Carbon dioxide responses at extreme conditions: opioid effects and tolerability

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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.

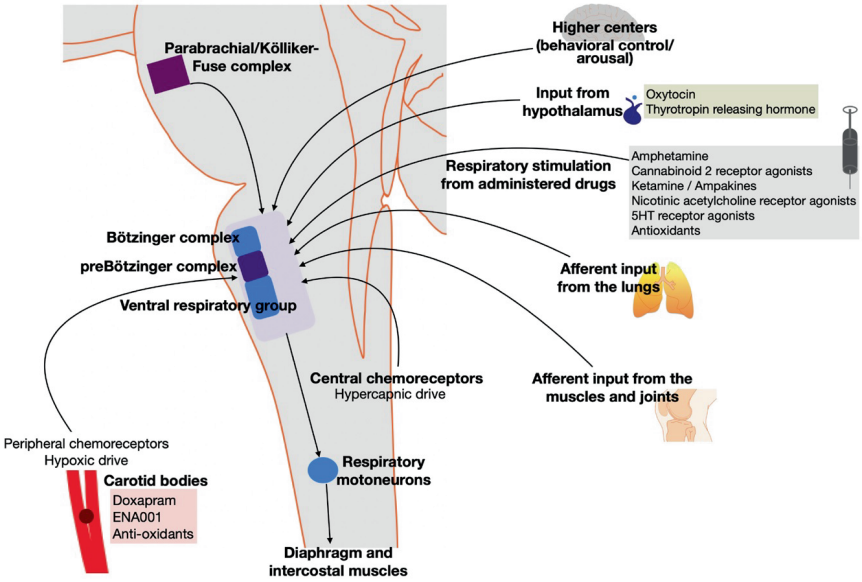

Figure 1: Schematic overview of the input to the brainstem respiratory centers. In purple, the parabrachial/Kölliker–Fuse complex, located in the pons, and pre-Bötzinger complex, located in the brainstem, that show high respiratory sensitivity to exogenously administered opioids and consequently are 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

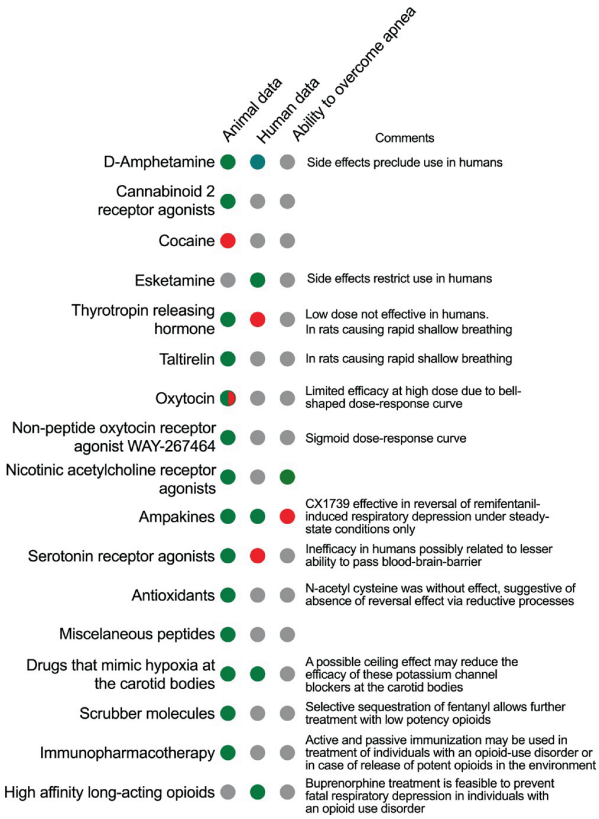

Figure 2: List of agents used to treat or prevent opioid-induced respiratory depression. Green circles indicate effective reversal/prevention or ability to reverse an apneic event; red circles indicate ineffective reversal/prevention or inability to reverse an apneic event; gray circles indicate that 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.

References

1. Albert Dahan, Leon Aarts, and Terry W Smith. “Incidence, reversal, and prevention of opioid-induced respiratory depression”. In: *The Journal of the American Society of Anesthesiologists* 112.1 (2010), pp. 226–238.
2. Kyle TS Pattinson. “Opioids and the control of respiration”. In: *British journal of anaesthesia* 100.6 (2008), pp. 747–758.
3. Adrienn G Varga et al. “Differential impact of two critical respiratory centres in opioid-induced respiratory depression in awake mice”. In: *The Journal of physiology* 598.1 (2020), pp. 189–205.
4. Barbara Palkovic et al. “Dose-dependent respiratory depression by remifentanil in the rabbit parabrachial nucleus/Kölliker–Fuse complex and pre-Bötzinger complex”. In: *Anesthesiology* 135.4 (2021), pp. 649–672.
5. Nathan A Baertsch et al. “Dual mechanisms of opioid-induced respiratory depression in the inspiratory rhythm-generating network”. In: *Elife* 10 (2021), e67523.
6. Albert Dahan et al. “Averting opioid-induced respiratory depression without affecting analgesia”. In: *Anesthesiology* 128.5 (2018), pp. 1027–1037.
7. David T Yeung et al. “National institutes of health (NIH) executive meeting summary: Developing medical countermeasures to rescue opioid-induced respiratory depression (a transagency scientific meeting)—August 6/7, 2019”. In: *Journal of Medical Toxicology* 16 (2020), pp. 87–105.
8. Martijn Boon et al. “Combining opioids and benzodiazepines: effects on mortality and severe adverse respiratory events”. In: *Ann Palliat Med* 9.2 (2020), pp. 542–557.
9. Eveline LA van Dorp, Ashraf Yassen, and Albert Dahan. “Naloxone treatment in opioid addiction: the risks and benefits”. In: *Expert opinion on drug safety* 6.2 (2007), pp. 125–132.
10. Carroll A Handley and Dorrence L Ensberg. “A comparison of amphetamine sulfate with other stimulants of the central nervous system in morphine respiratory depression”. In: *The Journal of the American Society of Anesthesiologists*. Vol. 6. 6. The American Society of Anesthesiologists. 1945, pp. 561–564.
11. Olivia A Moody et al. “D-amphetamine accelerates recovery of consciousness and respiratory drive after high-dose fentanyl in rats”. In: *Frontiers in Pharmacology* 11 (2020), p. 585356.
12. Peter M Lalley. “D1-dopamine receptor agonists prevent and reverse opiate depression of breathing but not antinociception in the cat”. In: *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 289.1 (2005), R45–R51.

13. Peter M Lalley. “Dopamine1 receptor agonists reverse opioid respiratory network depression, increase CO2 reactivity”. In: *Respiratory physiology & neurobiology* 139.3 (2004), pp. 247–262.
14. Carmen A Zavala et al. “Cannabinoid CB2 receptor activation attenuates fentanyl-induced respiratory depression”. In: *Cannabis and Cannabinoid Research* 6.5 (2021), pp. 389–400.
15. Beth M Wiese et al. “Cannabinoid-2 agonism with AM2301 mitigates morphine-induced respiratory depression”. In: *Cannabis and Cannabinoid Research* 6.5 (2021), pp. 401–412.
16. Peter F Weed, Lisa R Gerak, and Charles P France. “Ventilatory-depressant effects of opioids alone and in combination with cannabinoids in rhesus monkeys”. In: *European journal of pharmacology* 833 (2018), pp. 94–99.
17. Ernesto Solis Jr, Anum Afzal, and Eugene A Kiyatkin. “Intravenous cocaine increases oxygen entry into brain tissue: critical role of peripheral drug actions”. In: *ACS chemical neuroscience* 10.4 (2018), pp. 1923–1928.
18. Shruthi A Thomas, David Perekopskiy, and Eugene A Kiyatkin. “Cocaine added to heroin fails to affect heroin-induced brain hypoxia”. In: *Brain research* 1746 (2020), p. 147008.
19. L Mildh et al. “The effect of low-dose ketamine on fentanyl-induced respiratory depression”. In: *Anaesthesia* 53.10 (1998), pp. 965–970.
20. K Jonkman et al. “Esketamine counters opioid-induced respiratory depression”. In: *British Journal of Anaesthesia* 120.5 (2018), pp. 1117–1127.
21. Denise R Cook-Snyder et al. “The contribution of endogenous glutamatergic input in the ventral respiratory column to respiratory rhythm”. In: *Respiratory physiology & neurobiology* 260 (2019), pp. 37–52.
22. Elise Sarton et al. “The involvement of the μ-opioid receptor in ketamine-induced respiratory depression and antinociception”. In: *Anesthesia & Analgesia* 93.6 (2001), pp. 1495–1500.
23. Eleonore Fröhlich and Richard Wahl. “The forgotten effects of thyrotropin-releasing hormone: Metabolic functions and medical applications”. In: *Frontiers in neuroendocrinology* 52 (2019), pp. 29–43.
24. Gary W Kraemer et al. “Thyrotropin releasing hormone: antagonism of pentobarbital narcosis in the monkey”. In: *Pharmacology Biochemistry and Behavior* 4.6 (1976), pp. 709–712.
25. Dmitry A Kharkevich, Boris A Chizh, and Sergey A Kasparov. “Stimulant effect of thyrotropin-releasing hormone and its analog, RGH 2202, on the diaphragm respiratory activity, and their antagonism with morphine: possible involvement of the N-methyl-D-aspartate receptors”. In: *Brain research* 551.1-2 (1991), pp. 110–115.

26. James D Boghosian, Anita Luethy, and Joseph F Cotten. “Intravenous and intratracheal thyrotropin releasing hormone and its analog taltirelin reverse opioid-induced respiratory depression in isoflurane anesthetized rats”. In: *Journal of Pharmacology and Experimental Therapeutics* 366.1 (2018), pp. 105–112.

27. Kaye E Dandrea and Joseph F Cotten. “A comparison of breathing stimulants for reversal of synthetic opioid-induced respiratory depression in conscious rats”. In: *Journal of Pharmacology and Experimental Therapeutics* 378.2 (2021), pp. 146–156.

28. S Tagliavini et al. “Influence of TRH on regional blood flow and metabolic acidosis in a model of volume-controlled hemorrhagic shock in rats”. In: *Neuropeptides* 20.4 (1991), pp. 233–238.

29. Allison Doyle Brackley and Glenn M Toney. “Oxytocin receptor activation rescues opioid-induced respiratory depression by systemic fentanyl in the rat”. In: *Journal of Pharmacology and Experimental Therapeutics* 378.2 (2021), pp. 96–107.

30. Vivek Jain et al. “Intranasal oxytocin increases respiratory rate and reduces obstructive event duration and oxygen desaturation in obstructive sleep apnea patients: a randomized double blinded placebo controlled study”. In: *Sleep Medicine* 74 (2020), pp. 242–247.

31. Yoshiyuki Meguro et al. “Neuropeptide oxytocin enhances μ opioid receptor signaling as a positive allosteric modulator”. In: *Journal of Pharmacological Sciences* 137.1 (2018), pp. 67–75.

32. Jun Ren, Xiuqing Ding, and John J Greer. “Activating $\alpha 4\beta 2$ nicotinic acetylcholine receptors alleviates fentanyl-induced respiratory depression in rats”. In: *Anesthesiology* 130.6 (2019), pp. 1017–1031.

33. Jun Ren, Xiuqing Ding, and John J Greer. “Countering opioid-induced respiratory depression in male rats with nicotinic acetylcholine receptor partial agonists varenicline and ABT 594”. In: *Anesthesiology* 132.5 (2020), pp. 1197–1211.

34. Jun Ren et al. “Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats”. In: *The Journal of the American Society of Anesthesiologists* 110.6 (2009), pp. 1364–1370.

35. Amanda R Lorier, Gregory D Funk, and John J Greer. “Opiate-induced suppression of rat hypoglossal motoneuron activity and its reversal by ampakine therapy”. In: *PloS one* 5.1 (2010), e8766.

36. BG Oertel et al. “Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia”. In: *Clinical Pharmacology & Therapeutics* 87.2 (2010), pp. 204–211.

37. Dian Xiao et al. “The impact and mechanism of ampakine CX1739 on protection against respiratory depression in rats”. In: *Future Medicinal Chemistry* 12.23 (2020), pp. 2093–2104.

38. Anna J Haw et al. “Ampakine CX 1942 attenuates opioid-induced respiratory depression and corrects the hypoxaemic effects of etorphine in immobilized goats (Capra hircus)”. In: *Veterinary anaesthesia and analgesia* 43.5 (2016), pp. 528–538.

39. Wei Dai et al. “A brain-targeted ampakine compound protects against opioid-induced respiratory depression”. In: *European Journal of Pharmacology* 809 (2017), pp. 122–129.

40. Wei Dai et al. “The impact and mechanism of a novel allosteric AMPA receptor modulator LCX001 on protection against respiratory depression in rodents”. In: *Frontiers in Pharmacology* 10 (2019), p. 105.

41. A Krystal et al. “0571 opioids and sleep apnea: antagonism of remifentanil-induced respiratory depression by cx1739 in two clinical models of opioid induced respiratory depression”. In: *Sleep* 40 (2017), A212.

42. David Cavalla et al. “Tianeptine prevents respiratory depression without affecting analgesic effect of opiates in conscious rats”. In: *European Journal of Pharmacology* 761 (2015), pp. 268–272.

43. Madalee M Gassaway et al. “The atypical antidepressant and neurorestorative agent tianeptine is a μ-opioid receptor agonist”. In: *Translational psychiatry* 4.7 (2014), e411–e411.

44. Nadine Tod et al. “Investigation of cardiorespiratory effects of the selective 5-HT4 agonist BIMU-8 in etorphine-immobilised goats (Capra aegagrus hircus) in a randomized, blinded and controlled trial”. In: *Veterinary Record* 189.1 (2021), no–no.

45. BG Oertel et al. “The partial 5-hydroxytryptamine1A receptor agonist buspirone does not antagonize morphine-induced respiratory depression in humans”. In: *Clinical Pharmacology & Therapeutics* 81.1 (2007), pp. 59–68.

46. Jörn Lötsch et al. “The 5-hydroxytryptamine 4 receptor agonist mosapride does not antagonize morphine-induced respiratory depression”. In: *Clinical Pharmacology & Therapeutics* 78.3 (2005), pp. 278–287.

47. Wulf Hildebrandt et al. “Effect of N-acetyl-cysteine on the hypoxic ventilatory response and erythropoietin production: linkage between plasma thiol redox state and O2 chemosensitivity”. In: *Blood, The Journal of the American Society of Hematology* 99.5 (2002), pp. 1552–1555.

48. Luc J Teppema, Raymonda R Romberg, and Albert Dahan. “Antioxidants reverse reduction of the human hypoxic ventilatory response by subanesthetic isoflurane”. In: *The Journal of the American Society of Anesthesiologists* 102.4 (2005), pp. 747–753.

49. James Mendoza et al. “l-Cysteine ethyl ester reverses the deleterious effects of morphine on, arterial blood–gas chemistry in tracheotomized rats”. In: *Respiratory physiology & neurobiology* 189.1 (2013), pp. 136–143.

50. Benjamin Gaston et al. “D-Cystine di (m) ethyl ester reverses the deleterious effects of morphine on ventilation and arterial blood gas chemistry while promoting antinociception”. In: *Scientific reports* 11.1 (2021), p. 10038.

51. Michael W Jenkins et al. “Glutathione ethyl ester reverses the deleterious effects of fentanyl on ventilation and arterial blood-gas chemistry while prolonging fentanyl-induced analgesia”. In: *Scientific reports* 11.1 (2021), p. 6985.

52. Santhosh Baby et al. “Systemic administration of tempol attenuates the cardiorespiratory depressant effects of fentanyl”. In: *Frontiers in Pharmacology* 12 (2021), p. 690407.

53. Piotr Wojciechowski, Kryspin Andrzejewski, and Katarzyna Kaczyńska. “Intracerebroventricular neuropeptide FF diminishes the number of apneas and cardiovascular effects produced by opioid receptors’ activation”. In: *International Journal of Molecular Sciences* 21.23 (2020), p. 8931.

54. Xiaonan Liang, Zheng Yong, and Ruibin Su. “Inhibition of protein kinase A and GIRK channel reverses fentanyl-induced respiratory depression”. In: *Neuroscience Letters* 677 (2018), pp. 14–18.

55. Medge D Owen et al. “Glycyl-glutamine inhibits the respiratory depression, but not the antinociception, produced by morphine”. In: *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 279.5 (2000), R1944–R1948.

56. Michael J Welsh, Donald D Heistad, Francois M Abboud, et al. “Depression of ventilation by dopamine in man: evidence for an effect on the chemoreceptor reflex”. In: *The Journal of clinical investigation* 61.3 (1978), pp. 708–713.

57. Stuart M Tomares et al. “Effects of domperidone on neonatal and adult carotid chemoreceptors in the cat”. In: *Journal of applied physiology* 77.3 (1994), pp. 1274–1280.

58. Rikki H Chokshi et al. “Breathing stimulant compounds inhibit TASK-3 potassium channel function likely by binding at a common site in the channel pore”. In: *Molecular pharmacology* 88.5 (2015), pp. 926–934.

59. Kevin P Cunningham et al. “Effects of the ventilatory stimulant, doxapram on human TASK-3 (KCNK9, K2P9. 1) channels and TASK-1 (KCNK3, K2P3. 1) channels”. In: *Acta physiologica* 228.2 (2020), e13361.

60. Peadar B O’Donohoe et al. “A1899, PK-THPP, ML365, and Doxapram inhibit endogenous TASK channels and excite calcium signaling in carotid body type-1 cells”. In: *Physiological Reports* 6.19 (2018), e13876.

61. C Spencer Yost. “A new look at the respiratory stimulant doxapram”. In: *CNS drug reviews* 12.3-4 (2006), pp. 236–249.

62. Akira Haji, Satoko Kimura, and Yoshiaki Ohi. “Reversal of morphine-induced respiratory depression by doxapram in anesthetized rats”. In: *European Journal of Pharmacology* 780 (2016), pp. 209–215.

63. Margot Roozekrans et al. “Two studies on reversal of opioid-induced respiratory depression by BK-channel blocker GAL021 in human volunteers”. In: *Anesthesiology* 121.3 (2014), pp. 459–468.

64. M Roozekrans et al. “Reversal of opioid-induced respiratory depression by BK-channel blocker GAL021: A pharmacokinetic-pharmacodynamic modeling study in healthy volunteers”. In: *Clinical Pharmacology & Therapeutics* 97.6 (2015), pp. 641–649.

65. Joseph F Cotten. “The latest pharmacologic ventilator”. In: *Anesthesiology* 121.3 (2014), pp. 442–444.

66. James R Riches et al. “Analysis of clothing and urine from Moscow theatre siege casualties reveals carfentanil and remifentanil use”. In: *Journal of analytical toxicology* 36.9 (2012), pp. 647–656.

67. Tharusan Thevathasan et al. “Calabadion 1 selectively reverses respiratory and central nervous system effects of fentanyl in a rat model”. In: *British Journal of Anaesthesia* 125.1 (2020), e140–e147.

68. Daniel Diaz-Gil et al. “A novel strategy to reverse general anesthesia by scavenging with the acyclic cucurbit [n] uril-type molecular container calabadion 2”. In: *Anesthesiology* 125.2 (2016), pp. 333–345.

69. Brian P Mayer et al. “Solution-state structure and affinities of cyclodextrin: fentanyl complexes by nuclear magnetic resonance spectroscopy and molecular dynamics simulation”. In: *The Journal of Physical Chemistry B* 120.9 (2016), pp. 2423–2433.

70. Matthew L Banks, Margaret E Olson, and Kim D Janda. “Immunopharmacotherapies for treating opioid use disorder”. In: *Trends in pharmacological sciences* 39.11 (2018), pp. 908–911.

71. Rebekah D Tenney et al. “Vaccine blunts fentanyl potency in male rhesus monkeys”. In: *Neuropharmacology* 158 (2019), p. 107730.

72. U.S. Government Accountability Office (GAO). *Science & Tech Spotlight: Opioid vaccines*. Accessed: July 21, 2021. 2019. URL: `https://www.gao.gov/products/gao-19-706sp`.

73. Marco Pravetoni and Sandra D Comer. “Development of vaccines to treat opioid use disorders and reduce incidence of overdose”. In: *Neuropharmacology* 158 (2019), p. 107662.

74. Michael D Raleigh et al. “A fentanyl vaccine alters fentanyl distribution and protects against fentanyl-induced effects in mice and rats”. In: *Journal of Pharmacology and Experimental Therapeutics* 368.2 (2019), pp. 282–291.

75. Paul T Bremer et al. “Combatting synthetic designer opioids: a conjugate vaccine ablates lethal doses of fentanyl class drugs”. In: *Angewandte Chemie International Edition* 55.11 (2016), pp. 3772–3775.

76. Christine Robinson et al. “Therapeutic and prophylactic vaccines to counteract fentanyl use disorders and toxicity”. In: *Journal of medicinal chemistry* 63.23 (2020), pp. 14647–14667.

77. MD Raleigh et al. “Pharmacological mechanisms underlying the efficacy of antibodies generated by a vaccine to treat oxycodone use disorder”. In: *Neuropharmacology* 195 (2021), p. 108653.

78. Michael D Raleigh, Claudia Accetturo, and Marco Pravetoni. “Combining a candidate vaccine for opioid use disorders with extended-release naltrexone increases protection against oxycodone-induced behavioral effects and toxicity”. In: *Journal of Pharmacology and Experimental Therapeutics* 374.3 (2020), pp. 392–403.

79. Lauren C Smith et al. “Monoclonal antibodies for combating synthetic opioid intoxication”. In: *Journal of the American Chemical Society* 141.26 (2019), pp. 10489–10503.

80. Carly Baehr et al. “Monoclonal antibodies counteract opioid-induced behavioral and toxic effects in mice and rats”. In: *Journal of Pharmacology and Experimental Therapeutics* 375.3 (2020), pp. 469–477.

81. CiBots, Inc. *NarcoBond™: A novel opioid overdose countermeasure*. Cell Cure. Available at: `http://www.cellcured.com/pipeline/opioid-overdose-countermeasures`. Accessed July 22, 2021. 2019. URL: `http://www.cellcured.com/pipeline/opioid-overdose-countermeasures`.

82. National Academies of Sciences, Engineering, and Medicine. *Medications for Opioid Use Disorder Saves Lives*. Washington, D.C.: The National Academies Press, 2019.

83. Ashraf Yassen et al. “Mechanism-based PK/PD modeling of the respiratory depressant effect of buprenorphine and fentanyl in healthy volunteers”. In: *Clinical Pharmacology & Therapeutics* 81.1 (2007), pp. 50–58.

84. Albert Dahan et al. “Respiratory effects of the nociceptin/orphanin FQ peptide and opioid receptor agonist, cebranopadol, in healthy human volunteers”. In: *Anesthesiology* 126.4 (2017), pp. 697–707.

85. K. Wiest et al. “High plasma buprenorphine concentrations decrease respiratory effects of intravenous fentanyl”. In: *Annual Conference of the American Society of Addiction Medicine*. Paper presented at: Annual Conference of the American Society of Addiction Medicine, April 4–7. Orlando, Florida, 2019. URL: `https://www.eventscribe.com/2019/posters/ASAM/SplitViewer.asp?PID=MzcwNzkzNTM2MDA`.

86. Nasim Zamani, Nicholas A Buckley, and Hossein Hassanian-Moghaddam. “Buprenorphine to reverse respiratory depression from methadone overdose in opioid-dependent patients: a prospective randomized trial”. In: *Critical Care* 24 (2020), pp. 1–9.

87. Anna J Haw, Leith CR Meyer, and Andrea Fuller. “Nalbuphine and butorphanol reverse opioid-induced respiratory depression but increase arousal in etorphine-immobilized goats (Capra hircus)”. In: *Veterinary anaesthesia and analgesia* 43.5 (2016), pp. 539–548.

88. Leith CR Meyer et al. “Use of butorphanol and diprenorphine to counter respiratory impairment in the immobilised white rhinoceros (Ceratotherium simum)”. In: *Journal of the South African Veterinary Association* 89.1 (2018), pp. 1–8.

89. Nobuo Umezawa et al. “Orexin-B antagonized respiratory depression induced by sevoflurane, propofol, and remifentanil in isolated brainstem-spinal cords of neonatal rats”. In: *Respiratory Physiology & Neurobiology* 205 (2015), pp. 61–65.

90. Iris Bachmutsky et al. “Opioids depress breathing through two small brainstem sites”. In: *Elife* 9 (2020), e52694.