19 to 1.36)	< 0.001

Abbreviations: AUC, area under the curve; CV, coefficient of variation; GM, geometric mean; GMR, geometric mean ratio; PCO2, end-tidal partial pressure of carbon dioxide.

[a] *A 1-sided P value <.025 was considered significant for the primary and secondary ventilation outcomes.*

[b] *A difference in exposure was concluded if the 2-sided 90% CI of the geometric mean ratio excluded 1 (2-sided P < .1).*

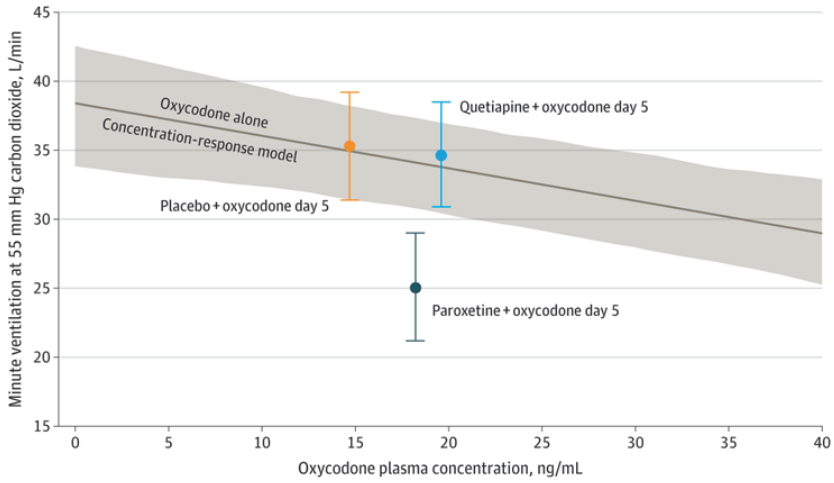


Figure 3: The oxycodone concentration–response model is based on a linear mixed-effect model with all data from oxycodone alone. The downward sloping black line indicates the prediction; the shaded region, 95% CI (mean slope, -0.29 L/min per ng/mL [95% CI, $-0.47 to -0.11$); mean intercept, 39.8 L/min [95% CI, 34.0 to 45.7]). Data points represent the observed data from the 5-hour time point on day 5 (primary end point) for mean ventilation at 55 mmHg carbon dioxide (values in Table 2) and geometric mean oxycodone plasma concentration with placebo plus oxycodone was 14.7 ng/mL (coefficient of variation [CV], 31%); oxycodone concentration with paroxetine, 18.2 ng/mL (CV, 21%); and oxycodone concentration with quetiapine, 19.6 ng/mL (CV, 21%).

Discussion

In this randomized, double-blind, crossover clinical trial involving healthy participants, paroxetine (40mg daily for 5 days) combined with oxycodone (10mg on days 1 and 5) compared with oxycodone alone decreased ventilation when end-tidal carbon dioxide was 55 mmHg. In contrast, quetiapine (increasing daily doses from 100mg to 400mg) combined with oxycodone did not decrease ventilation when end-tidal carbon dioxide was 55 mmHg.

The finding that paroxetine combined with oxycodone, compared with oxycodone alone, decreased the ventilatory response to hypercapnia is concerning because this is the primary feedback mechanism for the body to rescue itself from opioid-induced respiratory depression.[2,6] The secondary outcomes supported that paroxetine decreased the ventilatory response to hypercapnia through a direct pharmacodynamic effect rather than by a pharmacokinetic interaction because paroxetine had a similar effect on its own compared with placebo on day 4. Furthermore, exploratory concentration response modeling supported that the increase in oxycodone

concentration with paroxetine did not explain the observed effect of paroxetine on the primary outcome (Figure 3). This study included exploratory outcomes of resting respiratory measures while participants breathed room air for 5 minutes prior to the rebreathing procedure. When performing post hoc comparisons at the primary end point time on day 5, neither drug combination significantly decreased resting minute ventilation; however, both drug combinations significantly increased resting end-tidal carbon dioxide (by $\approx 3-4$ mm Hg) and decreased resting oxygen saturation (by $\approx 1.1\%-1.4\%$).

In the nonclinical study that motivated this clinical trial,[8] quetiapine caused a substantially larger increase in oxycodone maximum plasma concentration than what was observed in this clinical trial, likely explaining the different respiratory effects observed with the quetiapine-oxycodone combination in the nonclinical study vs this clinical trial. This was likely due to interspecies differences in pharmacokinetics and that substantially higher doses of each drug were administered. The nonclinical study findings with paroxetine were similar to those observed in this trial. Review of older literature identified additional nonclinical studies supporting a relationship between certain systemically administered drugs that affect serotonin and ventilatory depression.[15–20] Inhibition of serotonin synthesis increased baseline ventilation and the ventilatory response to carbon dioxide, which was reversed by administering a serotonin precursor.[18–20] Furthermore, morphine induced respiratory depression was enhanced by drugs that increase serotonin, including monoamineoxidase inhibitors and the SSRI fluoxetine.[18,19] Other studies identified a relationship between paroxetine or fluoxetine alone and decreased ventilation.[21–24] Additional studies have shown that specific types of serotonin neurons increase their firing rate in response to hypercapnia and that activation of specific serotonin receptor subtypes stimulates ventilation.[25] However, paroxetine does not bind to serotonin receptors at clinically relevant concentrations but rather is highly selective for inhibiting the serotonin transporter, leading to its SSRI properties.[26] Regarding clinical data, a retrospective analysis of patients referred to a sleep clinic found that SSRIs, compared with a norepinephrine-dopamine reuptake inhibitor, were associated with impaired breathing and worse nocturnal oxygen saturation.[27] Several previous studies involving patients with panic disorder used inhalation of carbon dioxide as a trigger for anxiety and panic symptoms. In addition to finding that multiple SSRIs[28–31] and certain tricyclic antidepressants[28,29] decreased hypercapnia-induced anxiety, a subset of studies using the carbon dioxide rebreathing method found that chronic treatment with SSRIs or certain tricyclic antidepressants decreased the ventilatory response to hypercapnia in this population.[32,33] In overdose, paroxetine and other SSRIs are not known to cause severe respiratory depression or death on their own,[34] suggesting that ventilatory depressant effects may plateau after exceeding a certain exposure, which is consistent with the findings from the nonclinical study with paroxetine alone.[8]

Sound data regarding concomitant medications can be difficult to obtain on patients who overdose while taking opioids because information often relies on death certificates, which vary by death investigation practice (eg, performing comprehensive postmortem drug testing) and

reporting practice (eg, focusing ona single lethal drug or listing multiple drugs).[35] Retrospective analyses of administrative health care data that grouped all antidepressants together identified prior antidepressant prescription as a predictor of opioid overdose or serious opioid-induced respiratory depression, and antidepressant use was included in a developed risk index.[36,37] However,these studies[36,37] did not evaluate the causal link between antidepressants and overdose and were limited by potential treatment and outcome misclassification. An additional recent retrospective analysis with similar limitations and the potential for unmeasured confounding variables found that use of SSRIs that inhibit oxycodone metabolism (paroxetine or fluoxetine; inhibit CYP2D6) at the time of oxycodone initiation was associated with a small but significantly higher risk of opioid overdose compared with the use of other SSRIs.[38] The results from this clinical trial confirmed that paroxetine caused a relatively small increase in oxycodone concentration; however, quetiapine, which inhibits CYP3A4, also increased oxycodone plasma concentration without affecting the primary outcome.

This clinical trial is a part of the FDA's proactive work to address the opioid crisis and help reduce opioid overdoses and deaths and more specifically to determine whether drugs that might be used in place of benzodiazepines may also exacerbate opioid-induced respiratory depression.8 The findings may have important clinical implications for patients taking paroxetine, or potentially other SSRIs, who concomitantly use opioids, but further research is needed to determine this. SSRIs take approximately 3 weeks to reach maximal therapeutic effect, which correlates with the time required for presynaptic inhibitory serotonergic receptors to desensitize.[39,40] Some prior nonclinical studies suggest different effects of SSRIs on respiration over a similar time frame.[21] Further clarifying the potential time-dependent risks of SSRIs when combined with opioids will be important because treating co-occurring mental health conditions is a critical part of addressing the opioid crisis.

Limitations

This study has several limitations. First, it is not known if the findings with paroxetine will extend to other SSRIs; however, as reviewed in this article, the effects may be due to paroxetine's primary mechanism of action common among SSRIs. Second, the study was conducted in a controlled setting with procedures to increase end-tidal carbon dioxide. Although this differs from what patients would experience, the method allows testing drug combinations at doses that do not lead to severe respiratory depression when breathing room air while still assessing ventilatory effects as carbon dioxide increases, which reflects the physiology of severe respiratory depression seen with opioid overdoses.[2,6] Third, the study involved healthy participants with 5 days of dosing; thus, it is not known if the paroxetine effect on ventilation would persist with longer term treatment. However, clinical studies discussed earlier that involved patients referred to a sleep clinic and with panic disorder suggest that SSRIs affect ventilation after longer term treatment.[27,33]

Conclusions

In this preliminary study that involved healthy participants,paroxetine combined with oxycodone, compared with oxycodone alone, significantly decreased the ventilatory response to hypercapnia on days 1 and 5, whereas quetiapine combined with oxycodone did not cause such an effect. Additional investigation is needed to characterize the effects after longer-term treatment and to determine the clinical relevance of these findings.

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Chapter 4

Advances in Reversal Strategies of Opioid-induced Respiratory Toxicity

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Introduction

Opioids produce respiratory depression and are consequently potentially lethal. Activation of the μ-opioid receptors expressed within the respiratory neuronal network of the brainstem causes irregular breathing, followed by periodic breathing and eventually the cessation of rhythmic breathing activity.[1–5] Recent studies show that although μ-opioid receptors are widely expressed within the respiratory network, the pre-Bötzinger complex, the brainstem respiratory rhythm generator, and the Kölliker–Fuse nucleus are two crucial areas in the brainstem for development of opioid-induced respiratory depression but also for reversal or prevention of respiratory depression by naloxone and nonopioid respiratory stimulants (fig. 1).[1,3–5]

In the perioperative and emergency setting, the opioid antagonist naloxone remains the first choice of treatment of respiratory depression from an opioid overdose, mainly due to its efficacy.[1,6] In case of an emergency, all that matters is that the patient resumes rhythmic breathing, and naloxone will effectively reverse the opioid effect, although sometimes high doses are required.[1] However, there are a number of circumstances where administration of naloxone may be inadequate or undesired.[7] Such circumstances include (1) an individual overdose with potent, high-affinity, and long-acting opioids, such as carfentanil or high-dose fentanyl; (2) opioid use or abuse in combination with other depressants of the central nervous system such as alcohol, cannabis, benzodiazepines, antidepressants, or antipsychotics, which synergistically enhance respiratory depression but are not reversed by naloxone[8]; (3) conditions in which naloxone reversal will cause effects that are undesired such as loss of analgesia, precipitation of withdrawal, agitation, and sympathetic stress[1,6,9]; (4) mass accidental or intentional poisoning with opioids, where supplies of naloxone may be exhausted or where naloxone is ineffective[7]; and finally (5) in case of an opioid use disorder.[9] For these reasons, in recent years there has been an increased interest in the development of novel reversal strategies aimed at providing efficacy close to that of naloxone but without its drawbacks. One important disadvantage of naloxone is its short duration of action due to rapid metabolism (elimination half-life, 30 min) and rapid clearance from the brain compartment (blood-effect-site equilibration half-life, 5 to 8 min).[9] It may even be advantageous to combine opioid therapy with nonopioid respiratory stimulants to prevent fatal respiratory depression.

To give a narrative overview of this highly relevant topic, we systematically discuss new and predominantly experimental (immuno)pharmacotherapies, published in the last 5 yr, that are aimed at reversal of opioid-induced respiratory depression, whether successful or not, as alternatives to naloxone or related compounds. The discussion of naloxone and its analogs is beyond the scope of the current review. We do acknowledge that novel opioid-receptor antagonists are being developed with longer half-lives (e.g., methocinnamox, nalmefene, biohybrid nanoparticles encapsulated naloxone) than naloxone,[7] but their effect is based on antagonistic activity at the opioid receptor,[3,7] which is distinct from developments that we present here.

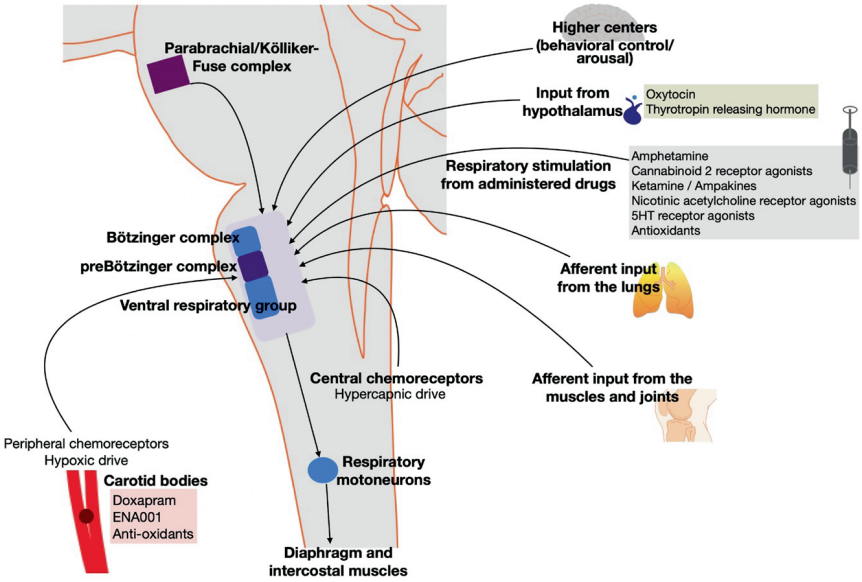

Figure 1: Schematic overview of the input to the brainstem respiratory centers. In purple, the parabrachial/Kölliker–Fuse complex, located in the pons, and pre-Bötzinger complex, located in the brainstem, that show high respiratory sensitivity to exogenously administered opioids and consequently are the target of reversal of opioid-induced respiratory depression. Excitatory drives are shown from various areas within the central and peripheral nervous system (hypercapnic and hypoxic drive, arousal from activation of higher centers), receptors from the lungs and muscles or joints, hypothalamus, and administration of exogenous respiratory stimulants.

Materials and Methods

We performed a search in PubMed on August 2, 2021, with main search terms “opioid-induced respiratory depression” and “non-opioid respiratory stimulants.” We retrieved 2,082 studies, of which 311 were published since August 2016. Based on the abstract and full text of these papers and the references they listed, we included 34 papers in our primary analysis. The search strategy was developed in collaboration with information specialists of the Walaeus Library of Leiden University Medical Center (Leiden, The Netherlands) and is available from the authors. The respiratory stimulants are grouped based on their characteristics and mechanism of action: nonopioid controlled substances, hormones, nicotinic acetylcholine receptor agonists, ampakines, serotonin receptor agonists, antioxidants, miscellaneous peptides, drugs acting at the carotid bodies, sequestration techniques, and opioids.

Controlled Substances: Nonopioids

Amphetamine

In the early 1940s, the respiratory stimulatory effect of amphetamine was already recognized. For example, in 1945, Handley and Ensberg compared the effect of amphetamine to other respiratory stimulants including caffeine and ephedrine to reverse morphine-induced respiratory depression.[10] In 14 human subjects, they observed that amphetamine sulfate (benzedrine) produces a brisk reversal of morphine-induced respiratory depression exceeding the effects of all other tested stimulants. In 2020, the ability of D-amphetamine was examined to accelerate recovery from high-dose fentanyl in rats.[11] It was shown that D-amphetamine shortened recovery from unconsciousness and enhanced respiratory drive in terms of improvement of hypercapnia and hypoxia within 5 min of D-amphetamine administration. Amphetamine inhibits synaptic reuptake of monoamine including dopamine, serotonin, and norepinephrine. It is hypothesized that enhancement of dopaminergic neurotransmission and activation of D1-dopamine receptors cause arousal and respiratory stimulation. Activated D1-dopamine receptors increase cyclic adenosine monophosphate (cAMP) in respiratory neurons and consequently increased breathing activity. Earlier studies showed that D1-dopamine receptor agonists are able to overcome fentanyl- and enkephalin-induced respiratory depression without affecting analgesia.[12,13] Additionally, increased levels of serotonin within the pre-Bötzinger complex may be involved as well in D-amphetamine–induced respiratory stimulation.[11] It is important to realize, however, that D-amphetamine has other effects within the central nervous system, and it is doubtful whether D-amphetamine, currently available for the treatment of attention deficit hyperactivity disorder, is sufficiently selective to be useful as medical countermeasure to rescue or prevent opioid induced respiratory depression.

Cannabinoid 2 Receptor Agonists

The endocannabinoid system consists of cannabinoid type 1 and type 2 receptors, their endogenous ligands, so-called endocannabinoids, and enzymes that control formation and degradation of these ligands.[14,15] Endocannabinoids play a modulatory role in various physiologic systems including the ventilatory control system. Recent studies indicate the presence of cannabinoid receptors in respiratory centers in the brainstem, including the pre-Bötzinger complex.[15] Activation of cannabinoid type 1 receptors by Δ9-tetrahydrocannabinol produces respiratory depression, while cannabinoid type 2 receptors activated by endocannabinoids have a tonic excitatory respiratory effect.[15,16] Given this, it seems attractive to determine whether activation of cannabinoid type 2 receptors reverses opioid induced respiratory depression. Two studies addressed this issue. Zavala et al.[14] tested the ability of the G-protein biased cannabinoid type 2 agonist LY2828360, which does not recruit the β-arrestin signaling pathway, to attenuate fentanyl-induced respiratory depression in wild-type and cannabinoid type 2 knockout mice. While LY2828360 fully reversed fentanyl respiratory depression in wild-type animals, no effects

were observed in cannabinoid type 2 knockout mice. In an independent study, Wiese et al.[15] demonstrated that cannabinoid type 1 and cannabinoid type 2 agonist Δ9-tetrahydrocannabinol produces respiratory depression and that the selective cannabinoid type 2 receptor agonist AM2301 reversed morphine respiratory depression. However, the effect was observed only when 10 mg/kg morphine was reversed by 10 mg/kg AM2301; at higher morphine doses, AM2301 was insensitive, even after increasing the AM2301 dose to 100 mg/kg, suggestive of a saturation in effect of AM2301.

Cocaine

Cocaine is a psychostimulant that induces sympathetic activation by enhancing monoamine neurotransmission. When administered to rodents, cocaine increases oxygen entry into brain tissue by 10 to 15%.[17] Thomas et al.[18] studied whether cocaine is able to reverse the decrease in brain oxygen levels that occurs after heroin administration. To determine the oxygen levels, oxygen sensors were placed in the nucleus accumbens, as a measure of the functional output of breathing activity. While modest cocaine effects were observed after low-dose heroin administration, no cocaine effect was observed in an attempt to reverse the 50% drop in oxygen content from a heroin overdose. These data indicate no protective effect when cocaine is abused simultaneously with potent opioids such as heroin. In fact, the high prevalence of cocaine found in blood of heroin overdose deaths suggests that cocaine increases the likelihood of opioid induced respiratory depression. The cerebral vasodilation and blood redistribution toward the brain induced by cocaine is unable to offset the neuronal depression and consequent oxygen dynamics induced by an opioid overdose.

Ketamine

In 1998, Mildh et al.[19] showed in healthy volunteers that a single subanesthetic bolus dose of racemic ketamine attenuated mild fentanyl-induced respiratory depression, but did not prevent a decrease in blood oxygenation. Jonkman et al.[20] tested the effect of escalating doses (4, 8, 12, and 16 mg, each dose given during 15 min) of esketamine, the S(+)- isomer of ketamine, and observed dose-dependent, albeit partial, reversal of remifentanil-induced respiratory depression in healthy volunteers. Ketamine reduced the depression in ventilation to about 50% of baseline. No effect on breathing was observed when esketamine was administered without opioid. Since esketamine is a potent analgesic, these data suggest that esketamine may be used to stabilize respiration, for example in the postoperative period, and simultaneously reduce opioid consumption, further improving respiratory activity. Several mechanisms may be involved in the stimulatory effects of ketamine including enhancement of monoaminergic neurotransmission, or agonist activity of ketamine and its metabolites at the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor.[20] Blockade of glutamatergic neurotransmission has been proposed as well,[20] but there are data showing that loss of glutamate drive to the Bötzinger complex reduces inspiratory and expiratory duration as well as peak phrenic ampli-

tude, and the subsequent reduced glutamatergic drive to the pre-Bötzinger complex causes the complete loss of the respiratory pattern.[21] Interestingly, Jonkman et al.[20] showed that esketamine only stimulates carbon dioxide– dependent ventilation, very similar to the ampakines, suggestive of a common mechanistic pathway. Finally, at a high dose, ketamine produces respiratory depression that is naloxone- sensitive, indicative of an effect at the opioid receptor system.[22]

In summary, the majority of the nonopioid scheduled substances discussed here show a respiratory stimulatory effect, and further studies are needed to determine their use in opioid overdose toxicity. We need to realize that all of them come with unwanted side effects ranging from a high risk of abuse and addiction to schizotypical experiences that may be frightful to the patient. In this context, low-dose ketamine may offer the best clinical utility of all of these agents, where enhancement of respiratory activity and a reduction of opioid consumption in the perioperative setting outbalances its side effects.

Hormones

Thyrotropin-releasing Hormone Receptor Agonists

Thyrotropin-releasing hormone is predominantly produced in the hypothalamus. It regulates the release of thyroid-stimulating hormone and prolactin from the pituitary gland. Thyrotropin-releasing hormone mediates its effects by binding to the G-protein–coupled thyrotropin releasing hormone receptor that is ubiquitously expressed in the brain and in peripheral tissues, indicative of a broad functionality.[23] Thyrotropin-releasing hormone has a dose-dependent excitatory effect on breathing activity that coincides with an increase in blood pressure and heart rate and is able to overcome opioid-induced respiratory depression in various species including nonhuman primates.[24,25]

Exogenously administered thyrotropin-releasing hormone has evident neuroendocrine effects, has a short halflife of less than 5 min, and poorly passes the blood–brain barrier due its low lipophilicity. Various analogs have been developed with an improved therapeutic selectivity and a longer duration of action. One such analog is taltirelin, which is registered in Japan for treatment of spinal cerebral degeneration.[26] In a series of experiments, Cotten's research group studied the effect of thyrotropin-releasing hormone and taltirelin on opioid-induced respiratory depression in the rat.[26,27] Intravenous thyrotropin-releasing hormone and taltirelin reversed morphine-induced respiratory depression in the isoflurane-anesthetized rat. Reversal was due to an effect on respiratory rate, which exceeded premorphine respiratory rates by 200 to 300% after treatment with thyrotropin-releasing hormone or taltirelin.[26] While taltirelin normalized blood gas values, thyrotropin-releasing hormone decreased arterial carbon dioxide concentration but failed to normalize arterial oxygen concentrations; taltirelin caused lactic acidosis. Interestingly, also after intratracheal administration, thyrotropin-releasing hormone caused rapid reversal of morphine-induced respiratory depression. Overall, these data indicate

that thyrotropin-releasing hormone and taltirelin cause rapid, shallow breathing after morphine administration in the anesthetized rat.[26] As stated by the investigators, this pattern of breathing is undesired because of increased dead space ventilation and a high probability of atelectasis and ensuing hypoxia. Possibly the inability to correct arterial oxygen concentration and development of lactic acidosis may be related to increased work of breathing, which causes anaerobic metabolism, reduced oxygen uptake, or both.

In a second set of experiments, Dandrea and Cotten tested the effect of intravenous taltirelin on morphine and sufentanil-induced respiratory depression in conscious rats.[27] Similar to the experiments in anesthetized rats, taltirelin reversed respiratory depression by an increase in respiratory rate. Blood gas analysis revealed the inability to restore arterial oxygen concentration and worsening of lactic acidosis. The two studies by Cotten and coworkers suggest that the state of inhalational anesthesia allows for an improved reversal of opioid toxicity due to some muscle relaxation and reduced oxygen consumption due to anesthesia-suppressed metabolism. This is an important observation and warrants further study in awake animals and humans.

In an exploratory study, we tested the effect of a bolus and continuous infusion of thyrotropin-releasing hormone in six human volunteers after remifentanil-induced respiratory depression (A. Dahan, 2021, verbal communication). In intravenous doses ranging from 0.8 to 8 mg, which corresponds to a maximum dose of 0.1 mg/kg, thyrotropin-releasing hormone did not reverse remifentanil-induced respiratory depression. The dose range was based on earlier human studies that showed respiratory stimulation at 0.4 mg thyrotropin-releasing hormone. Further studies have to explore higher doses of thyrotropin-releasing hormone in humans. Finally, in a rat model of hemorrhagic shock, thyrotropin-releasing hormone improved circulatory and respiratory functions, but due to the release of acid metabolites, it worsened acidosis.[28] This may hamper the utility of thyrotropin-releasing hormone in patients with compromised organ perfusion.

Oxytocin Receptor Agonists

Another hypothalamic hormone, which has been studied for its ability to reverse opioid-induced respiratory depression, is the neuropeptide oxytocin.[29] In chloralose/urethane anesthetized, paralyzed, vagotomized, 100% oxygen-ventilated rats, the effect of intravenous oxytocin and the nonpeptide oxytocin receptor agonist and weak vasopressin receptor antagonist WAY-267464 were assessed on phrenic nerve activity after a fentanyl dose sufficient to silence phrenic nerve activity. Oxytocin displayed a bell-shaped response curve in its ability to reverse phrenic nerve activity with maximal reversal at low dose but absence of reversal at high dose. The return of respiratory depression was related to cross-activation of vasopressin receptors at high oxytocin levels, possibly from activation of the baroreceptor reflex by high blood pressure. Blockade of the vasopressin receptor during oxytocin exposure by the vasopressin receptor-1a receptor antagonist V1aRX resulted in reversal of opioid respiratory depression at high-dose oxytocin.

Interestingly, similar to the ampakines and esketamine, oxytocin receptor activation without opioid exposure did not stimulate breathing. However, there are reports that oxytocin ameliorates respiratory rates in patients with sleep-disordered breathing.[30] The mechanism through which oxytocin stimulates opioid-depressed respiratory activity remains unknown. Oxytocin is a positive allosteric modulator of the μ-opioid receptor and enhances μ-opioid receptor signaling induced by fentanyl and other opioids.[31] While this suggests that opioid respiratory depression would be worsened by oxytocin, its respiratory excitatory effects at oxytocin receptors within the brainstem respiratory network seem to overcome such a negative effect. Whether oxytocin is able to reverse opioid-induced respiratory depression in humans overdosed on potent opioids remains unknown and may be hampered by oxytocin's bell-shaped response curve and the limited and slow passage of oxytocin across the blood–brain barrier. Further studies into WAY-267464 are warranted as this drug does not seem to have these same restrictions.

Nicotinic Acetylcholine Receptor Agonists

Nicotinic acetylcholine receptors are expressed within the respiratory network in the brainstem and are present in carotid bodies. These receptors are made up of subunits, and those present within respiratory networks contain subunits α4, α7, and β2.1 Ren et al. studied the effect of nicotinic acetylcholine receptor agonists and partial agonists on their ability to rescue rats from opioid-induced respiratory depression.[32,33] Selective α4β2 nicotinic acetylcholine receptor agonist A85380 (but not α7 nicotinic acetylcholine receptor agonist PNU282987) did not have an effect on ventilation by itself, but countered respiratory depression induced by fentanyl in conscious adult rats; the effect was by increasing respiratory rate.[32] Additionally, A85380 reduced apnea duration and increased ventilation after remifentanil infusion. Importantly, the α4β2 nicotinic acetylcholine receptor agonist was antinociceptive and enhanced fentanyl analgesia. In an independent study by Dandrea and Cotten, A85380 was unable to reverse opioid-induced toxicity, but this may be dose-related.[27]

In a next study, Ren et al.[33] showed that using two partial α4β2 nicotinic acetylcholine receptor agonists, varenicline, which was developed for the treatment of nicotine addiction, and ABT954, which is under development for the treatment of diabetic peripheral neuropathic pain, countered fentanyl-induced respiratory depression. Similar to A85380, varenicline and ABT954 increased respiratory rate but not tidal volume. This is probably related to the muscle rigidity induced by opioids, which affects tidal volume, which is not alleviated by the nicotinic acetylcholine receptor agonists. Varenicline combined with low-dose naloxone (1 μg/kg) was able to overcome lethal apneas induced by high-dose fentanyl whereas either drug on its own, at the same doses, was unable to initiate breathing after fentanyl. This indicates a synergistic interaction between naloxone and varenicline. ABT954 on its own was able to reinitiate respiratory activity after high-dose fentanyl. Finally, both nicotinic acetylcholine receptor agonists were able to overcome lethal apneas after the combination of fentanyl and diazepam.[33]

Combined, these data provide strong evidence that α4β2 nicotinic acetylcholine receptor

(partial) agonists effectively counter opioid-induced respiratory depression in conscious rats. The observation that analgesia is enhanced or not reduced and the fact that these drugs have a long half-life are advantages over naloxone. Further studies in humans using the clinically available varenicline will shed light on its efficacy in opioid overdose victims and whether the drug has a stimulatory effect on tidal volume as well as on respiratory rate. Furthermore, the combined use of naloxone and varenicline is promising and may serve as a model for the use of low-dose naloxone combined with other respiratory stimulants that show limited or partial reversal of opioid-induced respiratory depression.

Ampakines

With respect to reversal of opioid-induced respiratory depression, the ampakines are by far the most studied drugs. We earlier reported on four ampakines, CX717, CX546, CX614, and XD-8-17C, that all reversed respiratory depression induced by a variety of opioids in rodents.6 For example, CX717 reversed fentanyl- and DAMGO ([DAla, N-MePhe, Gly-ol]-enkephalin)–induced respiratory toxicity in the rat and partly prevented alfentanil-induced depression of the ventilatory response to hypercapnia in human volunteers.[34–36] Ampakines are benzamine compounds that allosterically modulate the α-amino- 3-hydroxy-5-methyl-4-isoxazole propionic acid receptor–mediated synaptic response in a positive fashion.[1] The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors are ubiquitously present in the central nervous system and are expressed within the respiratory network, and their activation stimulates breathing activity under conditions of hypoventilation by increasing respiratory rate and, to a lesser extent, tidal volume. Since 2016, four new studies on ampakines in animal models of respiratory depression were published aimed at the development of a respiratory stimulant for human or veterinary medicine (CD-8-17C, LCX001, CX1739, and CX1942).[37–40] All show promising results counteracting the effect of potent opioids such as etorphine and fentanyl. Particularly compound CX1739, the precursor of LCX001, showed promising results in a phase 2 human study.[41] In a preliminary report, CX1739 reduced remifentanil-induced respiratory depression at a steady-state plasma concentration of 2 ng/ml, without affecting analgesia or pupil diameter. However, CX1739 did not counteract respiratory depression after a remifentanil 1 µg/kg bolus dose. This exemplifies the limit of ampakines in their ability to activate respiratory drive in the respiratory rhythm generator after high-dose opioid-induced respiratory depression.

Finally, the atypical antidepressant tianeptine also acts at the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor system by enhancing α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid–mediated glutamatergic neurotransmission.[42] After an animal study showed that tianeptine mitigates morphine-induced respiratory depression,[42] we tested its effect in two independent studies on alfentanil- and remifentanil-induced respiratory depression (R. van der Schrier, 2013, verbal communication; A. Dahan, 2021, verbal communication) and could not detect any respiratory stimulatory effects from oral or intravenous tianeptine. Interestingly, intravenous tianeptine worsened remifentanil-induced respiratory depression, possibly related to

its agonistic activity at the μ-opioid receptor.[43]

We envision further studies on ampakines and particularly CX1739 to determine whether higher doses may overcome bolus dose–related respiratory depression, for example in combination with naloxone.

Serotonin Receptor Agonists

The neurotransmitter serotonin (5HT) plays an important role in inspiratory and expiratory respiratory control with actions in the opposite direction from the μ-opioid receptors. We earlier summarized data on seven different serotonin agonists aimed at receptor subtypes 1a, 4a, and 7a.[6] Irrespective of the targeted subtype, all agonists caused reversal or prevention of μ-opioid–induced respiratory depression in animal studies. In two recent studies, the effect of the 5HT4a receptor agonist BIMU8 was tested on etorphine- and sufentanil-induced respiratory depression.[26,44] In etorphine-immobilized goats, BIMU8 reduced etorphine toxicity with a reversal of the drop in respiratory rate and normalization of blood gasses.[44] In the animals, blood pressure dropped after infusion of BIMU8, and some goats developed muscle rigidity or muscle spasms. In conscious rats, however, BIMU8 failed to counter sufentanil-induced respiratory depression, although this may be dose-related.[23] This stands in contrast to 8-OH-DPAT, a 5HT1a and weak 7a receptor agonist that does reverse sufentanil-induced respiratory depression in the conscious rat by increasing respiratory rate and tidal volume.[26] The development of muscle rigidity may be an additional cause of respiratory depression. Reducing muscle rigidity by adding the α1-adrenoreceptor agonist prazosin to 8-OH-DPAT treatment further improved tidal volume and oxygenation, albeit respiratory rate decreased by about 25%.[26] This again highlights the importance of addressing muscle rigidity, the so-called wooden chest syndrome, in the light of opioid-induced respiratory depression. Importantly, and in contrast to the animal studies, in humans, the two studies that tested the effect of serotonin agonists selectively targeting the 1a and the 4a-receptor subtypes failed to show efficacy in morphine-induced respiratory depression.[45,46] This may be related to insufficient dosing or due to insufficient exposure of the 5HT agonists at their receptor within the brainstem due to inability to pass the blood–brain barrier.[1] Further studies on selective and permeable 5HT agonists are warranted.

Antioxidants

Ventilatory drive is modulated by redox-sensitive pathways. For example, the hypoxic ventilatory response, originating at the carotid bodies, is modulated by changes in redox state. Oral intake of the antioxidant N-acetyl-cysteine, which elevates intracellular cysteine levels, enhances the magnitude of the hypoxic ventilatory response, suggestive of a role for the thiol redux state in hypoxic chemosensitivity.[47] A combination of the antioxidants ascorbic acid and α-tocopherol effectively reversed the blunted hypoxic response due to a low dose of inhalational anesthetics.[48] Hence, it seems attractive to determine the ability of antioxidants to counter opioid-induced respiratory depression. In a first study, Lewis's research group showed that

L-cysteine-ethyl ester reverses respiratory acidosis and arterial hypoxemia after morphine administration, but only in tracheotomized rats, not in nontracheotomized animals.[49] This is possibly related to an increase in upper airway resistance and subsequent occurrence of negative intrathoracic pressure in the latter group of animals. L-cysteine and L-serine-ethyl ester were ineffective, which highlights the importance of ability to penetrate relevant neurons and the essential role for the sulfur moiety in causing the respiratory stimulatory effects. In a subsequent study, Lewis's group showed that D-cysteine-diethyl and D-cysteine-dimethyl ester offset moderate morphine-induced respiratory depression in freely moving, nontracheotomized rats due to increases in respiratory rate and tidal volume, while enhancing morphine analgesia.[50] D-cysteine was without effect. In a third study, in awake rats, the investigators show that pretreatment with glutathione ethyl ester offsets fentanyl-induced respiratory depression through effects on respiratory rate and tidal volume, and consequently stabilized breathing and enhanced analgesia.[51] In summary, these studies show that ethyl esters increase respiratory rate and sometimes also tidal volume, and that the increase is sustained during moderate opioid-induced respiratory depression, offsetting the opioid effect on respiratory rate. Finally, in awake and isoflurane-anesthetized rats, pretreatment with the antioxidant Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) prevented fentanyl-induced respiratory depression, while the potent antioxidant N-acetyl-L-cysteine methyl ester was without effect.[52] In all of these studies, the sedative opioid effects remained unaffected by the antioxidants.

Various mechanisms have been postulated to explain the ability of these antioxidants to reverse or prevent opioid-induced respiratory depression, including reduction of enhanced production of reactive oxygen species by opioids, enhancement of nitrosyl derivatives in the carotid body and nucleus tractus solitarius, enhancement of skeletal muscle contractility, or alterations in opioid bias toward the G-coupled transduction pathway. All can theoretically increase respiratory drive after opioid exposure. However, the absence of efficacy of the potent antioxidant N-acetyl-L-cysteine methyl ester on opioid toxicity suggests that an effect on reductive processes seems less plausible.[52] We are probably observing specific redox-independent actions of these agents outside of the opioid transduction pathway as no effects were observed on opioid-induced sedation and analgesia remained uncompromised. Further studies are warranted both mechanistically and clinically to determine whether such agents have a role in preventing opioid overdose toxicity in humans.

Miscellaneous Peptides

Neuropeptide FF is a mammalian amidated peptide with antiopioid analgesic activity.[46] Since it modifies opioid analgesia, Wojciechoski et al.[53] tested its effect on respiratory depression induced by endomorphin-1 in urethaneanesthetized rats. Before any opioid challenge, at a high intravenous dose, neuropeptide FF reduced minute ventilation. Lower doses were unable to systematically reduce the number and duration of endomorphin-1–induced apneas, possibly related to poor permeability across the blood–brain barrier. In contrast, intracerebroventric-

ular administration caused a dose-dependent reduction of apneic events, an effect that was blocked by the neuropeptide FF antagonist RF9. The mechanisms of these effects on opioid-induced respiratory depression remain unknown, but are possibly related to reversal of vagal-mediated opioid-induced apneas via neuropeptide FF receptor activation in the nucleus tractus solitarius.

Another approach to influence opioid-induced respiratory depression has been to focus on peptides that directly interact with the signaling pathway(s) of the opioid receptor. Liang et al.[54] show that intracerebroventricular pretreatment with protein kinase A inhibitor H89 slowly reversed fentanyl-induced respiratory depression by increasing respiratory rate. Similarly, G-protein gated inwardly rectifying potassium channel blocker Tertiapin-Q dose-dependently reversed fentanyl effects through increasing respiratory rate but without affecting minute ventilation.[54] Somewhat surprisingly, phosphodiesterase-4 inhibitor rolipram and cAMP analogs were ineffective in this model.[54] These data suggest that H89 interacts with the ventilatory control system in a cAMP-independent fashion.

These observations that various peptides, including, for example, the endogenous dipeptide glycyl-L-glutamine,[55] may counter opioid-induced respiratory depression increase our knowledge on the mechanisms of opioid-induced respiratory depression. The viability of such targets in the reversal and prevention of a lethal opioid overdose is still far off, but merits further study.

Drugs Acting at the Carotid Bodies

The carotid bodies, located in between the internal and external carotid arteries, just above the bifurcation, contain the peripheral chemoreceptors. These receptors respond to acidosis and low oxygen concentrations in blood with the release of neurotransmitters that activate the sinus nerve, a branch of the glossopharyngeal nerve, which results in a brisk hyperventilatory response. A variety of neuromodulators and receptors within the carotid body involved in the transduction of low oxygen partial pressure into a ventilatory response may be a possible target for reversal or prevention of opioid induced respiratory depression. For example, while dopamine blunts ventilatory responses originating at the carotid bodies, dopamine antagonists enhance carotid body output, albeit more pronounced at low oxygen levels.[56,57] More viable targets are the so-called background potassium channels of the K2P potassium channel family, i.e., hypoxia and acid-stimulated TASK-1, TASK-3, and heterodimer TASK-1/TASK-3 channels, which provide hypoxia-sensitive background potassium conductance in the carotid body type 1 cell.[58–60] In response to hypoxia, these channels mediate the depolarization of the type 1 carotid body cell.[61] Exogenous blockers of these potassium channels, i.e., drugs that mimic hypoxia within the carotid body, produce respiratory stimulation and may be used to overcome centrally mediated opioid-induced respiratory depression.[6,62] Research development into the efficacy of potassium channel blockers in reversing opioid toxicity has been slow in the last 5 yr with just two published investigations, one on the analeptic drug doxapram and another on the experimental drug PK-THHP, both of which inhibit TASK-1 and TASK-3 channels and stimu-

late breathing.[26,38] In etorphine-immobilized goats, doxapram effectively reversed respiratory depression but with adverse effects such as excitation and arousal.[38] These findings agree with human data showing that doxapram, at doses causing respiratory stimulation, induces adverse events including hypertension, dyspnea, headache, dizziness, flushing, sweating nausea/vomiting, muscle spasms, and sometimes severe anxiety.[61] The enhanced pressor response is probably related to an enhanced afferent input from the doxapram-activated carotid bodies to pressure centers in the brainstem.[61]

Interestingly, another carotid body stimulant, ENA001, previously known as GAL021, and an analog of the respiratory stimulant almitrine, acts at large-conductance calcium voltage activated potassium channels, BKCa channels, formerly known as maxi-K channels, stimulates breathing, and partly counters opioid-induced respiratory depression in humans, without causing significant adverse effects.[63,64] It remains unknown why these two stimulants, doxapram and ENA001, acting via the same target organ but at different receptor subtypes, have such different side effect profiles. Finally, PK-THPP, yet another TASK-1 and TASK-3 channel inhibitor, did not enhance breathing or improve arterial blood gas values in rats treated with sufentanil.[26] These data indicate that selectivity in carotid body channel targets is important in countering opioid-induced respiratory depression. It remains unknown whether drugs like ENA001 are able to overcome opioid-induced apnea.[65] Modeling studies based on human data suggest a ceiling in the ability of ENA001 to reverse alfentanil-induced respiratory depression.[64] Possibly combining ENA001 with naloxone may enhance its ability to effectively treat overdose with potent opioids, where ENA001 initially is ineffective.

Sequestration of Opioid Molecules in the Circulation

The techniques mentioned thus far all produce respiratory stimulation via activation or inhibition of systems that do not interfere with the opioid load at the opioid receptors. A radically different method is to sequester the exogenous opioids in blood in such a way that few opioid molecules cross the blood–brain barrier into the brain compartment or cause the rapid redistribution of the opioids back into the blood compartment due to the drop in nonbound opioid concentration in blood. Sequestration may be done by administration of container or scrubber molecules, or by immunopharmacotherapy, in which the opioids are bound to and consequently "neutralized" by antibodies. In both cases, the opioid is unavailable to interact with the central opioid receptor system as the complex cannot cross the blood–brain barrier due to its size and polarity. Similar to the administration of naloxone, the reduction in activated opioid receptors will uncover underlying symptoms such as pain and opioid craving and may cause withdrawal and agitation. This is different from the aforementioned therapies that leave the opioid-receptor interaction intact. However, in contrast to naloxone therapy, opioids that are not the target of the sequestration may be used to treat these secondary symptoms. Opioid sequestration may be used at the end of surgery to counteract the residual effect of

potent opioids or treat an inadvertent opioid overdose, prevent renarcotization after naloxone treatment, or treat opioid toxicity in case of accidental or intended exposure, for example in case of a mass chemical attack with opioids as occurred in the 2002 Moscow theater hostage rescue attack.[66] Additionally, immunopharmacotherapy may be used to prevent a fatal opioid overdose in individuals with an opioid use disorder after a drug-free period for example, due to incarceration or stay in a drug rehabilitation center or as part of their treatment.

Container Molecules

The container molecule calabadion 1 is an acyclic cucurbit[*n*]uril that selectively encapsulates ammonium cations, such as the phenylammonium ion moiety of fentanyl.[67] In awake and isoflurane-anesthetized rats, calabadion 1 is able to dose-dependently reverse fentanyl-induced respiratory depression and muscle rigidity with correction of impaired blood gasses.[67] The calabadion–fentanyl complexes are rapidly eliminated via renal clearance, avoiding the risk of renarcotization. Calabadion 1 binds fentanyl with high affinity but is less effective in binding other opioids such as morphine, hydromorphone, or pethidine. It is able to encapsulate these bigger molecules, but due to a conformational change of the molecule, the binding capacity is reduced. This may be advantageous in perioperative care when high-dose potent opioids such as fentanyl are replaced by morphine or hydromorphone for postoperative pain management. A similar container molecule, calabadion 2, is able to encapsulate the anesthetics ketamine and etomidate, but binds fentanyl at lower affinity than calabadion 1.[68] Also, other container molecules are being developed to bind fentanyl and fentanyl analogs, such as β-cyclodextrin, which binds the amide phenyl ring of the vast majority of fentanyl analogs.[69]

Immunopharmacotherapy

Immunopharmacotherapy is the use of specific antibodies that target and bind specific drugs, e.g., opioid molecules such as fentanyl, heroin, or oxycodone, in the bloodstream.[70–73] The antibodies may be obtained after active immunization with a conjugate vaccine or by passive immunization through administration of monoclonal antibodies. While the former requires some time before sufficient antibodies have been created by immune cells, the latter leads to immediate sequestration of the targeted opioids. Since the opioid molecules are small, the immune system is "blind" to them, and the vaccine must contain an opioid analog (a hapten) that is linked to an immunogenic carrier (e.g., adenoviruses). After immunization, the opioid–antibody complex is too large to cross the blood–brain barrier. While many studies describe the development of opioid vaccines, few of them test their ability to overcome the respiratory effects of potent opioids. Results of these studies are predominantly positive. For example, Raleigh et al.[74] show that a conjugate fentanyl vaccine is effective in the prevention of overt respiratory depression in the rat after fentanyl administration as measured by the decrease in oxygen saturation. Brain fentanyl concentrations were 73% lower in vaccinated rats compared to control animals after fentanyl injection. Similarly, a conjugate fentanyl vaccine tested in mice showed

a reduction in fentanyl lethality compared to control mice with no fatalities in vaccinated mice versus 18 to 55% in control mice after fentanyl administration.[75] In an independent study, immunization against fentanyl reduced respiratory depression, and showed cross-reactivity with sufentanil, albeit only for its analgesic effects, but not with alfentanil or remifentanil.[76] In this same study, immunization did not interfere with propofol, dexmedetomidine, or isoflurane anesthesia. In contrast, a rat vaccine to treat oxycodone use disorder that produced high and sustained antibody titers failed to reduced oxycodone-induced respiratory depression but prevented antinociception and reduced the self-reinforcing effects of intravenous oxycodone.[77] Finally, Raleigh et al. combined a vaccine against oxycodone with extended-release naltrexone and observed greater efficacy than just the vaccine regarding antinociception and respiratory depression.[78]

A different method is passive immunization by administration of monoclonal antibodies, generated in mouse hybridomas after vaccination of the mice with a conjugate opioid vaccine.[70,79,80] Smith et al.[79] showed that passive immunization is effective in increasing fentanyl survival after administration of high-dose fentanyl (above the 50% lethal dose). In fact, the specific antibody tested was as effective as naloxone in the reversal of fentanyl and carfentanil antinociception but with a much longer half-life of 6 days. Importantly, these authors performed a pharmacokinetic–pharmacodynamic simulation study to extrapolate their data to fentanyl-induced respiratory depression in a human. The simulations revealed that a 60-kg individual who received a lethal dose of 3,000 µg intravenous fentanyl as a bolus will show a sharp reduction in ventilation toward apnea followed by a slow restoration of ventilation with return to 40% of baseline ventilation after 20 min. It is reasonable to assume that this individual would have died in the meantime. After treatment with a 500-mg dose of fentanyl antibodies, 24 h before the fentanyl dose, fentanyl caused a similar initial drop in ventilation, which, however, was rapidly followed by a return to 50% of baseline ventilation after about 3 min and 80% after 5 min. The authors calculated that the antibody needs to have a binding association rate constant of at least $1\,\mathrm{nmol\,s^{-1}}$ and a dissociation constant of 0.7 h-1 or less to be effective in rapidly reversing fentanyl toxicity.[79]

These findings are promising and may result in effective treatment and prevention options in a variety of conditions that might induce opioid-induced respiratory depression. The advantages of targeted and specific opioid sequestration, i.e., long duration of action, ability to effectively treat pain and withdrawal with nontargeted opioids, are evident. Still, some challenges remain such as less efficacy in immune-compromised patients, possibly loss of effective treatment over time and with higher opioid doses, and need for multiple vaccines in case of polydrug abuse/overdose.[65]

Finally, we would like to mention another sequestration technique, i.e., the development of a nano-sponge holding purified human opioid receptors that will buffer opioids in the circulation (NarcoBond; CellCure, USA).[7,81] The nano-sponge consists of a nanoparticle coated with lipid bilayer cell membrane containing µ-opioid receptors. After intravenous injection, the

nano-sponge binds and traps opioid molecules and effectively lowers the unbound opioid concentration at the receptor site. No studies have been published on its ability to rescue animals after an overdose from respiratory depression.

Controlled Substances: Opioids

Buprenorphine, among other drugs, is registered by the U.S. Food and Drug Administration (Silver Spring, Maryland) for treatment of opioid use disorder. Buprenorphine is a long-acting opioid that displaces the abused shorter-acting opioid from the μ-opioid receptor and dampens withdrawal symptoms and craving.[82] Eventually, illicit narcotic use will diminish. The question is whether long-acting opioid treatment also prevents respiratory depression from potent short-acting opioids such as fentanyl, heroin, and oxycodone that rapidly cross the blood–brain barrier. Particularly, the characteristics of buprenorphine are such that it may prevent lethal apneas in individuals with an opioid use disorder who overdose on potent opioids. Buprenorphine is a partial agonist at the μ-opioid receptor, antagonist at the κ-opioid receptor, and agonist at the nociception protein receptor. Partial agonism prevents full development of respiratory depression, while nociception protein receptor (NOP) activation stimulates breathing.[83,84] Both reduce the probability of severe respiratory depression or a fatal apneic event. Moreover, buprenorphine has slow receptor kinetics with high affinity for the μ-opioid receptor.[83] This indicates that buprenorphine will prevent binding of potent opioids to the μ-opioid receptor. We tested this assumption in individuals with an opioid use disorder and observed that buprenorphine at concentration in plasma greater than 2 ng/ml prevented respiratory depression and apnea from intravenous fentanyl, even when administered at high dose, a cumulative dose 1,800 μg in individuals with an opioid use disorder.[85] Simulation studies revealed that the best result is observed at a buprenorphine concentration of 5 ng/ml to prevent respiratory depression at the highest fentanyl dose simulated (5,000 μg), a dose not uncommon in the abuse scene.

Buprenorphine may also be used to treat opioid-induced respiratory depression. In opioid-dependent patients who were brought to the emergency room because of respiratory depression or developed respiratory depression during hospitalization, in the context of an opioid overdose, buprenorphine was superior to a continuous infusion of naloxone in reversal of respiratory depression as measured by blood gas values, intubation, and death.[86] Additionally, buprenorphine precipitated less withdrawal than naloxone, although some withdrawal was seen after high-dose buprenorphine. These results warrant further studies to address the interaction between buprenorphine and potent opioids as one needs to realize that buprenorphine, particularly at high dose, produces respiratory depression by itself, and interactions with potent short-acting opioids and sedatives are not well described in humans.

Finally, other opioid receptor agonists/antagonists have been studied in veterinary medicine to prevent lethal opioidinduced respiratory depression from potent opioids such as etorphine.[87,88] For example, in etorphine-immobilized goats, the partial agonists at the μ-opioid receptor nalbuphine and butorphanol improve respiratory parameters but at the cost of excitatory re-

sponses.[80] Given the many side effects that these agents produce, such as sedation, confusion, dizziness, and hallucinations, their use in humans is limited. Another example is diprenorphine,[88] which is used as a veterinary antidote after etorphine or carfentanil immobilization of large animals (e.g., rhinoceros). Its long duration of action, high receptor affinity, and partial agonist activity have prevented its use in humans.

Discussion and Future Perspectives

We systematically reviewed three dozen often still experimental approaches to reduce or prevent opioid-induced respiratory depression (fig. 2). We envision even more potential targets that stimulate breathing and may overcome respiratory depression from opioids or any other cause, derived from existing drugs, such as the carbonic anhydrase inhibitor acetazolamide, the antioxidants ascorbic acid and α- tocopherol, the cholinesterase inhibitor physostigmine, and the hormones progesterone or orexin.[6,89] We subdivided the countermeasures by mode of action or molecule characteristics to give a clear insight into their mechanism of action and side effect profile. Some drugs, such as the majority of controlled substances, have a limited indication, mostly due to their adverse effects such as high probability of abuse and addiction and development of schizotypal adverse events.

Irrespective of mechanism, all countermeasures, apart from sequestration techniques, have in common that their goal is the pharmacologic strengthening or reactivation of rhythmogenesis within the respiratory neuronal network by providing tonic input to the respiratory neurons that remain depressed by the opioids due to their enduring presence within the network. The main conclusion of our scoping review is that, compared to naloxone, most if not all of these medical countermeasures are insufficiently viable to be used in daily clinical practice to treat an acute highdose (synthetic) opioid-induced respiratory depression that causes apnea, let alone to successfully address mass casualties from public health or military events due to the release of potent high-affinity opioids such as carfentanil in the environment. There are various reasons why these therapeutic or preventive interventions fail. The main reason lies within the respiratory neuronal network itself in that, as long as respiratory drive is depressed due to activation of the μ-opioid receptor system, the degree of activity that is being generated by nonopioid stimulants is insufficient to overcome depression of respiratory neurons in, for example, the pre-Bötzinger and parabrachial/Kölliker–Fuse complexes, two small brain areas with high respiratory sensitivity to opioids.[3–5,90] Baertsch et al.[5] showed that opioids have a dual mechanism of opioid-induced respiratory depression at the pre-Bötzinger complex within the inspiratory rhythm-generating network (fig. 1). While stimulants such as ampakines may compensate one mechanism, i.e., opioid- induced impairment of excitatory presynaptic neurotransmission, they are unable to compensate the second mechanism, i.e., opioid-induced intrinsic hyperpolarization of respiratory neurons. Consequently, the overall efficacy of ampakines may

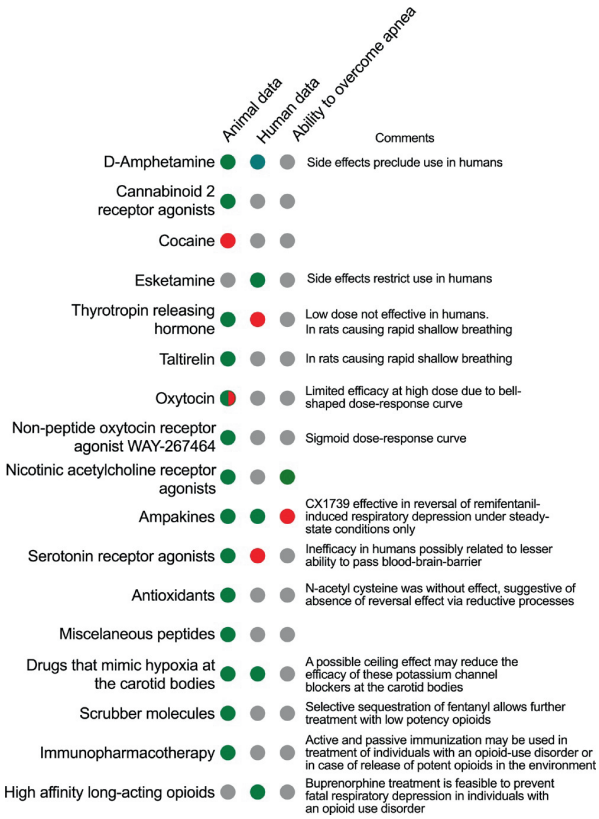

Figure 2: List of agents used to treat or prevent opioid-induced respiratory depression. Green circles indicate effective reversal/prevention or ability to reverse an apneic event; red circles indicate ineffective reversal/prevention or inability to reverse an apneic event; gray circles indicate that no studies have been published.

be limited and only be useful in case of low-dose opioid-induced mild to moderate respiratory depression. Additionally, due to their specific mechanism of action, some drugs act at the Kölliker–Fuse complex, while others target the pre-Bötzinger complex, with a net insufficient effect on severe respiratory depression leading to apnea.[1] This applies to most stimulants with possibly the exception of the nicotinic acetylcholine receptor agonists, which are able to overcome opioid-induced apnea, at least in rodents.[32,33] Stimulants that act at the carotid bodies are limited in their ability to reverse opioid-induced apnea due to a ceiling in afferent input from the carotid body to the respiratory network in the brainstem.[64]

In summary, it is evident from recent experimental data and our systematic review that at higher opioid doses, the level at which the disruption of the respiratory rhythmogenesis is restored by reversal agents or respiratory stimulants is limited.[3–5] An additional reason for therapy failure may be that an insufficient amount of drug reaches the brainstem respiratory neurons.[6] While the mechanism of action may be appropriate, this pharmacokinetic drawback may be overcome by designing more lipophilic reversal agents that readily cross the blood–brain barrier. This may, for example, apply to serotonin receptor agonists.[1] Approaches that sequester opioid molecules within the bloodstream will effectively lower the opioid load within the brainstem respiratory network. While this is a desired mechanism under some circumstances, these countermeasures are still insufficiently tested with respect to efficacy, speed of onset and offset, and safety.

Considering these limitations, we suggest altering current research approaches and initiating research programs that specifically test drug combinations. Ren et al. already showed that combining the nicotinic acetylcholine receptor agonist varenicline with low-dose naloxone overcomes fentanyl-induced apnea.[34] As stated earlier, this interaction of two drugs with different modes of action may serve as a model for other drug combinations that separately show limited or partial reversal of opioid respiratory depression but in combination might be highly potent with synergistic excitatory effects on respiration. Several combinations come to mind such as low-dose naloxone in combination with nicotinic acetylcholine receptor agonists, ampakines, or drugs acting at the carotid bodies. Particularly when opioids are overdosed in combination with other depressants, the combination of stimulants may be more effective. Whether low-dose naloxone needs to be part of such drug combinations requires further study, as possibly other combinations are viable as well.

Given the immediate need for alternatives to current therapy, the U.S. National Institute of Allergy and Infectious Diseases/National Institutes of Health (Bethesda, Maryland) recently (August 2019) organized a 2-day transagency scientific meeting and discussed the development of novel medical countermeasures and treatment strategies to mitigate opioid-induced respiratory toxicity.[7] One of the goals of the meeting was to provide a forum for networking and collaborative partnership. We encourage such collaborations aimed at optimizing treatment in the reversal and prevention of opioid-induced respiratory depression.

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Chapter 5

Carbon Dioxide Tolerability in Rat and Man: A Translational Study

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Introduction

Carbon dioxide (CO_2) is a product of the aerobic metabolism of energy containing nutrients (carbohydrates) in humans and animals and from industry- and transport-related combustion processes. The rise in global CO_2 emission ($37\,\mathrm{Gt}$ in 2018)[1] and consequently its accumulation in the atmosphere caused and continues to cause a global rise in temperature, which has deleterious effects on the climate.[2,3] Hence, there is the need for CO_2 reduction, for example through capture and storage[4,5] CO_2 is captured in industrial plants and transported via pipelines to underground (or undersea) storage facilities, such as depleted gas stores.[5] Large scale implementation of this technique in the near future could result in storage of CO_2 in the vicinity of populated areas. In case of incidents (e.g., pipeline failures and/or problems at storage facilities) point source releases of large quantities of CO_2 will result in a cloud with high CO_2 levels. Acute exposure to high levels of CO_2 may be hazardous for human health, both within the fence line (workers), as well as outside the fence line (general public). CO_2 exerts its acute toxicity through different mechanisms. Most importantly, at acute exposure to high levels (>30%), CO_2 induces the displacement of oxygen (CO_2 is heavier than air), causing a hypoxic environment and toxicity from asphyxia (the lack of oxygen combined with an increase in arterial CO_2 concentrations). Second, upon inhalation, CO_2 causes sympathoexcitation and acidosis[6], which may cause arrhythmias and tissue injury. Finally, CO_2 induces severe anxiety and fear due to cerebral acidosis[7,8], which may cause inability to take coherent decisions, an effect that is further aggravated by cognitive decline at high inspired CO_2 concentrations.[9]

Since its discovery in 1754, several scientific publications were dedicated to the effects of CO_2, but the number of dose escalation studies on the effect of acute exposure to CO_2 on changes in human physiology is sparse.[7,9–11] The highest inhaled CO_2 concentration studied was 40% and published almost a century ago.[10] While these extremely high concentrations were only applied for a few breaths, it did provide some information on CO_2-induced subjective symptoms. Here we present data from an exploratory and translational project, performed in humans and rats, that was aimed to improve our understanding of the physiological and behavioral effects of short-term (acute) exposure to high levels of carbon dioxide. This understanding will facilitate the safety design and emergency response procedures for CO_2 transport and storage facilities. We exposed healthy young adult humans to 6–12% inhaled CO_2 for 10–60 min, and exposed rats to 10–50% CO_2 for up to 60 min.

Since there is a lack of systematic examination of the effect of time-varying escalating concentrations of inhaled CO_2 in humans and translation between man and animal (rodent) studies is sparsely described, we performed the current study, with ultimate aim to provide data to reassess the current guidelines for CO_2 exposure. To these ends, we determined the effect of CO_2 inhalation on tolerability (in human and rats), lethality (in rats) and on the acute acid-base state as measured by arterial pH. Studies involved regular arterial blood sampling for blood gas analyses during and following exposure to CO_2 and objectivation of behavioral changes. In

humans, we further tested cognition, cerebral oxygen saturation, and measured hemodynamic parameters. In the animals, the lungs were examined to assess pathology and possible causes for CO_2-related death. Next, to correlate the results of the animal with those of the human studies, we developed a translational model of pH to provide insight in the effect of higher CO_2 concentrations than tested in our human study population on pH. We hypothesized that at least 50% of healthy volunteers are able to tolerate 9% CO_2 inhalation for periods up to 30 min.

Materials and methods

Ethics and registration

In this exploratory project both animal and human studies were performed. The animal protocol was approved by the University Animal Ethics Committee (Leiden University, Leiden, the Netherlands), the human protocol by the Human Ethics Committee (Commissie Medische Ethiek, Leiden, the Netherlands) and the Central Committee on Research Involving Human Subjects (CCMO, competent authority) in The Hague, the Netherlands, all in 2015. During the study we remained in close contact with the ethics committee and regularly reported on the progress and occurrence of adverse events of the study. The human protocol was registered in the trial register of the Dutch Cochrane Center trialregister.nl under identifier NL4955 on 1 August 2015. Since the trial register is no longer available, the protocol can be obtained from the authors (a.dahan@lumc.nl). The study was conducted in accordance with current Good Clinical Practice Guidelines and adhered to the principles of the Declaration of Helsinki. The study was performed from 1 Oct 2015-1 March 2018.

Study in humans

Participants

Healthy male volunteers were recruited to participate in the study. Inclusion criteria were age 18–25 years; body mass index in the range 18–25 kg/m2 (inclusive) with body weight between 50 and 100 kg; absence of any significant medical, neurological, or psychiatric illness as determined by the investigators; and willingness and competence to sign a written informed consent. Exclusion criteria were: a history of panic disorder; a history of hypertension; present or a history of any illicit drug use; present or a history of alcohol abuse (intake of more than 4 units per day); smoking of more than 10 cigarettes per day; participation in a drug trial in the 3 months prior to screening; any physical abnormality as determined by an independent physician; or any other issue/condition that in the opinion of the investigator would complicate or compromise the study or the well-being of the subject (these include claustrophobia, fear of needles,

car sickness, recurrent headaches, tinnitus, unwillingness to follow the instructions of the researchers).

CO_2 exposure and stopping rules

All subjects were studied once (i.e. they only were exposed to one concentration of CO_2 for just one duration). The first set of 54 subjects received one of three inhaled carbon dioxide concentrations, 6%, 7.5% or 9%, with adjusted inspired oxygen levels of 19.7%, 19.3% and 18.9%, respectively (these values simulate O2 displacement by CO_2). Exposure times were escalating with 6 subjects inhaling 6% CO_2 for 10 min, 6 others for 30min and finally 6 others for 60 min. When no stopping rules were met, we increased the CO_2 concentration to 7.5% and again exposed 6 subjects for 10 min, 6 others for 30 min and finally 6 subjects for 60 min. After successful completion of these cohorts,we amended the protocol to further expose 20 volunteers to 10% CO_2 (inspired O2 18.8%; n = 10) and 12% CO_2 (inspired O2 18.4%; n = 2) for a maximum duration of 10min. Stopping rules were: pH < 7.20, heart rate >180 beats per min, systolic blood pressure >200 mmHg, diastolic blood pressure >120 mmHg or subjectively experienced side effects warranting discontinuation (as decided by the subject or the investigators). In case stopping rules were met in cohorts 1 to 10, we discontinued the experiment in that subject but continued to the next subject; however, the study would be terminated when all subjects of a specific cohort would have reached stopping rules. For cohort 11, we decided to evaluate the advancement to a next subject based on the results of the previous subject.

Monitoring

To ensure the safety of the subjects and obtain as much information during CO_2 xposure we continuously measured the ECG, peripheral arterial oxygen saturation through a finger probe (SpO2), and regional cerebral oxygen saturation (rSO2) using an INVOSTM 5100C cerebral oxygen saturation monitor (Medtronic, Minneapolis, MI, United States) with the sensor applied to the left side of the forehead. An intravenous access line was placed in the cubital vein of the non-dominant arm, and a 22G arterial cannula was placed in the radial artery (at the level of the wrist) of the non-dominant arm. The arterial line was connected to a FloTrac sensor and Vigileo system (Edwards Lifesciences, Irvine, United States) for cardiac index monitoring. The oxygen and carbon dioxide concentrations were measured in the helmet close to the mouth using a capnograph (Capnomac Ultima, Datex-Ohmeda, Finland).

Gas delivery

An adult size continuous positive airway pressure helmet (Dimair ®) was used to deliver the gas mixture to the participants. The helmet was positioned over the head and closed around the neck, and allowed normal verbal communication with the research staff and permitted performance of cognition tasks without any constraints or respiratory efforts. A gas mixture was delivered to the helmet from a custom-build computer-controlled gas-mixing setup (the first-

generation Leiden gas mixer) containing three mass flow controllers (Bronkhorst High-Tech BV, Veenendaal, the Netherlands) for delivery of 50 L/min gas in any combination of O2, CO_2 and nitrogen.[12] The helmet had an outlet (∅ 44 mm) ensuring adequate drainage of gas flow and guaranteeing no pressure buildup within the helmet even at high respiratory rates. Prior to the exposure to increase levels of CO_2, the subjects breathed a gas mixture that mimicked room air (20.8% O2 in nitrogen).

Study sequence

After a period of relaxed breathing, the following baseline values were collected: arterial gas values (pH, arterial PO2, arterial PCO_2 and oxygen saturation; derived from the i-STAT blood gas analyzer (Abbott, United States) using CG8+ and CG4+ cartridges; in the 10 and 12% exposure cohorts, two devices were used to keep up with the frequent blood sampling; the device is able to measure pH values from 7.7-6.5), cardiac index, cerebral oxygen saturation (rSO2), and subjective experiences (sedation, nausea, headache) and a p-deletion test. Next, the subject was exposed to the preset gas mixture. During exposure arterial blood gas measurements were obtained at 5-min intervals (2-min intervals for the 10 and 12% CO_2 cohorts), subjectively experienced side effects at 10-min intervals and non-invasive blood pressure using an arm cuff at 10-min intervals. All other variables (O2 and CO_2 concentrations in the helmet, cardiac index and brain oxygen saturation) were logged continuously at 50 Hz.

The CO_2 exposure ended when the intended duration of the experiment was reached, in case stopping rules were met, upon request of the subject, or upon judgement of the attending physician. Upon termination, 100% oxygen was administered for 5 min; thereafter the helmet was removed and the subject breathed room air. In case the experiment ended prematurely, an attempt was made to obtain a final arterial blood gas sample was obtained. We queried the subjects immediately after CO_2-exposure for side effects including sedation, nausea and headache using a 11-point Likert scale ranging from 0 (not present) to 10 (maximum experience of symptom); querying continued at 30-min intervals for at least another hour. After removal of the helmet, the subject was monitored for 60-min. The subject was dismissed from the laboratory only if side effects (including subjective effects) had waned and the attending physician agreed that the subject was sufficiently recuperated to go home.

The p-deletion test

To determine the cognition of the subject during and following CO_2-exposure, the modified p-deletion test was performed.[13] The test consists of 19-lines on one page of 38 lower case letters b, d, q and p. A total of 45 letters p are distributed at 2 to 3 per line at a random location within the line. The test was performed at t = 10-, 30- and 60-min during CO_2 exposure (depending on the cohort) and at t = 30- and 60-min following CO_2 exposure. The test was scored by determining the number of successfully completed lines and the number of mistakes.

Study in rats

Animals and study design

CD® (Sprague Dawley) IGS adult male rats (250–270 g) were purchased from Charles River Laboratories (Leiden, the Netherlands). The animals were obtained with a femoral arterial catheter in place (Instech Laboratories Inc., PA, United States). The animals were exposed to one of five inspired CO_2 concentrations, 10, 20, 30, 40 or 50%, with adjusted inspired oxygen concentrations of 18.9, 16.7, 14.6, 12.5 or 10.4%, respectively, in cohorts of 8 animals (total number of animals used in the study is 41, including 1 control animal breathing just ambient air). Each animal was exposed for 60 min, after which they were euthanized by pentobarbital injection.

The arterial line allowed continuous access to arterial blood (100 µl) for blood gas analysis and glucose and [K+] measurement. In order to restrict blood loss from the animals two distinct sampling strategies were applied per cohort. The sampling regimen of the first group (n = 4) focused on acute changes in blood gas values following the initiation of CO_2 exposure (t = 0) with sampling at 2-min interval from t = 0 (just prior to exposure) to t = 10 min, and 2 final samples at t = 15 and 20 min. In the second group, the remaining 4 animals, sampling occurred at t = 0, 5, 10, 20, 30, 40, 50 and 60 min following the start of CO_2 exposure. Maintenance of the arterial catheter prior to the study was according to the guidelines of the Charles River laboratories. During exposure, all animals were monitored for changes in behavior and respiratory rate was counted at 5-min intervals. Animals that appeared in discomfort (e.g. because of epileptiform activity) or moribund (e.g. gasping) prior to the end of the CO_2 exposure were taken out of the inhalation box and euthanized.

CO_2 exposure occurred in a custom-build Perspex transparent inhalation box. The animals were unrestrained throughout the experiment. The air humidity and temperature in the box were maintained constant by using of a humidifier. The box was connected to the first-generation Leiden gas mixer, and the desired gas mixture flowed through the box at 20 L/min. Each CO_2 exposure was preceded by inhalation of a gas mixture that mimicked ambient air. The arterial line was accessible from the outside of the box. Gas concentrations within the box were constant until completion of the 1-h CO_2 exposure or in case the animal became moribund and was removed from the box. Following the death of the animals, macro and micropathological examination of the lungs was performed to determine exposure-induced lung damage by the department of animal pathology of the Leiden University Medical Center.

Macroscopic and microscopic inspection of the lungs

Obduction and macroscopic analysis was performed in all animals. A microscopic inspection of the lungs was performed in a random selection of the animals (n = 7), exposed to 10% CO_2 (n = 1), 20% CO_2 (n = 1), 30% (n = 1), 40% CO_2 (n = 1) and 50% CO_2 (n = 3). To serve as control, one additional animal was euthanized with pentobarbital after 1-h of air breathing (without CO_2 exposure). First, the lungs were inspected for subpleural hemorrhages. Next, five to six

lung sections were stained with hematoxylin and eosin to grade the number of hemorrhages (alveolar, peribronchial, perivascular), alveolar or perivascular emphysema and % total emphysematous area. A total of 46 observations were included in the analysis.

Data analysis

For sample size calculation, we focused on the tolerability of inhaling 6% CO_2 versus 9% CO_2 in the human population and assumed that all subjects in the 6% CO_2 arm of the study would tolerate 30 min of CO_2 inhalation, while in the 9% CO_2 arm this would be 5. We then calculated a minimum sample size of 8 to detect whether the stated difference exists between the two proportions. Given the uncertainties in the assumptions, we included 10 subjects in each group.[14] The data are described as mean ± SD, median (range) or number (percentages). No formal data comparisons were performed as this was an exploratory study. Additionally, group numbers were small, comparisons were hampered by data loss from discontinuations (human study) or premature death (animal study) and concentration-effect relationships were evident, making a post hoc comparison less relevant.

Translation between species

The translational model starts out with the CO_2 alveolar mass balance[15]:

$$\text{VOL}_{\text{ALV}} \times \frac{dPCO_2}{dt} = \dot{V} \times (P_ICO_2 - P_{\text{ALV}}CO_2) + k \times Q \times (C_vCO_2 - C_{\text{ALV}}CO_2) \quad (1)$$

where $VOL_{ALV} =$ is the volume of alveolar tissue, $\dot{V}$ is minute ventilation, P_ICO2 is the inspired CO_2 concentration, $P_{ALV}CO_2$ is the alveolar CO_2 concentration (we assume that this is equal to arterial and end-tidal CO_2 concentration), k is a constant that relates blood CO_2 content to concentration, Q cardiac output (minus pulmonary shunt), C_VCO_2 the CO_2 content of venous blood, and $C_{ALV}CO_2$ the alveolar CO_2 content, which we assume equals arterial CO_2 content. To simplify the calculations, we further assume that VOL_{ALV} is negligible over the time scale of interest, ventilation and cardiac output rapidly reached their increased values and remained constant, CO_2 concentration is linearly related to CO_2 content in blood, venous CO_2 concentration has a first-order delay relative to arterial CO_2 concentration, the production of CO_2 is negligible relative to the high inspired CO_2 concentration, and $[HCO3-]$ in blood is constant. Then:

$$\dot{V} \times (P_ICO_2 - P_{\text{ALV}}CO_2) + k \times (P_vCO_2 - P_{\text{ALV}}CO_2) = 0 \quad (2)$$

where P_VCO_2 is the venous CO_2 concentration. Mixing of CO_2 in the lungs was described as follows:

$$P_{ALV}CO_2 = \alpha \times P_ICO_2 + \beta \times P_vCO_2 \tag{3}$$

where α and β are mixing parameters with $\alpha = \dot{V}/(\dot{V} + k)$ and $\beta = 1 - \alpha$. Since CO_2 production is not included in the model, the sum of α and β might differ from 1.

The CO_2 mass balance of the body is modeled as:

$$\frac{dP_vCO_2}{dt} = (P_{ALV}CO_2) - P_vCO_2) \times \phi \tag{4}$$

where ϕ is the body CO_2 equilibration rate constant.

The Henderson-Hasselbalch equation equals:

$$pH = 6.1 + [^{10}\log(HCO_3^-)]/(0.23 \times P_{ALV}CO_2) \tag{5}$$

CO_2 exposure was tested as a covariate in the log domain of all parameters. All pH-time data of the human and rat data were fitted simultaneously to the model, while simulations were performed to predict the pH-time data in humans inspiring 10, 15, 20, 30, 40 and 50% CO_2. The analysis and simulations were performed in NONMEM, a software package for nonlinear mixed effects modeling, using a population approach.

Results

Study in humans

We intended to include seventy-four male subjects in the study. The study had eleven specific CO_2 inhalation cohorts. Cohorts 1–9: 6, 7.5 and 9% CO_2 inhalation for 10, 30 and 60 min

(with 6 subjects in each cohort) and after finalizing cohorts 1-9, cohorts 10 and 11 (included after ethics approval of a protocol amendment): 10 and 12% inhalation for 10 min, with 10 subjects per cohort. The actual number of treated subjects was 66, as the study was prematurely terminated after 2 subjects were exposed to 12% CO_2 for less than 10 min because of side effects (see below). The mean (±SD) age and mean body mass index of the treated subjects were 24 ± 3 years and 23 ± 2 kg/m2, respectively. Among the cohorts no differences were observed in age or body mass index distributions. After the start of CO_2 exposure, the intended inspired values were reached within 1 min, arterial PCO_2 and PO2 are given in Figures 1B,C.

CO_2 tolerability

Average inhalation times are given in Table 1. The inhalation of 6 and 7.5% CO_2 was well tolerated for up to 60 min by all subjects. The next cohort, 9% CO_2, was well tolerated by subjects inhaling the gas mixture for 10 min. Longer exposure was less well tolerated by 4 (out of 6) subjects that completed 30 min and 1 of 6 completed 60 min of inhalation. The causes of discontinuation were anxiety, panic or exhaustion due to heavy breathing. In the last 2 cohorts (#10 and 11), 3 of 10 subjects completed the 10-min inhalation of 10% CO_2, while none of the subjects completed the 10-min inhalation of 12% CO_2 (only two subjects were tested in this cohort). The causes of discontinuation were dissociation, blackout, anxiety and overwhelming dyspnea. The two subjects that were discontinued in the 12% CO_2 inhalation (after 7 ± 2 min) had pH values <7.2 and were unable to communicate with the investigators although they appeared awake. Additional symptoms observed in the 9, 10 and 12% cohorts were myoclonic twitches, restlessness, headache (average score ±SD 4.8 ± 2.2 on an 11-point Likert scale) and sedation (2.5 ± 1.9 on an 11-point Likert scale); these symptoms were not the reason for participant-initiated discontinuation. After consultation with the ethics committee, we decided not to proceed to a third subject in the 12% CO_2 cohort and prematurely ended the study. Interestingly, upon recovery, during inhalation of 100% oxygen, some subjects developed a headache, and some of these subjects vomited. We relate this to the occurrence of sudden vasoconstriction (due to hyperoxia) and cerebral hypoperfusion following maximal vasodilation (due to hypercapnia).[16]

CO_2 effect on cognition and brain oxygen saturation

Cognitive performance, as measured by the p-deletion test, was dose-dependently affected by CO_2 inhalation, with the worst performance at inhaled CO_2 concentrations of 10 and 12%, with respect to number of lines completed (Figure 1A) and number of errors (data not shown). These effects proved to be transient as a swift recovery was observed upon termination of the exposure. Inability to focus and the laborious breathing activity were the main causes for the decrease in performance. The decreased cognitive performance was unrelated to the oxygen concentration in the brain as brain oxygen concentration (measured by near-infrared spectroscopy on the forehead) increased from 75 to 80–90% within 10 min of inhalation (Figure 1D; recovery of $_rSO_2$ given in Figure 1E), most probably related to an increase in cerebral blood flow

associated with the increase in cardiac output and cerebral vasodilation.

CO_2 effect on hemodynamics variables

From 6 to 10% CO_2 exposure, mean arterial pressure and cardiac index dose-dependently increased to a maximum of 140 mmHg and 10 L/min per m2, respectively (Figures 1F,G). No further increase in mean arterial pressure was seen in the 12% CO_2 cohort, which we relate to the small number of subjects (n = 2) that remained in that cohort and the frequent blood sampling that limited the ability of the invasive blood pressure measurement device to obtain reliable blood pressure values.

CO_2 effect on blood gas values and estimated minute ventilation

Arterial PO2 increased over the first 5 min of exposure to 125 mmHg, in a dose-independent manner (Figure 1B). This was unexpected as at constant inspired oxygen fraction, water pressure and atmospheric pressure, alveolar PO2 is inversely related to alveolar PCO_2. At higher inspired CO_2 levels alveolar PO2, and subsequently arterial PO2 and oxygen saturation should decrease. Additionally, the right-shift of the hemoglobin-oxygen dissociation curve supports oxygen unloading from hemoglobin, causing a further drop in arterial PO2. Possibly a reduction of the ventilation/perfusion mismatch, related to an increase in ventilation and cardiac output, counteracted the expected decrease in arterial PO2 (see also below). In all cohorts, pH decreased rapidly to a plateau after 5min of CO_2 inhalation (Figure 1H). Dose-dependency was apparent until the 10% CO_2 cohort with no further decrease in the 12% CO_2 cohort, which we again attribute to the small number of subjects that remained in that cohort. Lowest pH was 7.18 in the subject that was subsequently discontinued from the 12% CO_2 cohort. Upon recovery, pHreturned to baseline values within 5 min; pHrecovery is given in Figure 1I. Finally, glucose levels (onlymeasured in the 6–9% CO_2 cohorts) remained constant over time within the normal range (range 5.5–6.3 mmol/L with normal values 4.0–6.1mmol/L). From the measured arterial PCO_2 values, we were able to estimate the minute ventilations at static pH values as observed in the 6–9% CO_2 cohorts, using the formula of the hypercapnic ventilatory response,

Table 1: Study Participant Demographics and Baseline Characteristics

Inhaled concentration	6%			7.5%			9%			10%	12%
Intended duration	10	30	60	10	30	60	10	30	60	10	10
No. subjects completing inhalation / total No. participants	6/6	6/6	6/6	6/6	6/6	6/6	6/6	4/6	1/6	3/10	0/2*
Actual inhalation-duration (min)	10+/-0	30+/-0	60+/-0	10+/-0	30+/-0	60+/-0	10+/-0	16+/-12	20+/-22	7+/-3	7+/-2

*Just 2 of the 10 planned subjects entered this cohort, after which the study was prematurely terminated. Inhalation duration: mean ± SD.

$V_E = S \times (PaCO_2 - B)$, where V_E is minute ventilation, S the slope of the hypercapnic ventilatory response, $PaCO_2$ arterial PCO_2 and B the extrapolated arterial PCO_2 at zero ventilation.[17] We used values of S and B estimated in similar study populations.[12,18,19] The results were for 6% CO_2 an estimated minute ventilation of 29 L/min, for 7.5% CO_2 48 L/min and for 9% CO_2 66 L/min.

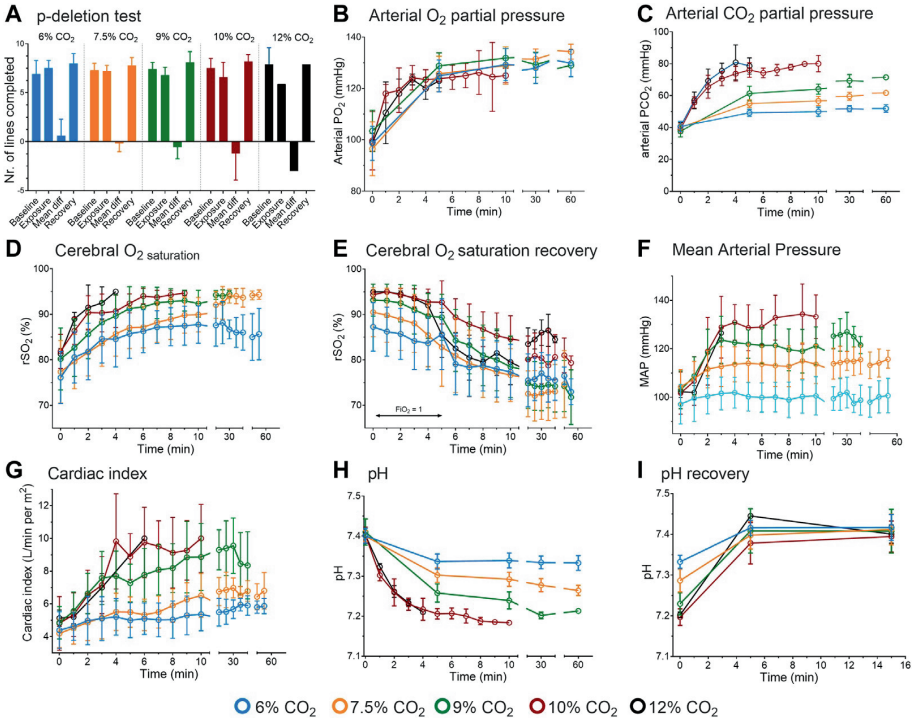

Figure 1: Results of the human experiments. (A) Cognitive function assessed by p-deletion test. Results are from before CO_2 inhalation, during inhalation and during recovery. The mean difference is the difference between tests obtained at baseline and during inhalation. (B) Arterial oxygen concentrations during $6 - 12\%$ CO_2 inhalation. (C) Arterial carbon dioxide concentrations during CO2 inhalation. (D) Cerebral oxygen concentration as measured by near-infrared spectroscopy during CO_2 inhalation. (E) Cerebral oxygen concentration following CO_2 inhalation while breathing 100% oxygen (for 5 min) and next while breathing room air. (F) Mean arterial pressure during CO_2 inhalation. (G) Cardiac index during CO_2 inhalation. (H). pH values during $6 - 12\%$ CO_2 inhalation. (I). pH data obtained during the first $15 - min$ following CO_2 exposure while breathing 100% oxygen. The data obtained at 12% inhalation are from 2 subjects; duration of 10 and 12% CO_2 inhalation was max. 10 min. All data are mean $\pm$ SD.

Study in rats

Forty adult male Sprague Dawley rats with mean weight 261 ± 33 g were exposed to increasing concentrations of inhaled CO_2 (10, 20, 30, 40 and 50%) with 8 animals per dosing cohort. Additionally, 1 animal served as control for pathology.

CO_2 tolerability

All sixteen animals completed the 1-h exposure to 10 and 20% CO_2 without serious discomfort. The animals in the 10% CO_2 cohort exhibited normal behavior throughout the 1-h exposure. The animals in the 20% CO_2 cohort displayed hyperactivity and excitation for the first 30–40 min of exposure and then transitioned slowly into hypo-activity during the remaining time in the inhalation box. Hyperactivity consisted of rapid irregular breathing, disorganized behavior and uncoordinated movements. During exposure to 30% CO_2, an initial 10-min period of hyperactivity was followed by pronounced hypoactivity (apparent CO_2 narcosis); in four animals sudden and severe epileptiform activity occurred and the animals were, as we wanted to prevent any further discomfort, immediately euthanized with pentobarbital. None of the other four animals showed any excitatory signs or irregular breathing. During exposure to 40% CO_2, an initial 1–2 min period of hyperactivity was followed by complete hypoactivity and rapid shallow regular breathing (apparent CO_2 narcosis); none of the animals died or displayed signs of epilepsy. During exposure to 50% CO_2, apparent narcosis rapidly developed and the animals showed slow shallow breathing. Five animals died after 14–25 min of exposure, the others survived until the end of exposure.

CO_2 effect on respiratory rate and blood gas values

During 10–40% CO_2 inhalation, respiratory rate increased from baseline values (80 breaths/min) to 150–175 breaths/min within 10-min of CO_2 exposure (Figure 2A). At 50% CO_2 inhalation, a decrease in respiratory rate was observed from baseline to 25 breaths/min. In all cohorts, the decline in pH was biphasic, with an initial rapid decrease followed by either no further decline (10% CO_2 cohort) or a further slower decline (Figure 2B). The magnitude of acidosis development was dose-dependent with lowest pH values observed in the 10% CO_2: pH = 7.26 ± 0.2 at t = 10 min, 20% CO_2 cohort: pH = 6.90 ± 0.4 at t = 30 min, 30% CO_2 cohort = 6.82 ± 0.01 at t = 20 min; 40% CO_2 cohort: pH = 6.63 ± 0.08 at 30 min; and in the 50% inhalation cohort: pH = 6.54 ± 0.02 at t = 20 min. Due to missing data from the loss of animals, device related limitation to measure pH below 6.5 or issues with sampling from the arterial line, these values may be an overestimation of the actual values in the highest CO_2 cohort.

In the low CO_2 inhalation cohorts (10 and 20%) arterial oxygen concentrations increased, despite reduced FiO2 values that were implemented to simulate O2 displacement by CO_2.(Figure 2C)[20] Similar to the observations in humans, we expect that this is the consequence of the reduced ventilation/perfusion mismatch (i.e. reduced shunting), the increase in breathing fre-

quency, hypercapnia-potentiated hypoxic pulmonary vasoconstriction and increased cardiac output. The increase in arterial PO2 was maintained throughout the 60-min of CO_2 inhalation.

In the 30–50% CO_2 inhalation cohorts, arterial oxygen concentrations significantly decreased (Figure 2B). Despite the pulmonary damage (see below), the PaO2/FiO2 ratio remained unaffected by the CO_2 level with relatively normal to supra-normal values irrespective of the inhaled CO_2 concentration (Figure 2C). Due to missing data from the loss of animals in the 30 and 50% CO_2 inhalation cohorts and sometimes sampling issues, the PaO2/FiO2 ratios are most probably largely overestimated.

CO_2 effect on potassium and glucose concentrations

Extracellular potassium concentration increased with decreasing pH due to the H+/K+ exchange across the cell membrane (Figure 2F). The increase in plasma K+- concentration may be associated with cardiac arrhythmias and death in the animals that succumbed to high dose CO_2 inhalation, although other causes of death are not excluded (see below). A dose-dependent increase in glucose concentration was observed with the highest glucose concentration measured in the 50% CO_2 cohort (24 mmol/L; Figure 2G). No increase in glucose was observed in the 10% CO_2 cohort.

CO_2 effect on macroscopic and microscopic changes to the lungs

Macroscopic inspection of the lungs revealed that the animals exposed to 30–50% CO_2 had signs of subpleural or pulmonary hemorrhage and edema (Figures 2H–K). The lung to body weight ratio dose-dependently increased indicative of accumulation of fluid and blood in the lungs (Figure 2E). An important finding was that the animals that had prematurely expired had 60% greater lung weights than animals that completed the exposure: 2.4 ± 0.3 g versus 1.4 ± 0.2 g. Microscopically, a dose-dependent increase in emphysematous pulmonary changes, edema and hemorrhages was observed (Table 2). The microscopic changes observed in the 30% and higher CO_2 cohorts were severe and most probably not compatible with (long-term) survival (Figures. 2L and 2M). These findings indicate that pulmonary damage may be the final and definite cause of death from CO_2 and/or acute hypoxia in these rats (at 50% CO_2 the arterial PO2 is around 75 mmHg, Figure 2C).

Translation between species

In order to predict the pH effect of higher CO_2 inhalations than tested in the human subjects, we constructed a translational physiological model of acidity (pH). To that end, we simultaneously analyzed the human and animal data, using a model that combined the CO_2 mass balance in the alveoli, the CO_2 mass balance in the body compartment and the Henderson-Hasselbalch equation using a population approach in the statistical package NONMEM. Inspection of the data fits as well as of the goodness of fit plots (data not shown) indicate that the data from two species were well described by the model. Examples of data fits are given in Figures 3A–E: Figures 3A–C

Table 2: Effect of CO_2 on lung damage in rats.

CO2 *** (sample size)	Hemorrhages *				Emphysema **	Perivascular edema and emphysema *
	Alveolar	Peri-bronchial	Peri-vascular	Sub-pleural		
Control (1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	30 %	1 (1-1)
10 % (1)	0 (0-1)	0 (0-0)	1 (1-1)	0 (0-0)	30 %	0 (0-0)
20 % (1)	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	30 %	1 (1-2)
30 % (1)	0 (0-0)	0 (0-0)	1 (1-1)	0 (0-1)	55 %	2 (2-2)
40 % (1)	0 (0-0)	0 (0-0)	1 (1-1)	0 (0-0)	65 %	2 (2-2)
50 % (1)	2 (1-2)	3 (1-3)	3 (2-3)	2 (1-2)	83 %	3 (3-4)

*Median number of observations per lung section (range); ** % total emphysematous area. ***The data are obtained from 5-6 lung sections with 1 animal per inhalational level, except for 50% CO_2 inhalation, where the results of 3 animals are given.

give examples human data fits with inhalations of 12, 10 and 9% CO_2; Figures 3D,E show two examples of rat data fits. Parameters estimates were similar for rat and human data, except for CO_2 mixing parameters α and β. Parameter values (median ± standard error of the estimate) at baseline, prior to any CO_2 exposure, were: $[HCO3^-] = 25.60.21 mmol/L$, $PCO_2 = 47.30.83$ mmHg and the body CO_2 equilibration rate constant $\phi = 1.980.56$; for humans $\alpha = 0.890.01$ and $\beta = 1 - \alpha$, for rats $\alpha = 0.670.12$ and $\alpha + \beta = 0.930.007$. CO_2 exposure was a significant covariate on ϕ with $\log\phi = -0.0310.007$. These results indicate a slower mixing of CO_2 in humans with a slower decrease in pH over time and reduction of ϕ at higher CO_2 exposures. Predictions of pH values at 10% inspired CO_2 or higher are given in Figures 4F (humans) and 4g (rats). The human simulations predict that pH will decrease below 7.2 after 6 min of 10% CO_2 inhalation and within 2 min after 15% CO_2 inhalation. At higher inhaled CO_2 concentration, pH decreased below 7.2 within 1 min of exposure. For example, at 20% inhaled CO_2 pH decreased to 7.25 within 50 s, and reached a value of 6.9 after 10 min of inhalation. At 50% CO_2 inhalation pH decreased initially to 7.08 and further towards 6.72 after 10 min of inhalation. The decrease in pH was slower in humans than in animals at similar simulated CO_2 inhalations, which we related to slower CO_2 mixing in humans than rats, as reflected by differences in values for α and β between the two species.

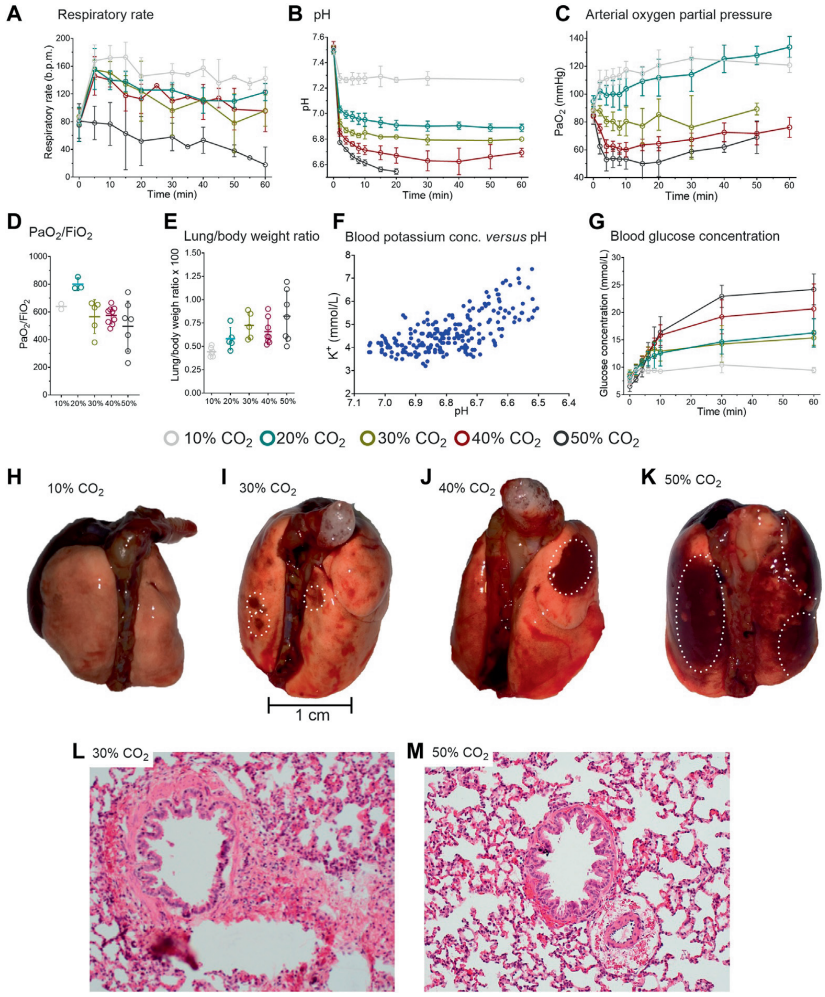

Figure 2: Results of the rat experiments. (A) Respiratory rate during the 1-h inhalation of 10, 20, 30, 40 or 50% CO_2. (B) pH during CO_2 inhalation. During 50% CO_2 inhalation 5/8 animals died after 14–25 min. In the remainder of animals, no pH samples were obtained beyond 20 min (C) Arterial oxygen concentrations. (D) Arterial PO2/FiO2 ratio in the different CO_2 exposure groups. (E) Lung/body weight ratio in the different CO_2 exposure groups. (F) Blood potassium concentration versus pH. (G) Blood glucose concentration in the various CO_2 exposure groups. (H-K). Macroscopic aspect of the lungs of three distinct animals that inhaled 1-h of 10%, 30% and 50% CO2. The white circles indicate the location of hemorrhages. L and M. Microscopic aspect of lung sections of two distinct animals treated with 30% (I) and 50% (M) CO_2. Peribronchiolar and perivascular hemorrhages are present as well as emphysema throughout the section.
The slices in panels L and M are 200-times magnified and stained with hematoxylin and eosin. The data in panels A-C and G are mean ± SD, in panels D and E median (50–75% interquartile) are given.

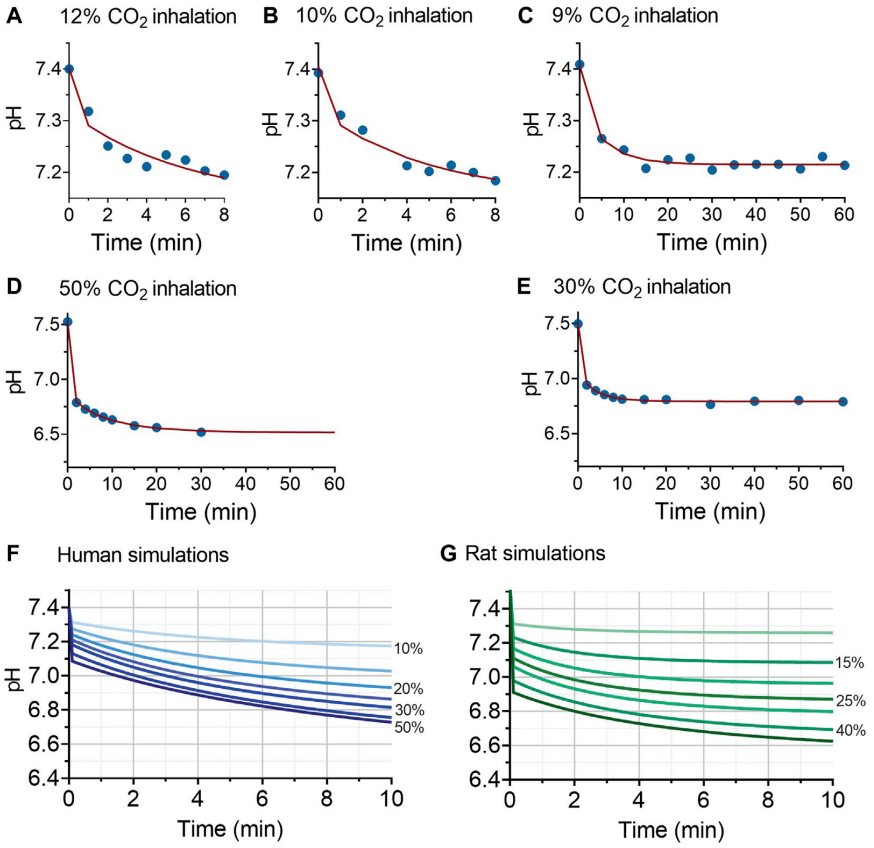

Figure 3: Results of the translational model analysis of acidosis (pH). Panels (A–E) given examples of the pH model fits in humans (A–C) and (D and E) in rats. The blue circles are the measured data, the red lines the data fits. Panels (F and G) give the simulations at inhalation values of 10% (top lines in the two panels), 15%, 20%, 25%, 30%, 40% and 50% (bottom lines in the two panels) CO_2 inhalation. The human simulations indicate that at inhalation levels of 15% CO_2 or higher, pH values <7.2 are readily reached.

Discussion

The main findings of this translational project were that in a population of heathy young volunteers, the inhalation of 6 and 7.5% CO_2 was well tolerated for up to 1-h, while tolerance to 9% CO_2 was limited to the short exposure time (10 min). Longer duration of 9% CO_2 inhalation and higher CO_2 concentrations (10 and 12%) were not tolerated, with causes for discontinua-

tion: exhaustion, anxiety, acidosis (pH < 7.2, which was one of the stopping rules), dissociation or blackout (both led to the inability to communicate with the subject). The oxygenation of the subjects remained intact with an increase in arterial PO2 and brain oxygen saturation. In rats, all animals survived the 10% and 20% CO_2 inhalation, while at 30% CO_2, 4 animals developed epileptiform activity and 5 animals died during 50% CO_2 exposure. These deaths were related to the high CO_2 aggravated by the presence of hypoxia, and were associated with severe lung damage, sympathoexcitation (as deduced from the blood glucose levels) and possibly also acidosis-induced hyperkalemia. Oxygenation of the animals worsened at higher CO_2 concentrations with a reduction in arterial PO2 to about 50 mmHg at 50% CO_2 inhalation. We were able to connect the human and rat data by constructing a translational model of pH allowing the prediction of pH values over time over the CO_2 concentration range of 10–50%.

The difference in the CO_2-level at which the human volunteers and the rats lost tolerance to further exposure was evident but CO_2-related physiological changes were such that we could perform an interpolation between species. Irrespective of species, we may conclude that up to 20% CO_2 inhalation, arterial PO2 increases and only decreases at higher inhalation levels. Possibly the initial increase in arterial PO2 is related to an increase in cardiac output, reduced pulmonary vascular resistance, CO_2-induced bronchodilation, acidosis-related improvement of hypoxic pulmonary constriction, that all combined led to an improvement of the ventilation/perfusion ratio. These effects counteract the expected reduction in arterial PO2 because of the lower inspired oxygen faction (reflecting oxygen dispersion by CO_2), right-ward shift of the oxygen dissociation curve, increased dead space ventilation due to tachypnea, and recruitment of poorly perfused lung areas. At higher inhaled CO_2 concentrations, the decrease in arterial PO2 is explained by these later factors and by lung damage (hemorrhages, edema and emphysema, Table 2 and Figure 2I–2M). The maintained PaO2/FiO2 ratio, particularly in the higher CO_2 inhalation cohorts, was not expected, but we relate these to that fact that measurements were restricted to animals that survived with possibly less pulmonary damage (attrition bias). Additionally, the FiO2 values in our experiments were low, in contrast to the high FiO2 in ventilated patients with respiratory distress disorders. Another relevant observation was the development of hyperglycemia that occurred at CO_2 concentrations of 10% and higher. Hyperglycemia is a sign of severe stress and is also observed during hemorrhagic shock and critical illness, and is related to sympathoadrenergic activity.[21,22] Importantly, hyperglycemia is related to poor outcome in critical illness.[22] The absence of hyperglycemia in the human experiments may be related to the restriction of measurements to the 3 lowest CO_2 concentration cohorts. Overall, these results indicate the validity of translational studies in the extrapolation of physiological responses from one species (rat) to the other (human), in this case the response to high inhaled concentrations of CO_2.

The development of a translational model to describe pH values at higher inhaled CO_2 than tested in our human population was successful. The results (Figure 1H, Figure 2B, Figure 3F,G) indicate that the decrease in pH was more rapid in rats than in humans upon initiation of in-

halation, a steady-state in pH developed earlier in the rat, and buffering capacity at steady state was greater in the rat. We relate this in part to a faster CO_2 mixing (as reflected by the species differences in model parameters α and β, see Eq. 3), possibly related to the smaller body size and relatively higher spontaneous ventilation and cardiac output in the rats. The greater tolerability to CO_2 in the rat may be an indication of the large phylogenetic distance between rat and man, in contrast to the distance between rat and other animals with a high tolerability to hypercapnia such as the naked mole rat.[23–25] In our human study, we did not allow continuation of CO_2 inhalation when pH values decreased below 7.2. This was an arbitrary level, as we are aware that lower pH levels (pH < 6.9) are sometimes observed in humans without deleterious consequences.[26] Still eventually extreme pH values (pH < 6.6) do compromise cellular and protein function and consequently will affect cardiac and brain function.[27–29]

The causes for discontinuation (apart from the low pH observed in two subjects during 12% CO_2 inhalation and death in the rats inhaling 50% CO_2) were related to neuropsychiatric effects with dissociation, blackout, anxiety or exhaustion in the human subjects, and epileptiform activity during CO_2 narcosis in the animals. Several mechanisms may be involved in the inability to tolerate CO_2. Cerebral hyperperfusion, increased intracranial pressure, cerebral edema and/or encephalopathy may have occurred during CO_2 inhalation. To detect possible structural brain damage in the animals, we removed the brains of the animals at obduction for a gross examination but observed no signs of edema or hemorrhage (data not shown). Possibly this is related to the fact that arterial PO2 remained above 75 mmHg throughout the hypercapnic exposure.[30,31] Still, we cannot exclude some deleterious effect of CO_2 on the function of specific brain centers at levels up to 12% CO_2 in humans and at higher concentrations in the animals. The coupling between local blood flow and synaptic activity may be severed at extreme levels of hypercapnia possibly due to a CO_2 effect at the acid sensing ion channels (ASICs), which are important in regulating the coupling between local blood flow and synaptic activity.[32,33] Further studies are needed to disentangle the complex interaction of arterial and brain tissue CO_2 concentrations and reversible and irreversible cerebral damage.

The injury to the rat lungs was of such severity at the 30% and higher CO_2 cohorts that they were considered (eventually) lethal. Still, we need to realize that a small part of the emphysema may have been related to the pentobarbital injection as also in the control animal some emphysema was observed (Table 2). Irrespective, these results suggest that the pulmonary damage was the main cause of death of the animals in the 50% CO_2 inhalation cohort. This is also consistent with and aggravated by the effects of acute hypoxia as oxygen levels at 50% CO_2 were around 10.5% (approx. 75 mmHg; Figure 2C).[34] However, we cannot exclude other contributing factors such as heart failure, cardiac arrhythmias or cerebral damage. Our findings agree with earlier animal studies in which acute exposure to 40% CO_2 in oxygen in 8 rats was associated with dyspnea and fulminant pulmonary edema in all animals.[35]

The combined rat and human data enable human risk assessment for CO_2 transport and storage facilities, where CO_2 is stored or transported in large quantities. In addition, the data

can support emergency response measures for these facilities.When individuals are exposed to an excess of CO_2 in ambient air, an important question is at what levels of CO_2 inhalation does the human body maintain its ability to adequately function to, for example, escape from the incident scene or to perform a cognitively challenging task. An answer to this question is not only dependent on the results of our current study in healthy young volunteers but is certainly also dependent on the age and more importantly the physical condition of the exposed individual as well as presence of underlying cardiac or pulmonary disease. Our current results indicate that during exposure to 9% CO_2 the body retains its ability to function for 10 min, albeit with large variability in tolerance with some subjects able to withstand 30min and one subject 60min of exposure. Still at this inhaled concentration all subjects experienced some form of discomfort, anxiety and reduced cognitive performance. Hence, it remains questionable whether at this inhaled concentration, the individual will be able to coherently perform a complex task. We expect that fleeing the scene will remain possible. Note that we expect CO_2 tolerance to decrease rapidly in older individuals with lower resilience and those with existing cardiac or pulmonary disease. The translational model predicts that at inhaled CO_2 concentrations greater than 9%, pH will rapidly decrease to values that further hamper the capacity to function adequately.

Risk assessment for CO_2 storage and transport facilities also includes estimates of probability of incidents, and probability of death in these incidents. Probability of death is typically estimated from acute lethality data in animals or anecdotal information form incidents. To support risk assessment for CO_2 storage and transport facilities, CO_2 lethality in rats has been investigated by Muijser et al. (2014).[36] Their lethality data are consistent with the data presented in this paper. When considering only rat lethality as endpoint, the CO_2 dose-response curve is too steep to allow for derivation of a reliable estimate of probability of death in humans. Our study has included additional physiological parameters to allow translation of the animal data to the human situation. Human tolerability was demonstrated here to levels of 9% CO_2 for short durations (up to 10 min) and these data support a reconsideration of the current CO_2 risks determined by e.g. the United Kingdom Health and Safety Executive and United States Environmental Protection Agency, which mention the unconsciousness can result within a few minutes of exposure to 7% CO_2.[37,38] Further analysis of the data in this study could provide essential insight into the probability of human effects and fatality after acute exposure to high levels of CO_2 and will drive land-use planning, setting of risks management measures, and emergency response planning.

Finally, in laboratory animals, a CO_2 overdose is the most commonly used practice for euthanasia.[39–41] Our rat data indicate that this will coincide with various neurological symptoms, indications of stress and tissue damage. Additionally, CO_2 at high concentrations activates nociceptors in the nasal cavity which is associated with severe pain sensations.[42–44] Hence, from an animal welfare perspective it is questionable whether this form of euthanasia is harmless as our findings as well as those of others indicate that a CO_2 overdose is highly distressful and will cause damage to lung tissue.[39–41]

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Chapter 6

Summary, conclusions and future perspectives

Summary, conclusions and future perspectives

The common thread that runs through this thesis is the safety of individuals exposed to potent opioids or to carbon dioxide (CO_2) and our ability to reverse devastating opioid-induced respiratory depression (OIRD). Within our health care system and particularly within the field of anesthesia, safety is a major concern and guidelines and measures have been adopted to reduce patient harm from pharmacological and non-pharmacological interventions. Consequently, anesthesia is currently considered safe, safer than, for example, riding a bike or driving a car, flying in an airplane or space shuttle, or engaging in any other high-tech activity. Still, beyond the strict supervision of anesthesia care givers or pain specialists, the relentless consumption of strong opioids has reached the level of a global crisis. Their addictive nature together with the opioid effect on the ventilatory control system is a lethal combination and the cause of many opioid deaths. With thousands of deaths every month, the United States, Canada and to a lesser extent some European countries such as Scotland, were struck exceptionally hard by the opioid crisis. The cause of the opioid crisis has been debated in several publications both in the scientific and lay press and has been depicted in the 2021 TV/internet mini-series Dopesick and various documentaries (see below). The opioid crisis per se is not the topic of this thesis. In this thesis, the effect of opioids on the ventilatory control system, quantified by their effect on the ventilatory response to inhaled CO_2, is studied. The thesis consists of three parts. In the first section, the impact of the potent opioid oxycodone on respiration is studied in healthy volunteers with and without the addition of alcohol (Chapter 2) or paroxetine and quetiapine (Chapter 3). In the second section (Chapter 4) the efficacy of a series of potential reversal agents on OIRD is extensively discussed. Notably, our research team recently evaluated a wide range of such agents in our research unit, including naloxone, buprenorphine, the BK-channel blocker GAL-021 (now known as ENA-001), doxapram, ketamine, thyrotropin releasing hormone, orexin and the atypical antidepressant tianeptine, with variable outcomes (see below).[1–8] Finally, in the last section we performed a series of studies on CO_2 in rat and man to establish tolerability and toxicity of escalating concentrations CO_2. Below, I give a summary of the results of each study.

Chapter 2. In this 3-way sequential crossover dose-escalating trial, the effect of co-administration of oral oxycodone and intravenous ethanol on resting ventilation, apneic events and the hypercapnic ventilatory response in healthy young and older volunteers was evaluated. Twelve young (21-28 years) and twelve elderly (66-77 years) individuals who were opioid-naïve, were given one oxycodone 20 mg tablet along with an intravenous infusion of ethanol 0, 0.5 or 1 g/L. Throughout the course of the study day, resting respiratory variables and the primary outcome, minute ventilation at isohypercapnia (end-tidal PCO_2 55 mmHg or VE55), were obtained at regular intervals. Oxycodone reduced baseline minute ventilation by 28% ($p < 0.001$ versus control); the addition of ethanol caused a further decrease of oxycodone-induced respiratory depression

by another 19% at ethanol 1g/L + oxycodone ($p < 0.01$ versus oxycodone). Only when combined with oxycodone did ethanol cause a significant increase in the number of apneic events measured in a 6-min window with a median (range) increase from 1 (0-3) at 0 g/L ethanol to 1 (0-11) at 1 g/L ethanol ($p < 0.01$). Mean (95% confidence interval) VE55 decreased from 33.4 (27.9-39.0) L/min (control) to 18.6 (15.6-21.6) L/min (oxycodone; $p < 0.01$ versus control) and to 15.7 (12.7-18.6) L/min (oxycodone combined with ethanol 1g/L; $p < 0.01$ versus oxycodone). In conclusion, ethanol combined with oxycodone induces greater ventilatory depression than either drug alone, the magnitude of which is clinically relevant. Elderly participants were more affected than younger volunteers.

Chapter 3. In collaboration with the US Food and Drug administration (FDA), we studied whether combining paroxetine or quetiapine with oxycodone, compared to oxycodone alone, decreases the ventilatory response to inhaled CO_2. This randomized, double-blind, crossover clinical trial, was performed at a the clinical pharmacology unit of a clinical research organization, Spaulding Clinical (West Bend, Wisconsin). Twenty-five healthy participants were enrolled in the study that was performed from in the first 5 months of 2021. Subjects were dosed with paroxetine 40 mg daily or quetiapine twice daily (increasing daily doses from 100 mg to 400 mg) for 5 days, combined with oral oxycodone 10 mg or placebo on days 1 and 5. The main outcome of the study was ventilation at end-tidal carbon dioxide of 55 mmHg (hypercapnic ventilation or VE55) using a CO_2 rebreathing technique. Nineteen participants completed the trial: median age, 35 [interquartile range 30 to 40] years, of which 11 were women [44%]. Mean VE55 was significantly decreased with paroxetine + oxycodone versus placebo + oxycodone on day 1 (29.2 versus 34.1 L/min; mean difference, -4.9 L/min, 1-sided 97.5% CI $-\infty$ to -0.6, p = 0.01) and day 5 (25.1 versus 35.3 L/min; 63 mean difference, -10.2 L/min, 1-sided 97.5% CI $-\infty$ to -6.3, $p < 0.001$) but was not significantly decreased with quetiapine + oxycodone versus placebo + oxycodone on day 1 (33.0 versus 34.1 L/min; mean difference, -1.2 L/min, 1-sided 97.5% CI $-\infty$ to 2.8, p = 0.28) or day 5 (34.7 versus 35.3 L/min; mean difference, -0.6 L/min, 1-sided 97.5% CI $-\infty$ to 3.2, p = 0.37). As a secondary outcome, mean VE55 was significantly decreased on day 4 with paroxetine alone versus placebo (32.4 versus 41.7 L/min; mean difference, -9.3 L/min, 1-sided 97.5% CI $-\infty$ to -3.9, $p < 0.001$), but not with quetiapine alone vs placebo (42.8 versus 41.7 L/min; mean difference, 1.1 L/min, 1-sided 97.5% CI $-\infty$ to 6.4 , p = 0.67). No drug-related serious adverse events were reported. In conclusion, in this preliminary study with healthy participants, paroxetine combined with oxycodone, compared to oxycodone alone, significantly decreased the ventilatory response to hypercapnia on days 1 and 5, while quetiapine combined with oxycodone had no such impact. Additional investigation is needed to characterize the effects after longer-term treatment and to determine the clinical relevance of these findings.

Chapter 4. Opioids may produce life-threatening respiratory depression and death from their actions at the opioid-receptors within the brainstem respiratory neuronal network. Since there

is an increasing number of conditions where the administration of the opioid receptor antagonist naloxone is inadequate or undesirable, there is an growing interest in the development of novel reversal and prevention strategies aimed at providing efficacy close to that of the opioid receptor antagonist naloxone but with fewer of its drawbacks such as its short duration of action, lesser ability to reverse high affinity opioids (e.g carfentanil) or drug combinations. We systematically reviewed the literature and discuss predominantly experimental pharmacotherapies, published in the last 5 years, that attempt to reverse or prevent opioid-induced respiratory depression as alternatives to naloxone. The respiratory stimulants are discussed on the basis of their pharmacological characteristics and mechanism of action: non-opioid controlled substances (e.g., amphetamine, cannabinoids, ketamine), hormones (thyrotropin releasing hormone, oxytocin), nicotinic acetylcholine receptor agonists, ampakines, serotonin receptor agonists, antioxidants, miscellaneous peptides, potassium channel blockers acting at the carotid bodies (doxapram, ENA001), sequestration techniques (scrubber molecules, immunopharmacotherapy), and opioids (partial agonists/antagonists). We argue that none of these often-still experimental therapies are adequately evaluated in terms of efficacy and safety, and that many of the agents presented have a lower efficacy at deeper levels of respiratory depression, and consequently are not able to overcome apnea, and a substantial number of these drugs have ample adverse effects. We propose to develop reversal strategies that combine respiratory stimulants with naloxone. Furthermore, we urge collaboration between research groups to expedite development of viable reversal strategies of potent synthetic opioid-induced respiratory depression.

Chapter 5. We examined the effect of increasing CO_2 concentrations on humans (6-12%) and rats (10-50%) at varying CO_2 inhalation times (10-60 min). In human volunteers, a continuous positive airway pressure helmet was used to deliver the gas mixture to the participants. Unrestrained rats were exposed to CO_2 in a transparent chamber. From both species regular arterial blood gas samples were obtained. After the studies, the lungs of the animals were examined for macroscopic and microscopic abnormalities. In humans, CO_2 concentrations of 9% inhaled for more than 10 min, and higher concentrations inhaled for less than 10 min were poorly or not tolerated due to fatigue, anxiety, dissociation or acidosis ($pH < 7.2$), despite intact oxygenation. In rats, concentrations of 30% and above were associated with CO_2 narcosis, epilepsy, poor oxygenation and, at 50% CO_2, sudden death. Lung hemorrhage and edema were detected in rats at inhaled concentrations of 30% and above. This study provides crucial insight into the occurrence of physiological changes in humans and fatalities in rats during and following acute exposure to high levels of CO_2. Humans tolerate 9% CO_2 and retain their ability to function coherently for up to 10 min. These data support reconsideration of the current CO_2 levels ($< 7.5\%$) that pose a risk to exposed individuals ($< 7.5\%$) as established by governmental agencies to $\leq$ 9%.

Conclusions

The main conclusions drawn from these studies are that:

1. Oxycodone produces greater respiratory depression when combined with ethanol or paroxetine. The effect of ethanol was greatest in an elderly population, indicative of the enhanced opioid and ethanol potency in elderly individuals. Although none of the intervention studies that we conducted were designed to study underlying mechanisms, they do suggest that the biological actions of ethanol, most likely via activation of GABA receptors, or paroxetine, via activation of specific 5HT receptors, share a common pathway with opioids, acting at mu-opioid receptors, within the pontine and brainstem respiratory networks. These findings are clinically relevant and should serve as caution against combining these drugs. How paroxetine and oxycodone behave when either drug has been used for longer periods ($> 5days$) is unknown and the subject of ongoing research in collaboration with the FDA (clinicaltrials.gov: NCT05470465).

2. Studies on the reversal or prevention of OIRD by non-opioid respiratory stimulants have yet to demonstrate consistent efficacy in humans, despite occasional promising results in animal studies. At first glance, this seems rather disappointing. Indeed, progress in this field has not yet led to the registration of viable non-opioid reversal agents, and we therefore have to continue to rely on the opioid antagonist naloxone. Still, naloxone has limited efficacy in a number of situations, including (i) overdoses with high affinity opioids (e.g. carfentanil), (ii) abuse of opioids combined with other centrally acting drugs such as benzodiazepines (e.g. benzodope), alpha-2-agonists (e.g. Tranq), ethanol, antidepressants, etc.; see for a documentary on Vice on Benzodope and Tranq: The next wave of the overdose crisis: https;//www.youtube.com/watch?v=82QhIOgJy1c'. (iii) conditions in which naloxone will cause undesired effects, such as precipitation of withdrawal, agitation or excited delirium, (iv) mass poisoning with potent opioids where naloxone is either ineffective or supplies may be exhausted, and (v) in case of an opioid use disorder in which individuals abuse weaker opioids (such as oxycodone or heroine) laced with fentanyl or carfentanil. However, research performed in our laboratory over the last few years provides some insight into potential alternative treatment approaches to naloxone; see below. Importantly, also new naloxone administration modes such as the intranasal administration of 4 mg naloxone seems able to reverse OIRD from potent opioids such as fentanyl and sufentanil (Fig. 1); we are currently studying intranasal naloxone in a collaboration with the FDA.

3. Humans tolerate inhaled CO_2 concentrations up to 9% for brief exposure times ($< 10min$). This later observation confirms our conviction that it is safe to use 7-9% inhaled CO_2 as a challenge to the ventilatory control system in humans, even in the elderly. Our studies also indicate that using high concentrations of CO_2 are distressing to animals, thus

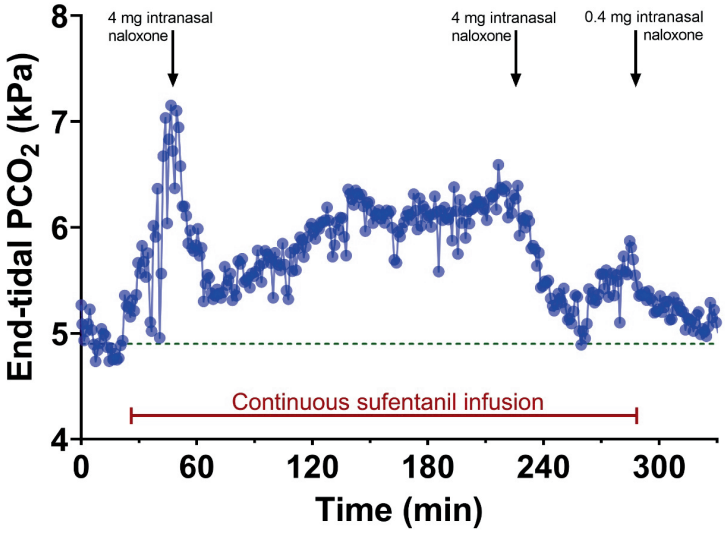

Figure 1: Effect of intranasal naloxone (4 mg) on end-tidal PCO2 during a continuous sufentanil infusionin one subjects. Data from van Lemmen et al. (unpublished observation).

caution should be exercised when euthanizing laboratory animals with CO_2.

Future perspectives In light of these conclusions, future research should focus on the development of analgesics that are highly effective but are without significant adverse events, such as addiction or respiratory depression. Since opioids for acute pain continue to be most efficacious of all currently available analgesics, a second line of research should be aimed at the development of respiratory stimulants that reverse or prevent OIRD without interfering with the opioid analgesic component. It may well be that a single stimulant is insufficiently effective, necessitating the combination of such stimulants with low-dose naloxone. If we focus on novel drug developments, an example of a potential new painkiller may be a drug that operates via the alpha-2A-adrenergic receptor (A2AR). In a recent publication in Science, Fink et al.[9] focus on this specific receptor, which is targeted by clonidine and dexmedetomidine, two drugs that are frequently used in anesthesia and in the intensive care unit to induce pain relief or an anesthesia-like sedation state. The authors searched for molecules that are chemically distinct from clonidine and dexmedetomidine because they reasoned that such molecules might separate sedation from analgesia. They discovered several agonists of the A2AR that activate a more selective set of cellular pathways than existing A2AR agonists. In animal experiments these agents were effective in relieving neuropathic, inflammatory and acute nociceptive pain

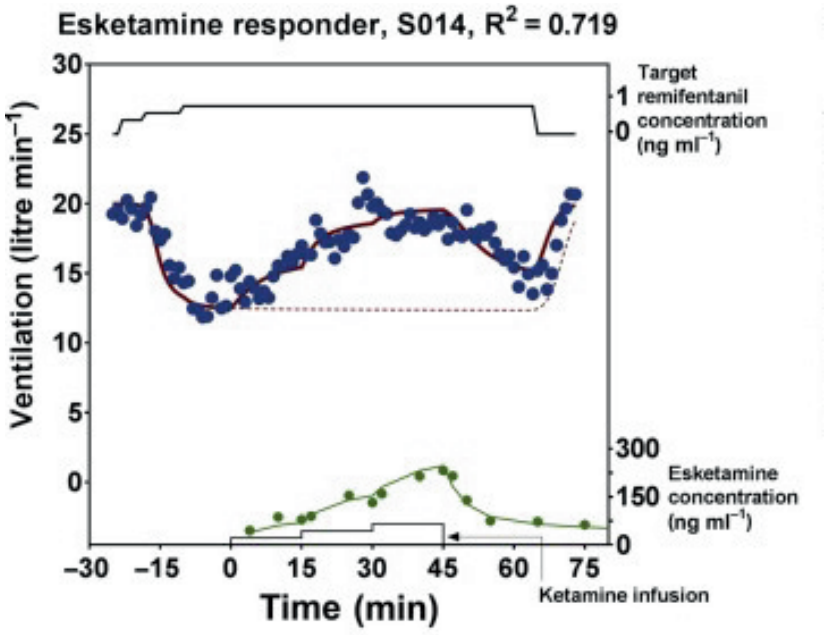

Figure 2: Effect of esketamine on remifentanil-induced respiratory depression in one volunteer. Data from Jonkman et al. 2018.[5]

without causing any sedation. Evidently studies in humans are needed before we can draw any meaningful conclusions but these data indicate that these specific agonists may well be an attractive alternative to opioids. These studies should certainly focus on efficacy and side effect profile as it is known that drugs acting at the A2AR also produce respiratory depression. Regarding respiratory stimulants, recent human studies conducted in our research unit showed efficacy from three drugs with a rather diverse mechanism of action: the N-methyl- D-aspartate receptor antagonist esketamine (Fig. 2), the "wakefulness" hormone orexin and the potassium-channel blocker ENA001 (Fig. 3). Negative results were obtained from thyrotropin-releasing hormone and the atypical tricyclic antidepressant and cognitive enhancer tianeptine. The use of esketamine as respiratory stimulant is clinically valuable because its use in, for example, the post-anesthesia care unit, will improve pain relief, reduce opioid consumption and stabilize or even stimulate breathing. Interestingly, the respiratory stimulation observed with esketamine was only evident in the presence of an opioid, suggestive that esketamine had an impact on areas of the brain involved in CO_2 sensing. ENA-001 operates at the carotid bodies, the primary sensor of hypoxia in mammalians. Recent findings demonstrate that ENA001 is effective in restoring the blunted hypoxic ventilatory response during propofol infusion.

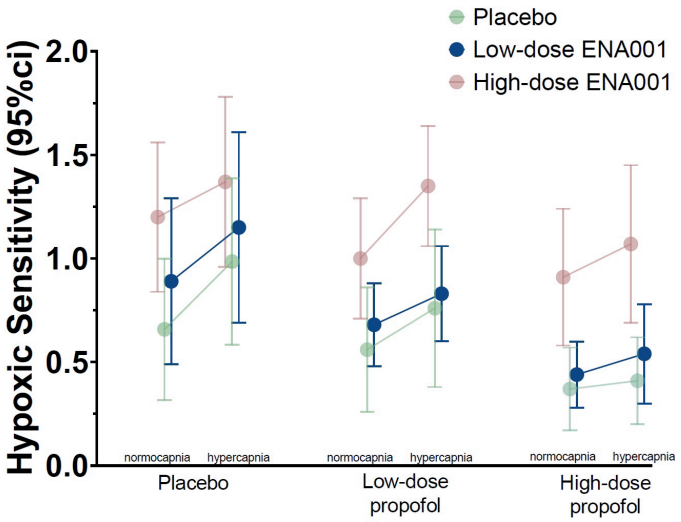

Figure 3: Effect of ENA-001 on propofol-induced blunting of the hypoxic ventilatory response in healthy male and female volunteers (unpublished data). Hypoxic sensitivity is defined by ΔVentilation/ΔSpO_2.

A drawback of this drug is that it acts at the carotid bodies and hence may show ceiling in its efficacy in restoring centrally-mediated (severe) respiratory depression. As stated above, it is likely that combining ENA001 with naloxone may overcome this issue and will cause more effective reversal of even very deep levels of respiratory depression including apnea.

At this point it is important to realize that reversal of OIRD with an exogenously administered drug (including naloxone) is only possible when the circulation is intact and the drug can reach the site of action (brainstem or carotid body). The FDA in collaboration with our group, developed a translational model to determine the impact of an opioid overdose on respiratory depression and cardiac output.[10] The data indicate that respiratory depression leading to hypoxia will cause cardiac arrest that is hard to reverse with even high doses of naloxone, particularly when high affinity opioids such as carfentanil are overdosed. Naloxone on its own is only effective when the opioid dose is low to moderate (Fig. 4). At higher doses effective resuscitation (chest compressions), intubation and manual ventilation, combined with effective respiratory stimulants are then needed. Finally, to examine the efficacy of drug combinations, we are currently planning a study on high-dose opioid-induced respiratory depression in which we will attempt to restore ventilation by using ENA-001 in combination with naloxone.

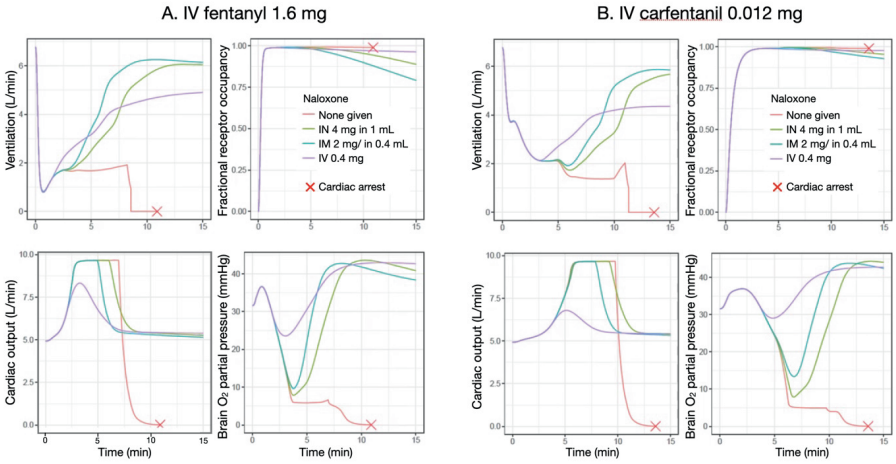

Figure 4: Simulations of the ability of naloxone to restore breathing and cardiac output (blood flow) and brain oxygen partial pressure following moderate-dose fentanyl (left 4 panels) and carfentanil (right 4 panels). Naloxone was given intranasally (IN), intramuscularly (IM) or intravenously (IV). Data from the FDA, with permission.

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Chapter 7

Nederlandse samenvatting en conclusies

Nederlandse samenvatting en conclusies

De rode draad door dit proefschrift, is de veiligheid van mensen die worden blootgesteld aan potente opioïden of aan koolstofdioxide (CO_2), en mogelijkheden om potentieel fatale opioïd geïnduceerde ademdepressie (OIRD) om te keren. In de gezondheidszorg, met name in het gebied van anesthesie, is veiligheid een speerpunt. Er zijn richtlijnen opgesteld om de schade die patiënten ondervinden als gevolg van farmacologische en niet-farmacologische interventies te verminderen. Als gevolg hiervan wordt anesthesie momenteel als zeer veilig beschouwd, veiliger dan bijvoorbeeld fietsen of autorijden, vliegen in een vliegtuig of ruimteschip, of het deelnemen aan andere hoogtechnologische activiteiten. Echter, buiten de strikte supervisie van zorgverleners op het gebied van anesthesie of pijnexperts, is de sterke toename van het gebruik van potente opioïden ontaard in een wereldwijde crisis. Het verslavingspotentieel alsmede het effect van opioïden op de ademhalingsregulatie is een dodelijke combinatie en heeft vele opioïdgerelateerde doden veroorzaakt. Met duizenden doden per maand zijn de Verenigde Staten, Canada en in mindere mate Europese landen zoals Schotland, hard getroffen door de opioïdencrisis. De oorzaak van de opioïdencrisis is in verschillende publicaties zowel in de wetenschappelijke als in de lekenpers aangehaald en is onder andere te zien in de TV/miniserie Dopesick uit 2021 en diverse documentaires. De opioïdencrisis op zich is niet het onderwerp van dit proefschrift. In dit proefschrift wordt het effect van opioïden op de ademhalingsregulatie, gekwantificeerd door het effect op de ventilatoire respons op ingeademde CO_2, bestudeerd. Het proefschrift bestaat uit drie delen. In het eerste deel wordt de impact van het potente opioïd oxycodon op de ademhaling bestudeerd bij gezonde vrijwilligers, met en zonder toediening van alcohol (Hoofdstuk 2) en paroxetine en quetiapine (Hoofdstuk 3). In het tweede deel (Hoofdstuk 4) wordt uitgebreid ingegaan op de effectiviteit van een reeks potentiële omkeermiddelen die kunnnen worden ingezet bij OIRD. Ons onderzoeksteam heeft onlangs een breed scala van dergelijke middelen geëvalueerd in ons laboratorium, waaronder naloxon, buprenorfine, de BK-kanaalblokker GAL-021 (nu bekend als ENA-001), doxapram, ketamine, thyrotropin-releasing hormone, orexine en het atypische antidepressivum tianeptine, met varierende uitkomsten.[1–8] Ten slotte hebben we in het laatste deel een reeks studies uitgevoerd naar CO_2 bij de rat en de mens om de tolerantie en toxiciteit van oplopende concentraties CO_2 vast te stellen. Hieronder geef ik een samenvatting van de resultaten van elke studie.

Hoofdstuk 2. In deze 3-weg sequentiële crossover dosis-escalatiestudie werd het effect van de gelijktijdige toediening van orale oxycodon en intraveneuze ethanol op de rustventilatie, apneu's en hypercapnische ventilatoire respons bij gezonde jonge en oudere vrijwilligers geëvalueerd. Twaalf jonge (21-28 jaar) en twaalf oudere (66-77 jaar) individuen die opioïd-naïef waren, kregen één tablet oxycodon 20 mg toegediend gecombineerd met een intraveneuze toediening van ethanol 0, 0,5 of 1 g/L. Gedurende de onderzoeksdag werden rust ademhalingsvariabelen en de primaire uitkomst, de minuutventilatie bij isohypercapnie (end-tidal PCO_2 55 mmHg of

VE55), op regelmatige tijdstippen verkregen. Oxycodon verminderde de basale minuutventilatie met 28% ($p < 0.001$ versus controle); de toevoeging van ethanol veroorzaakte een verdere afname van door oxycodon geïnduceerde ademhalingsdepressie met nog eens 19% bij ethanol 1g/L + oxycodon ($p < 0.01$ versus oxycodon). Alleen in combinatie met oxycodon veroorzaakte ethanol een significante toename van het aantal apneu-episodes gemeten in een 6-minuten tijdvenster met een mediane (bereik) toename van 1 (0-3) bij 0 g/L ethanol tot 1 (0-11) bij 1 g/L ethanol ($p < 0.01$). Gemiddeld (95% betrouwbaarheidsinterval) VE55 daalde van 33,4 (27,9-39,0) L/min (controle) naar 18,6 (15,6-21,6) L/min (oxycodon; $p < 0.01$ versus controle) en naar 15,7 (12,7-18,6) L/min (oxycodon gecombineerd met ethanol 1g/L; $p < 0.01$ versus oxycodon). Concluderend, ethanol gecombineerd met oxycodon veroorzaakt een grotere ventilatoire depressie dan elk van de afzonderlijke middelen, waarvan de omvang klinisch relevant is. Oudere deelnemers werden meer beïnvloed dan jongere deelnemers.

Hoofdstuk 3. In samenwerking met de Amerikaanse Food and Drug Administration (FDA) hebben we onderzocht of de combinatie van paroxetine of quetiapine met oxycodon, vergeleken met alleen oxycodon, de ventilatoire response op ingeademde CO_2 vermindert. Deze gerandomiseerde, dubbelblinde, crossover studie werd uitgevoerd in de klinisch farmacologische unit van een klinische onderzoeksorganisatie, Spaulding Clinical (West Bend, Wisconsin). Vijfentwintig gezonde deelnemers werden geincludeerd in de studie die werd uitgevoerd in de eerste 5 maanden van 2021. Deelnemers kregen paroxetine 40 mg dagelijks of quetiapine tweemaal daags (oplopende dagelijkse doses van 100 mg tot 400 mg) gedurende 5 dagen, gecombineerd met orale oxycodon 10 mg of placebo op dag 1 en 5. De belangrijkste uitkomst van de studie was ventilatie bij end-tidal koolstofdioxide van 55 mmHg (hypercapnische ventilatie of VE55) met behulp van een CO_2 rebreathing techniek. Negentien deelnemers voltooiden het onderzoek: mediane leeftijd, 35 [interkwartielafstand 30 tot 40] jaar, waarvan 11 vrouwen [44%]. De gemiddelde VE55 was significant verminderd bij paroxetine + oxycodon versus placebo + oxycodon op dag 1 (29,2 versus 34,1 L/min; gemiddeld verschil, -4,9 L/min, eenzijdige 97,5% CI $-\infty$ tot -0,6, p = 0,01) en dag 5 (25,1 versus 35,3 L/min; gemiddeld verschil, -10,2 L/min, eenzijdige 97,5% CI $-\infty$ tot -6,3, $p < 0,001$) dit was niet significant verminderd bij quetiapine + oxycodon versus placebo + oxycodon op dag 1 (33,0 versus 34,1 L/min; gemiddeld verschil, -1,2 L/min, eenzijdige 97,5% CI $-\infty$ tot 2,8, p = 0,28) of dag 5 (34,7 versus 35,3 L/min; gemiddeld verschil, -0,6 L/min, eenzijdige 97,5% CI $-\infty$ tot 3,2, p = 0,37). Als secundaire uitkomst was de gemiddelde VE55 significant verminderd op dag 4 voor paroxetine versus placebo (32,4 versus 41,7 L/min; gemiddeld verschil, -9,3 L/min, eenzijdige 97,5% CI $-\infty$ tot -3,9, $p < 0,001$), maar niet voor quetiapine versus placebo (42,8 versus 41,7 L/min; gemiddeld verschil, 1,1 L/min, eenzijdige 97,5% CI $-\infty$ tot 6,4 , p = 0,67). Er werden geen aan de geneesmiddelen gerelateerde ernstige bijwerkingen gemeld. Concluderend, in deze studie met gezonde deelnemers, verminderde paroxetine wanneer gecombineerd met oxycodon, vergeleken met alleen oxycodon de ventilatoire respons op hypercapnie sifnificant op dagen 1 en 5. Terwijl er voor quetiapine gecombineerd met oxycodon een dergelijke relatie

niet werd aangetoond. Verder onderzoek is nodig om de effecten na langdurige behandeling te karakteriseren en om de klinische relevantie van deze bevindingen te bepalen.

Hoofdstuk 4. Opioïden kunnen levensbedreigende ademdepressie en overlijden veroorzaken door hun werking op de opioïd-receptoren in het ademhalingscentrum gelocaliseerd in de hersenstam. Aangezien er een toenemende mate situaties ontstaan waarbij de toediening van de opioïd receptor antagonist naloxon ontoereikend of ongewenst is, is er een toenemende interesse in de ontwikkeling van nieuwe omkeer- en preventiestrategieën welke qua effectiviteit dichtbij die van de opioïd receptor antagonist naloxon ligt, maar minder van zijn nadelen heeft, zoals de korte werkingsduur, onvoldoende effect bij opioïden met een zeer hoge affiniteit voor de opioidreceptor (bijv. carfentanil) of combinaties van geneesmiddelen om te keren. We hebben een systematic review uitgevoerd en bespreken hoofdzakelijk experimentele farmacotherapie, gepubliceerd in de afgelopen 5 jaar, die in potentie opioïd-geïnduceerde ademdepressie om kunnen keren of als alternatief voor naloxon zouden kunnnen fungeren. De ademhalingsstimulantia worden besproken op basis van hun farmacologische eigenschappen en werkingsmechanisme: niet-opioïde gereguleerde stoffen (bijv. amfetamine, cannabinoïden, ketamine), hormonen (thyrotropine-vrijmakend hormoon, oxytocine), nicotinische acetylcholine receptor agonisten, ampakines, serotonine receptor agonisten, antioxidanten, diverse peptiden, kaliumkanaalblokkers die werken op de carotid bodies (doxapram, ENA-001), sequestratietechnieken (scrubmoleculen, immunofarmacotherapie) en opioïden (gedeeltelijke agonisten/antagonisten). We betogen dat geen van deze vaak nog experimentele therapieën adequaat zijn geëvalueerd op het gebied van effectiviteit en veiligheid, en dat veel van de gepresenteerde middelen een lagere effectiviteit hebben bij ernstige ademdepressie, en niet in staat zijn apneu effectief te behandelen, daarbij heeft een aanzienlijk deel van deze geneesmiddelen ernstige bijwerkingen. We stellen voor om omkeerstrategieën te ontwikkelen die ademhalingsstimulantia combineren met naloxon. Verder dringen we aan op samenwerking tussen onderzoeksgroepen om de ontwikkeling van bruikbare omkeerstrategieën van door krachtige synthetische opioïden geïnduceerde ademdepressie te versnellen.

Hoofdstuk 5. We onderzochten het effect van toenemende CO_2 concentraties op mensen (6-12%) en ratten (10-50%) bij variërende CO_2 inhalatietijden (10-60 min). Voor de experimenten bij mensen werd bij de vrijwilligers een CPAP helm gebruikt om het gasmengsel toe te dienen. De niet-gefixeerde ratten werden blootgesteld aan CO_2 in een transparante kamer. Gedurende beide experimenten werden regelmatig arteriële bloedgasmonsters genomen. Na de studies werden de longen van de dieren onderzocht op macroscopische en microscopische afwijkingen. Bij mensen werden CO_2 concentraties van 9% die langer dan 10 minuten werden ingeademd, en de hogere concentraties die gedurende 10 minuten of minder werden ingeademd, slecht of niet getolereerd vanwege vermoeidheid, angst, dissociatie of acidose ($pH < 7.2$), ondanks intacte oxygenatie. Bij ratten werden concentraties van 30% en hoger geassocieerd met CO_2 nar-

cose, epilepsie, verslechterende oxygenatie en, bij 50% CO_2, plotselinge dood. Longbloedingen en oedeem werden geobserveerd bij ratten bij geïnhaleerde concentraties van 30% en hoger. Deze studie biedt cruciaal inzicht in het optreden van fysiologische veranderingen bij mensen en sterfgevallen bij ratten tijdens en na acute blootstelling aan hoge niveaus van CO_2. Mensen verdragen 9% CO_2 en behouden hun vermogen om coherent te functioneren tot 10 minuten. Deze gegevens ondersteunen heroverweging van de huidige CO_2-niveaus ($< 7.5\%$) die een risico vormen voor blootgestelde individuen ($< 7.5\%$) zoals vastgesteld door overheidsinstanties tot $\leq$ 9%.

Conclusies

De belangrijkste conclusies die uit deze studies getrokken worden, zijn dat:

1. Oxycodon meer ademdepressie veroorzaakt wanneer het gecombineerd wordt met ethanol of paroxetine. Het effect van ethanol was het grootst bij de oudere populatie, wat wijst op een hogere potentie van opioïden en ethanol bij ouderen. Hoewel geen van de interventiestudies die we hebben uitgevoerd was ontworpen om onderliggende mechanismen te bestuderen, suggereren ze wel dat de biologische werking van ethanol, hoogstwaarschijnlijk via activatie van GABA-receptoren, of paroxetine, via activatie van specifieke 5HT-receptoren, een gemeenschappelijk pad delen met opioïden, die inwerken op mu-opioïdereceptoren, binnen de pontiene en medullaire ademhalingsnetwerken. Deze bevindingen zijn klinisch relevant en moeten dienen als waarschuwing tegen het combineren van deze geneesmiddelen. Hoe de combinatie van paroxetine en oxycodon zich gedragen wanneer een van beide geneesmiddelen voor langere perioden ($> 5dagen$) wordt gebruikt, is onbekend en het onderwerp van lopend onderzoek in samenwerking met de FDA (clinicaltrials.gov: NCT05470465).

2. Studies naar de omkering of preventie van OIRD door niet-opioïde ademhalingsstimulantia hebben nog geen consistente effectiviteit bij mensen aangetoond, ondanks af en toe veelbelovende resultaten in dierexperimentele studies. Op het eerste gezicht lijkt dit nogal teleurstellend. Inderdaad, vooruitgang in dit veld heeft nog niet geleid tot de registratie van werkzame niet-opioïde omkeermiddelen, en blijven daarom aangewezen op de opioïd antagonist naloxon. Naloxon heeft echter beperkte effectiviteit in een aantal situaties, waaronder (i) overdoses met opioïden een hoge affiniteit voor de opioid receptor (bijv. carfentanil), (ii) misbruik van opioïden gecombineerd met andere centraal werkende geneesmiddelen zoals benzodiazepines (bijv. benzodope), alpha-2-agonisten (bijv. Tranq), ethanol, antidepressiva, etc.; zie voor een documentaire op Vice over Benzodope en Tranq: De volgende golf van de overdosiscrisis: `https;//www.youtube.com/watch?v=82QhI0gJy1c`'. (iii) situaties waarin naloxon ongewenste effecten zal veroorzaken, zoals het veroorzaken van ontwenningsverschijnselen, waarbij agitatie of een excited delirium kunnen optreden, (iv) massavergiftiging met krachtige opioïden waar naloxon ineffec-

tief is of de voorraad kan opraken, en (v) in het geval van een opioïde gebruikstoornis waarbij individuen zwakkere opioïden (zoals oxycodon of heroïne) vermengd met fentanyl of carfentanil misbruiken. Onderzoek dat de afgelopen jaren in ons laboratorium is uitgevoerd, biedt echter enig inzicht in potentiële alternatieve benaderingen voor de behandeling met naloxon; zie hieronder. Belangrijk is dat ook nieuwe toedieningsvormen van naloxon, zoals de intranasale toediening van 4 mg naloxon, in staat lijken om OIRD van krachtige opioïden zoals fentanyl en sufentanil om te keren (Fig. 1); we bestuderen momenteel intranasale naloxon in samenwerking met de FDA.

3. Mensen verdragen geïnhaleerde CO_2-concentraties tot 9% voor korte blootstellingstijden ($< 10min$). Deze recente observatie bevestigt onze overtuiging dat het veilig is om 7-9% geïnhaleerde CO_2 te gebruiken als een challenge voor het ademregulatiesysteem bij mensen, zelfs bij ouderen. Onze studies geven ook aan dat het gebruik van hoge concentraties CO_2 stressvol is voor dieren, dus voorzichtigheid is geboden bij het euthanaseren van laboratoriumdieren met CO_2.

Toekomstperspectieven

Gezien deze conclusies zou toekomstig onderzoek zich moeten richten op de ontwikkeling van pijnstillers die zeer effectief zijn maar geen significante bijwerkingen hebben, zoals verslaving of ademdepressie. Aangezien opioïden voor acute pijn nog steeds het meest effectief zijn van alle momenteel beschikbare pijnstillers, zou een tweede onderzoekslijn gericht moeten zijn op de ontwikkeling van ademhalingsstimulantia die OIRD omkeren of voorkomen zonder te interfereren met het opioïde pijnstillende component. Het kan goed zijn dat een enkel stimulerend middel onvoldoende effectief is, wat noodzaakt tot de combinatie van dergelijke stimulantia met lage doses naloxon. Als we ons richten op nieuwe geneesmiddelontwikkelingen, kan een voorbeeld van een potentieel nieuwe pijnstiller een geneesmiddel zijn dat werkt via de alpha-2A-adrenerge receptor (A2AR). In een recente publicatie in Science richten Fink et al.[9] zich op deze specifieke receptor, die wordt getarget door clonidine en dexmedetomidine, twee geneesmiddelen die vaak worden gebruikt in de anesthesie en op de intensive care om pijnverlichting of een anesthesie-achtige sedatietoestand te induceren. De auteurs zochten naar moleculen die chemisch verschillen van clonidine en dexmedetomidine omdat ze redeneerden dat dergelijke moleculen sedatie van analgesie zou kunnen scheiden. Ze ontdekten verschillende agonisten van de A2AR die een meer selectieve set van cellulaire paden activeren dan bestaande A2AR-agonisten. In dierproeven waren deze middelen effectief in het verlichten van neuropathische, inflammatoire en acute nociceptieve pijn zonder enige sedatie te veroorzaken. Uiteraard zijn studies in mensen nodig voordat we conclusies kunnen trekken, maar deze gegevens geven aan dat deze specifieke agonisten wellicht een aantrekkelijk alternatief zijn voor opioïden. Vervolgstudies moeten zich zeker richten op effectiviteit en bijwerkingenprofiel, aangezien bekend is dat geneesmiddelen die inwerken op de A2AR ook

ademdepressie veroorzaken. Wat betreft ademhalingsstimulantia toonden recente menselijke studies uitgevoerd in ons laboratorium de effectiviteit van drie geneesmiddelen met een nogal uiteenlopend werkingsmechanisme: de N-methyl-D-aspartaatreceptor antagonist esketamine (Fig. 2), het 'waakzaamheidshormoon' orexine en de kaliumkanaalblokker ENA-001 (Fig. 3). Negatieve resultaten werden verkregen van thyrotropine-vrijmakend hormoon en het atypische tricyclische antidepressivum en 'cognitive enhancer' tianeptine. Het gebruik van esketamine als ademhalingsstimulans is klinisch waardevol omdat het gebruik ervan, bijvoorbeeld op de post-anesthesie zorgafdeling, de pijnverlichting zal verbeteren, het gebruik van opioïden zal verminderen en de ademhaling zal stabiliseren of zelfs stimuleren. Interessant is dat de waargenomen ademhalingsstimulatie met esketamine alleen evident was in aanwezigheid van een opioïd, wat suggereert dat esketamine een impact had op gebieden van de hersenen die betrokken zijn bij CO_2-detectie. ENA-001 werkt op de carotid bodies, de primaire sensor van hypoxie bij zoogdieren. Recente bevindingen tonen aan dat ENA-001 effectief is in het herstellen van de afgezwakte hypoxische ventilatoire respons tijdens propofol-infusie. Een nadeel van dit geneesmiddel is dat het werkt op de carotid bodies en daardoor mogelijk een maximum heeft in zijn effectiviteit bij het herstellen van centraal gemedieerde (ernstige) ademdepressie. Zoals hierboven vermeld, is het waarschijnlijk dat de combinatie van ENA-001 met naloxon dit probleem kan verhelpen en een meer effectieve omkering van zelfs zeer ernstige ademdepressie, inclusief apneu, kan bewerkstelligen.

Op dit moment is het belangrijk te beseffen dat het omkeren van OIRD met een exogeen toegediend geneesmiddel (inclusief naloxon) alleen mogelijk is wanneer de bloedsomloop intact is en het geneesmiddel de plaats van werking (hersenstam of carotid body) kan bereiken. De FDA, in samenwerking met onze groep, heeft een translationeel model ontwikkeld om de impact van een overdosis opioïden op ademhalingsdepressie en hartminuutvolume te bepalen.[10] De gegevens uit dit model geven aan dat ademdepressie die leidt tot hypoxie een hartstilstand zal veroorzaken die moeilijk om te keren is zelfs met hoge doses naloxon, met name wanneer opioïden met een zeer hoge affiniteit voor de opioidreceptor zoals carfentanil worden overgedoseerd. Naloxon op zich is alleen effectief wanneer de opioïdendosis laag tot matig is (Fig. 4). Bij hogere doses zijn effectieve reanimatie (borstcompressies), intubatie en handmatige ventilatie, gecombineerd met effectieve ademhalingsstimulantia nodig. Ten slotte, om de effectiviteit van geneesmiddelencombinaties te onderzoeken, zijn we momenteel van plan een studie uit te voeren naar ademhalingsdepressie geïnduceerd door hoge doses opioïden, waarin we zullen proberen de ventilatie te herstellen door ENA-001 te gebruiken in combinatie met naloxon.

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Addenda

Curriculum vitae
List of publications

Curriculum vitae

Rutger van der Schrier was born in Leiden on September 24th. He received his VWO degree at the Da Vinci College in Leiden. Following that, he pursued Psychology and earned a master's degree in clinical and health psychology at the University of Utrecht, before enrolling in Medicine at the University of Amsterdam. After graduating from medical school, he worked at the OLVG west's department of internal medicine before joining Albert Dahan's research team at the Leiden University Medical Center's Anesthesiology department. The three years that followed were devoted entirely to research. Then he enrolled in the same hospital's Anesthesiology residency program which was completed in 2021. He currently splits his time between practicing anesthesia in the operating room and conducting research in the lab.

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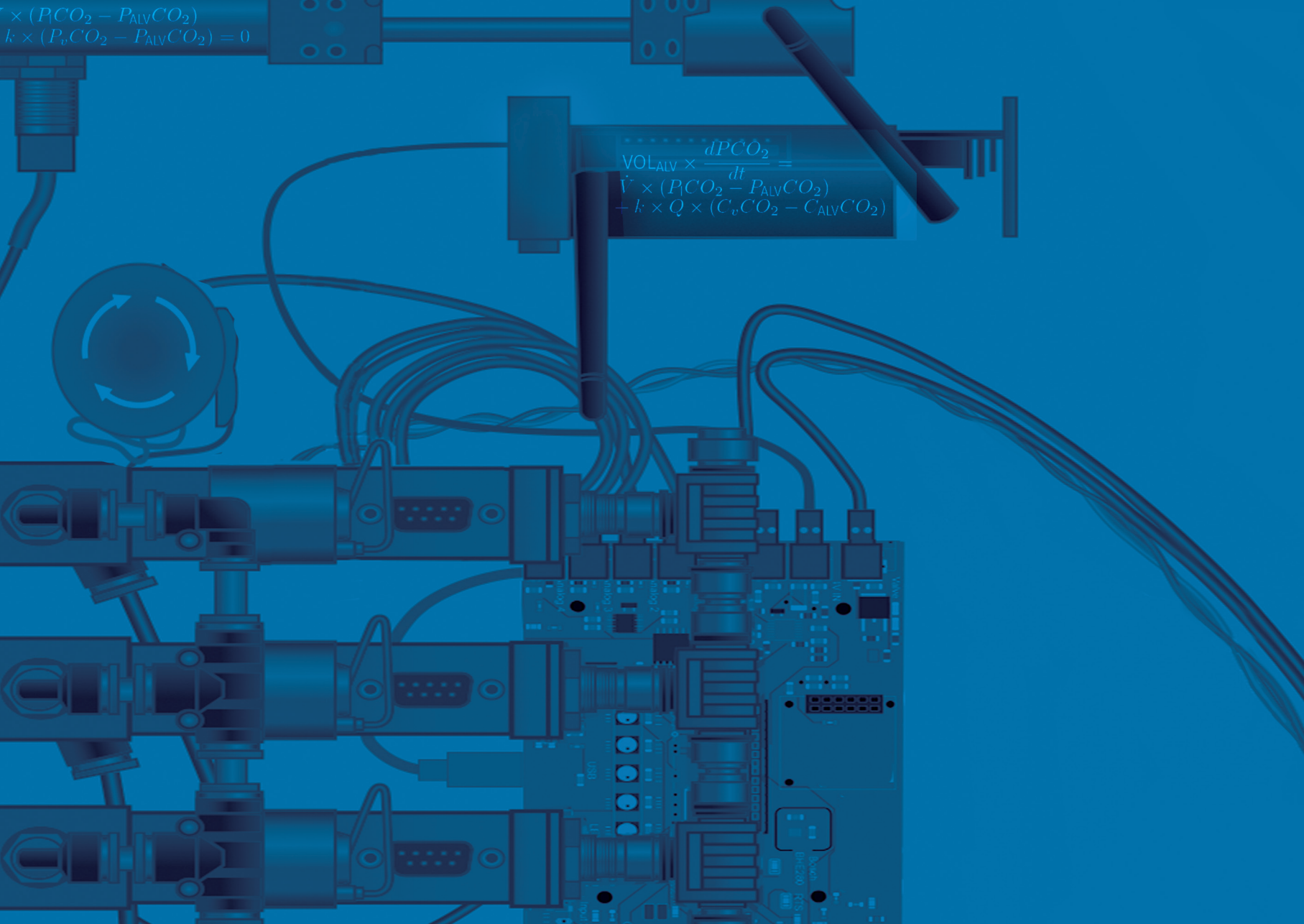
$\dot{V} \times (P_iCO_2 - P_{ALV}CO_2)$
$k \times (P_vCO_2 - P_{ALV}CO_2) = 0$
$\text{VOL}_{ALV} \times \frac{dPCO_2}{dt} =$
$\dot{V} \times (P_iCO_2 - P_{ALV}CO_2)$
$+ k \times Q \times (C_vCO_2 - C_{ALV}CO_2)$