Special Section on The Opioid Crisis

Noradrenergic Mechanisms in Fentanyl-Mediated Rapid Death Explain Failure of Naloxone in the Opioid Crisis

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ABSTRACT

In December 2018, the Centers for Disease Control declared fentanyl the deadliest drug in America. Opioid overdose is the single greatest cause of death in the United States adult population (ages 18–50), and fentanyl and its analogs [fentanyl/fentanyl analogs (F/FAs)] are currently involved in >50% of these deaths. Anesthesiologists in the United States were introduced to fentanyl in the early 1970s when it revolutionized surgical anesthesia by combining profound analgesia with hemodynamic stability. However, they quickly had to master its unique side effect. F/FAs can produce profound rigidity in the diaphragm, chest wall and upper airway within an extremely narrow dosing range. This clinical effect was called wooden chest syndrome (WCS) by anesthesiologists and is not commonly known outside of anesthesiology or to clinicians or researchers in addiction research/medicine. WCS is almost routinely fatal without expert airway management. This review provides relevant clinical human pharmacology and animal data demonstrating that the significant increase in the number of F/FA-induced deaths may involve α-adrenergic and cholinergic receptor–mediated mechanical failure of the respiratory and cardiovascular systems with rapid development of rigidity and airway closure. Although morphine and its prodrug, heroin, can cause mild rigidity in abdominal muscles at high doses, neither presents with the distinct and rapid respiratory failure seen with F/FA-induced WCS, separating F/FA overdose from the slower onset of respiratory depression caused by morphine-derived alkaloids. This distinction has significant consequences for the design and implementation of new pharmacologic strategies to effectively prevent F/FA-induced death.

SIGNIFICANCE STATEMENT

Deaths from fentanyl and F/FAs are increasing in spite of availability and awareness of the opioid reversal drug naloxone. This article reviews literature suggesting that naloxone may be ineffective against centrally mediated noradrenergic and cholinergic effects of F/FAs, which clinically manifest as severe muscle rigidity and airway compromise (e.g., wooden chest syndrome) that is rapid and distinct from respiratory depression seen with morphine-derived alkaloids. A physiologic model is proposed and implications for new drug development and treatment are discussed.

Introduction

Currently, opioid overdose is the number one cause of death in United States residents 18–50 years of age. The Centers for Disease Control reported that fentanyl (Sublimaze) and its analogs were the cause of death in >50% of United States

deaths related to opioids in 2016 and were estimated to be >70% for 2017 and 2018 (Hedegaard et al., 2018; Jannetto et al., 2019).

Belgian chemist Paul Janssen first synthesized the fully synthetic opioid, fentanyl, in 1960, which revolutionized the field of surgical anesthesia. Fentanyl has extremely high potency (e.g., ~150 greater than morphine) and hemodynamic stability (Stanley, 2014). Like all narcotics given in sufficient quantity, fentanyl/fentanyl analogs (F/FAs) can induce dose-dependent respiratory depression, hypoxia, and death (Stanley, 2014). Unlike morphine-derived alkaloids, F/FAs can rapidly induce a combination of vocal cord closure (laryngospasm) and severe muscle rigidity in the chest wall and diaphragm at doses well within therapeutic ranges used

ABBREVIATIONS: ACh, acetylcholine; CNS, central nervous system; CSF, cerebrospinal fluid; DRG, dorsal respiratory group; EMG, electromyography; EMS, emergency medical services; F/FA, fentanyl/fentanyl analog; FIMR, fentanyl-induced muscle rigidity; FIRMR, fentanyl-induced respiratory muscle rigidity; LC, locus coeruleus; M, muscarinic; N$_2$O, nitrous oxide; PCA, posterior cricoarytenoid; TA, thyroarytenoid; VCD, vocal cord dysfunction; VRG, ventral respiratory group; WCS, wooden chest syndrome.

for analgesia, and lower than doses known to cause severe respiratory depression. This phenomenon of fentanyl-induced respiratory muscle rigidity (FIRMR) and laryngospasm is clinically known as wooden chest syndrome (WCS), which occurs within 1 to 2 minutes after injection and lasts ∼8–15 minutes (Grell et al., 1970; Scamman, 1983; Streisand et al., 1993). Anesthesiologists are trained to manage WCS and airway compromise with intravenous muscle paralytics (e.g., succinylcholine) followed by endotracheal intubation. Janssen Pharmaceutica, the manufacturer of fentanyl, recognized that muscle rigidity induced by fentanyl should be treated with a paralytic, and respiratory depression should be treated with a μ-opioid receptor antagonist, such as naloxone (Grell et al., 1970; Streisand et al., 1993; Janssen Pharmaceutica, 2017).

WCS with F/FAs is not commonly known to the medical community or to vulnerable individuals using intravenous drugs in the community at large (Baumann et al., 2018), many of whom incorrectly believe that their heroin is not adulterated with F/FA (Ochalek et al., 2019). Rapidity of injection and the dose of F/FAs are the key determinants in the incidence, severity, and duration of fentanyl-induced muscle rigidity (FIMR)/WCS (Grell et al., 1970). Eyewitness and survivor accounts of intravenous overdoses involving F/FAs indicate a rapid onset of cyanosis, loss of consciousness, severe muscle rigidity, and seizure-like behavior associated with the rate of injection (Somerville et al., 2017). Autopsy and public health data all indicate a rapid onset of death via a mechanism that is differentiated from the respiratory depression associated with overdose from heroin and morphine-derived alkaloids (Burns et al., 2016; Somerville et al., 2017). Emergency medical services (EMS), emergency rooms, and first response personnel have reported that the normal dose ranges of the opioid reversal agent, naloxone, seem inadequate to reverse F/FA effects, and also report difficulty performing chest compressions/cardiopulmonary resuscitation or ventilation in F/FA overdose due to chest wall rigidity (Schumann et al., 2008; Fairbairn et al., 2017; Baumann et al., 2018). Emergency room admissions and survival rate studies of overdoses have shown that the ratios for emergency room visits to deaths are 1:1 for fentanyl versus 10:1 for heroin-related overdoses, indicating a narrow window for response to fentanyl overdose (Slavova et al., 2017).

Fentanyl undergoes rapid hepatic metabolism in humans and produces the inactive metabolite norfentanyl in 90–120 seconds (McClain and Hug, 1980). Autopsy data on deaths related to fentanyl indicate vascular compromise and cessation of liver perfusion and metabolism in less than 2 minutes, as indicated by the absence of tissue levels of norfentanyl (Burns et al., 2016).

Animal data from multiple studies indicate that one of the underlying molecular mechanisms for the rigidity of respiratory muscles induced by F/FAs involves the agonism of μ-opioid receptors in the locus coeruleus (LC), which appears to increase noradrenergic outflow from the LC via the activation of α1-adrenergic receptors in the LC and spinal cord (Lui et al., 1989; Fu et al., 1997). Supportive evidence of this mechanism is suggested by the demonstration of FIMR in peripheral skeletal muscle within 40–60 seconds after direct injection of fentanyl into the LC. This effect is inhibited by direct ablation of the LC, disruption of cerulospinal fibers connecting the LC with distal spinal cord motor efferents, and/

or pretreatment with α1-adrenergic antagonists. However, this interpretation of the data is controversial since it contradicts some of the existing ex vivo and in vivo voltammetry animal studies of the LC, which indicate that fentanyl decreases catecholamine metabolism, albeit on a slower time scale than FIMR (Aghajanian, 1982; Williams et al., 1984; Lui et al., 1989; Milne et al., 1989a; Fu et al., 1997). Conversely, evidence exists in both free-moving and anesthetized animal models supporting increased catecholamine release from both morphine and fentanyl analogs (e.g., sufentanil) in several regions of the brain, including the LC, on a time scale that overlaps with WCS (Rasmussen and Jacobs, 1985; Gaumann et al., 1988; Milne et al., 1989b). More specifically, Lalley (2003) demonstrated increased tonic firing in feline medullary nuclei controlling vagal innervation of vocal adductors on a time scale of ∼20 seconds after systemic fentanyl administration, presumably mediated by μ-opioid receptors and well within the clinical timing of WCS in humans (Lalley, 2003). These conflicting data indicate that interpretations of F/FA effects on the brain are complicated by the fact that these effects appear to be neuroanatomical and state dependent. A definitive experiment demonstrating these events (e.g., catecholamine release in the LC occurring with WCS) in an animal model of WCS that includes the observation of FIMR and laryngospasm on a corresponding time scale indicating causality has yet to be demonstrated. Additionally, F/FAs modulate cholinergic receptors in medullary motor centers that control respiratory mechanics and airway patency (Stoelting et al., 1975; Lui et al., 1989; Randich et al., 1992; Hustveit and Setekleiv, 1993; Hustveit, 1994; Fu et al., 1997; Lalley, 2003). Similarly, human and animal studies of coronary artery adrenergic receptor subtypes, μ-receptor–mediated cholinergic innervation of the cardiovascular system, and fentanyl binding at cholinergic receptors indicate the potential for a significant impact on coronary, hepatic, and cerebral perfusion by F/FAs (Willette and Sapru, 1982; Yamanoue et al., 1993; Lautt, 1996; Richardson et al., 1997; Tuñón et al., 1999; Griffioen et al., 2004; Møller and Henriksen, 2008; Schuppan and Afdhal, 2008; Jensen et al., 2009; Solis et al., 2017).

When considering these data, it is apparent that the ongoing increases in F/FA-related deaths may be a direct result of the lack of public and medical community awareness of F/FAs' lethal side-effect profile and a false sense of security provided by naloxone's past successes against conventional morphine-derived opioids (Baumann et al., 2018). However, conventional μ-receptor antagonist therapies (e.g., naloxone, nalmefene, and naltrexone) have little effect on the cholinergic or noradrenergic off-target sites that may be responsible for the rapid lethality of F/FAs (Daskalopoulos et al., 1975; Willette and Sapru, 1982; Willette et al., 1982, 1987). Pharmacologic interventions that target these receptors (e.g., α1-adrenergic receptor antagonists and cholinergic agents) in combination with μ-opioid antagonists must be further explored to develop more effective F/FA reversal and prophylaxis agents. Prophylaxis and immediate reversal agents that target rigidity, airway closure, and coronary perfusion may give the victims of these synthetic opioids a wider therapeutic window for life-saving treatment from F/FA overdose.
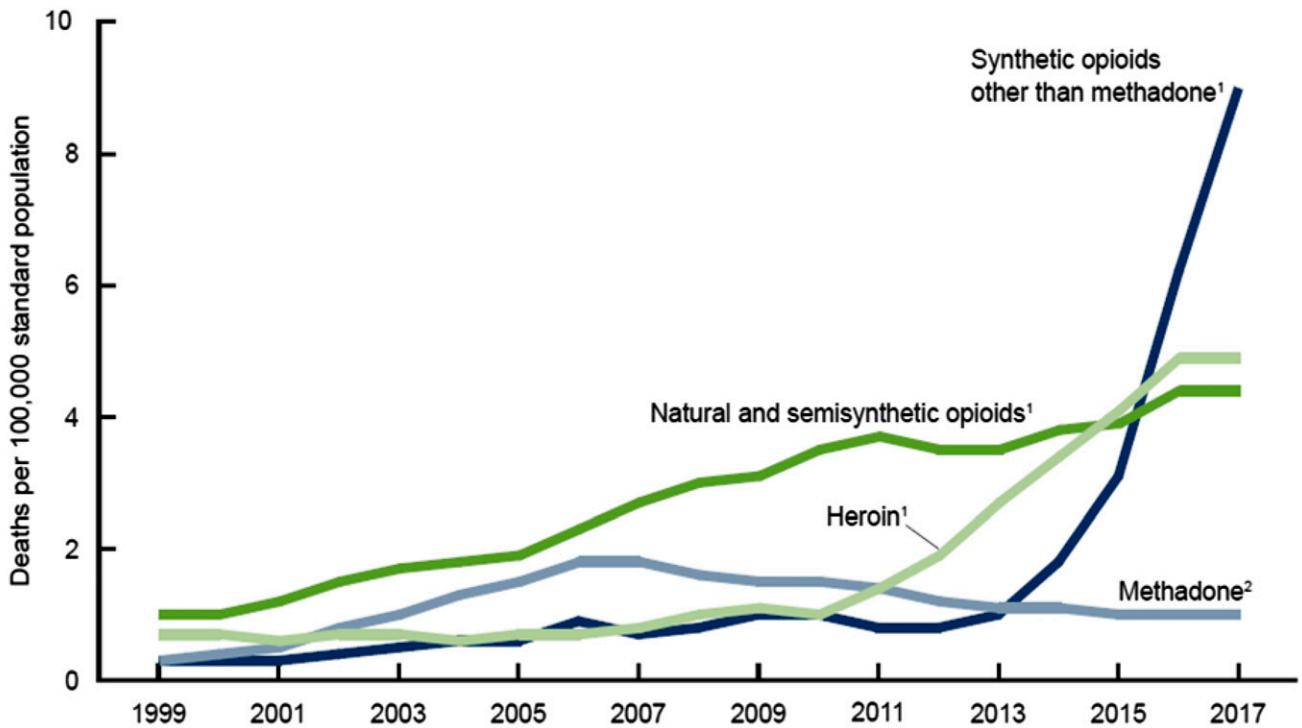
[1]Significant increasing trend from 1999 through 2017 with different rates of change over time, $p < 0.05$.
[2]Significant increasing trend from 1999 through 2006, then decreasing trend from 2006 through 2017, $p < 0.05$.
NOTES: Deaths are classified using the *International Classification of Diseases, 10th Revision*. Drug-poisoning (overdose) deaths are identified using underlying cause-of-death codes X40–X44, X60–X64, X85, and Y10–Y14. Drug overdose deaths involving selected drug categories are identified by specific multiple-cause-of-death codes: heroin, T40.1; natural and semisynthetic opioids, T40.2; methadone, T40.3; and synthetic opioids other than methadone, T40.4. Deaths involving more than one opioid category (e.g., a death involving both methadone and a natural and semisynthetic opioid) are counted in both categories. The percentage of drug overdose deaths that identified the specific drugs involved varied by year, with ranges of 75%–79% from 1999 through 2013 and 81%–88% from 2014 through 2017. Access data table for Figure 4 at: https://www.cdc.gov/nchs/data/databriefs/db329_tables-508.pdf#4.
SOURCE: NCHS, National Vital Statistics System, Mortality.

Fig. 1. Fentanyl (Sublimaze), and its analogs (F/FA) were the cause of death in >50% of United States deaths related to opioids in 2016 and estimated to be >70% for 2017 and 2018 (Hedegaard et al., 2018; Jannetto et al., 2019); data are from the Centers for Disease Control.

Current Statistics

Figure 1 shows the effects of the potency and the narrow therapeutic window of fentanyl and the most common drugs involved in United States overdose deaths as of 2017 (Hedegaard et al., 2018; Jannetto et al., 2019). The number of F/FA-related deaths have increased dramatically from ~1000 in 2010 and ~3000 fentanyl-related deaths in 2013 to currently over 30,000 (O'Donnell et al., 2017). Among the more than 70,000 drug overdose deaths estimated in 2017, the sharpest increase occurred among deaths related to F/FA with over 60,000 overdose deaths estimated in 2017 and 2018, and possibly more, due to the lack of access to analytical modalities capable of detecting other fentanyl analogs (Armenian et al., 2018; Hedegaard et al., 2018; Jannetto et al., 2019). Unfortunately, the F/FAs that are driving the current overdose rates are not from prescription sources or diverted pharmaceutical products, but are manufactured in other countries, sold over the internet, and delivered via private mail sources and conventional ports of entry (Ciccarone, 2017; Armenian et al., 2018). Further complicating the situation is the ease of transportability due to high potency, where fentanyl is ~1,000,000 lethal doses per kilogram and carfentanil is ~30,000,000 per kilogram (calculated based on an average lethal dose of 1 mg for fentanyl and the fact that carfentanil is

estimated to be 30 times more potent than fentanyl based on animal models) (Leen and Juurlink, 2019).

Fentanyl Pharmacology and Pharmacokinetics

Details of fentanyl pharmacology are described in several reviews (Planas, 2000; Barutell et al., 2004; Comer and Cahill, 2018). Points of emphasis for fentanyl in the context of the current opioid crisis can be reduced to a specific set of properties: potency, unique side effects, and cholinergic and noradrenergic receptor properties that will direct the development of more effective therapeutics for the treatment of F/FA overdose.

Fentanyl was first used clinically as an intravenous analgesic in Europe in 1963 and in the United States as a component of Innovar (a combination of fentanyl and droperidol) in 1968. Fentanyl effectively replaced morphine in major surgery due to its significantly greater potency and hemodynamic stability compared with high-dose morphine. Although fentanyl has not replaced morphine-derived alkaloids, F/FAs have become some of the world's most important and frequently used opioid analgesics (Stanley, 2014).

Fentanyl was the first of a family of opioids that later included sufentanil, alfentanil, and remifentanil for humans, and carfentanil for large animal veterinary medicine. Fentanyl's onset of

action and peak plasma concentrations are dependent on the dosage, method of delivery, and rapidity of injection; however, profound analgesic effects consistently appear within 90–120 seconds after intravenous administration and with plasma concentrations as low as 0.2–1.2 ng/ml in both opioid-naive and opioid-tolerant patients (Grell et al., 1970; Stanley, 2014).

Fentanyl, like morphine, meperidine, oxycodone, etc., can produce the usual μ-opioid receptor–mediated central nervous system actions such as analgesia, sedation, nausea, vomiting, respiratory depression (leading to apnea in higher doses), bradycardia (secondary to central vagal stimulating action), and unconsciousness in higher doses, irrespective of the mode of administration (Streisand et al., 1993). It is also apparent from human studies that WCS, which is the combination of chest wall and diaphragm rigidity and severe laryngospasm (e.g., vocal cord closure), is unique to F/FAs and can occur after intravenous, transdermal, or inhalational administration with incidence and severity related to the dose and speed of delivery (Grell et al., 1970; Sokoll et al., 1972; Freund et al., 1973; Yasuda et al., 1978). It should be clarified that although opioids such as morphine are known to cause abdominal wall muscle rigidity as a single agent in high doses, it has not been demonstrated in humans to cause upper airway compromise unless it is combined with the anesthetic gas nitrous oxide (N_2O), implying a separate mechanism from F/FA-induced laryngospasm (Sokoll et al., 1972; Freund et al., 1973; Yasuda et al., 1978). In fact, in all of morphine's long history of clinical use there is only a single case report of an association between morphine and laryngospasm. In the report, laryngospasm occurred in a neonate that had received both fentanyl and morphine for airway management in a hospital setting. As part of the report, PubMed and EMBASE searches of the medical literature revealed no prior cases (van der Lee et al., 2009). Upper airway complications with F/FAs will be discussed subsequently in greater detail in *Wooden Chest Syndrome* and *Naloxone*, providing compelling information for reexamining the efficacy of naloxone in F/FA overdoses.

WCS has occasionally been encountered following as little as 50 μg fentanyl given intravenously, can emerge in less than 2 minutes, last up to 15 minutes, and appears to cause death with a more accelerated time course than respiratory depression (Grell et al., 1970; Bailey et al., 1988; Stanley, 2014; Burns et al., 2016). It is important to note that WCS at low doses or doses given regularly in procedural sedation (<100 μg or 0.5–1 μg/kg) is extremely uncommon. Respiratory depression can begin during this same time period, but requires a significantly longer time for hypoxemia and cardiac arrest to occur. Compared with fentanyl, morphine takes approximately 15 minutes to achieve 80% of its peak effect and thus is much slower in onset of respiratory depression (Osborne et al., 1990; Burns et al., 2016). Heroin, on the other hand, gets into the brain quite rapidly but must be converted into morphine since heroin itself has very little intrinsic activity at μ-opioid receptors (Jenkins et al., 1994).

Regarding analgesia, fentanyl stimulates the μ-opioid receptor, activating the exchange of GTP for GDP on the G-protein complex, inhibiting adenylyl cyclase, and decreasing intracellular cAMP. This decrease in cAMP inhibits the release of neurotransmitters such as GABA, dopamine, acetylcholine, and noradrenaline (Childers, 1993; Al-Hasani and Bruchas, 2011) from the respective cells expressing the

μ-opioid receptor. The analgesic effects of F/FA are due to activation and opening of G-protein–coupled inwardly rectifying potassium channels that inhibit the opening of voltage-gated calcium channels. The inhibition of these calcium channels results in hyperpolarization of the cell reduced neuronal excitability and raised pain signaling threshold (Childers, 1993; Al-Hasani and Bruchas, 2011).

The very high lipophilicity of F/FAs enables rapid diffusion through membranes, including the blood-brain barrier and lipid-rich compartments of the central nervous system (CNS) (Hug and Murphy, 1979), thus allowing subjective effects to be experienced in one circulation time after injection. This rapid CNS access can produce analgesia and unconsciousness within minutes (Grell et al., 1970; Streisand et al., 1993; Poklis, 1995). However, these effects occur in spite of the fact that fentanyl and morphine have similar binding affinities at the μ-opioid receptor. Fentanyl has high lipophilicity, with octanol and water partition coefficients >9000 and >8000, respectively, for sufentanil compared with a partition coefficient for morphine of ~6, making fentanyl about 1000 times more lipid soluble than morphine (Roy and Flynn, 1988; Stone and DiFazio, 1988; Lötsch et al., 2013). In humans, serum concentrations for analgesia range from ~1 to 15 ng/ml, where a 6.9 ng/ml serum level corresponds to a calculated brain lipid concentration of 2.96 mM (Stone and DiFazio, 1988; Sohn et al., 2005; Rickli et al., 2018).

In addition to lipophilicity, fentanyl is more selective for μ-opioid receptors over δ- and κ-opioid receptors compared with morphine; however, it has similar binding affinity to morphine at μ-opioid receptors (Volpe et al., 2011). Also, it is important to mention that uniform assessment and ranking of K_i values and published measurements of binding affinities for selected opioids that include morphine and fentanyl are inconsistent due to a lack of uniformity in assay systems. This lack of uniformity has left a wide range of K_i values and difficulty in assigning meaningful ranking to drug potency based on these values. There is a clear need for accurate K_i data to understand fentanyl's underlying differences from morphine and to successfully develop effective reversal agents/drugs for synthetic opioids (Volpe et al., 2011).

In summary, fentanyl and its analogs revolutionized the field of surgery and anesthesia due to their remarkably high potency, rapid onset, and hemodynamic stability. However, shortly after their momentous introduction into mainstream medicine in the early 1970s, working with fentanyl's narrow therapeutic profile became a rite of passage for anesthesiologists who had to master its safe and efficacious administration while mitigating the onset of WCS. The following sections will examine the implications of WCS as an under-recognized cause of death in the context of the current fentanyl-driven opioid crisis and for the development of more effective therapeutics for F/FA overdose.

Wooden Chest Syndrome

WCS is the sudden onset of severe rigidity in key respiratory muscles of the chest wall, diaphragm, and upper airway that occurs within 90–120 seconds after the rapid intravenous injection of F/FAs, including sufentanil and alfentanil (Grell et al., 1970; Scamman, 1983; Streisand et al., 1993; Bennett et al., 1997). This contraction of chest wall, diaphragm, and abdominal muscles (i.e., FIRMR) is accompanied by vocal cord closure (laryngospasm) from centrally mediated activation of

vagal nerves (e.g., recurrent laryngeal nerve and external superior laryngeal nerve) that control adduction and abduction of the surrounding laryngeal muscles and the vocal cords themselves [Rex, 1970; Scamman, 1983; Lui et al., 1989; Lalley, 2003; see "Vocal Cord Dysfunction" (http://www.nuemblog.com/blog/vocal-cord-dysfunction/)]. For the purposes of this article, WCS will refer to the combined effects of F/FAs in humans on the large respiratory muscles (chest wall and diaphragm, i.e., FIRMR) and the laryngeal muscles and vocal cords (laryngospasm) that present as severe muscle spasm and/or rigidity with rapid failure of respiratory mechanics. WCS (FIRMR and laryngospasm) will be distinguished from FIMR as demonstrated in the animal model of opioid-induced rigidity. The term FIMR will be used only for the animal model and will refer strictly to muscle rigidity of the limbs and torso, since vocal cord effects (laryngospasm) and diaphragm have not been clearly demonstrated in the animal model. Other causes of neurotransmitter imbalance leading to muscle rigidity (e.g., serotonergic, neuroleptic, and central anticholinergic syndromes) will also be considered separately, since these clinical presentations tend to be more complex, insidious in onset, and less targeted to rapid respiratory failure.

Generally, the adductor muscles of the vocal cords (e.g., lateral cricoarytenoid and transverse arytenoid muscles) are relaxed during breathing at rest, allowing the sole abductor muscle of the vocal folds (e.g., posterior cricoarytenoid) to keep vocal cords and vocal folds open. Fentanyl activation of cholinergic and/or sympathetic innervation to these intrinsic laryngeal muscles may result in subsequent vocal cord closure (laryngospasm) (Willette et al., 1987; Lui et al., 1989; Lalley, 2003). Laryngospasm is generally managed in the operating or emergency room with the administration of muscle paralytics (succinylcholine). Without pharmacologic intervention, the glottic structures can remain closed for a period of 8–15 minutes (Grell et al., 1970; Scamman, 1983). This life-threatening reaction with F/FAs is distinct from side effects associated with nonsynthetic members of the opioid family, and is notable for its intensity and specificity. Other opioids, such as morphine, may incur abdominal muscle rigidity at high doses when administered with nitrous oxide, but can be easily managed with muscle relaxants and/or μ-opioid antagonists (Hamilton and Cullen, 1953; Freund et al., 1964, 1973). In contrast, the distinct adverse reaction of WCS, with its acute onset of laryngospasm and severe respiratory muscle rigidity, is life-threatening and only appears to occur with F/FAs in humans (Hamilton and Cullen, 1953; Freund et al., 1964, 1973; Grell et al., 1970; Yasuda et al., 1978). WCS can occur at doses as low as 0.5 μg/kg, but usually occurs in the dose range of >10 μg/kg and is based almost exclusively on the dose and rapidity of administration (Grell et al., 1970; Scamman, 1983; Streisand et al., 1993; Bennett et al., 1997).

It is important to note that increased thoraco-abdominal contraction may not be the most significant cause of WCS. The mechanical disruption of respiration in WCS may reside primarily in the closure of glottic structures and the upper airway (Scamman, 1983; Streisand et al., 1993; Bennett et al., 1997). Scamman (1983) observed that upper airway rigidity and glottis closure developed in a group of tracheostomy patients given high-dose fentanyl infusions. Subjects that were allowed to breathe via their tracheostomies

subsequently showed minimal increases in peak inspiratory pressures and relatively normal pulmonary mechanics compared with the control subjects without tracheostomy stomas. A more elaborate demonstration of this phenomenon used video laryngoscopy to measure response to high-dose fentanyl and revealed that consistent glottic closure occurred prior to significant changes in respiratory mechanics in the chest wall and abdomen (Bennett et al., 1997).

Although the effect is likely to be centrally mediated, smaller groups of muscles in the respiratory system are more sensitive and affected by fentanyl first, as is demonstrated by the order of muscle depolarization caused by the paralytic agent succinylcholine when used for endotracheal intubation. Smaller muscles in the face depolarize sequentially, with larger muscle groups such as chest wall and lower extremities depolarizing last (Chung and Rowbottom, 1993; Bennett et al., 1997).

Doses as small as 3–5 mg (e.g., 0.05–0.1 mg/kg in adults) of succinylcholine can relax the vocal cords without interrupting the mechanical capacity of respiration (Chung and Rowbottom, 1993); however, in the operating room, anesthesiologists routinely treat WCS with full doses of muscle paralytics (e.g., succinylcholine 1–1.5 mg/kg) followed by endotracheal intubation. Conversely, respiratory depression associated with F/FAs is treated directly with naloxone in incremental doses of 0.04–0.4 mg, and is clearly recommended by Janssen Pharmaceuticals, the manufacturer of fentanyl, in its package insert. Janssen Pharmaceuticals distinguishes muscle rigidity from respiratory depression and differentiates the treatment of each condition/presentation: "Respiratory depression can be reversed with the mu receptor antagonist naloxone. Muscle rigidity of the diaphragm and intercostal muscles can be eliminated by administration of the muscle paralytic succinylcholine" (Janssen Pharmaceutica, 2017).

Although exceedingly small doses of succinylcholine (e.g., 0.05–0.1 mg/kg) can effectively antagonize glottic closure (laryngospasm) induced by high-dose F/FAs, the treatment may still not be enough to overcome the accompanying masseter and jaw rigidity that can be associated with WCS, leaving the vocal cords open and vulnerable to aspiration but inaccessible to airway management (Grell et al., 1970; Chung and Rowbottom, 1993). The fact that vocal cord closure is a more significant component of WCS than previously realized must be considered in the management of patients found in acute respiratory failure due to overdose with F/FAs. Securing the airway by rapid sequence induction may be the safest solution in a patient who is unconscious and has relaxed airway reflexes, full gastric content, and high risk for aspiration, as is commonly seen in opioid overdose. This has significant implications for how first responders and EMS/paramedics can best manage the presentation of muscle rigidity and airway compromise with F/FAs. Whereas naloxone is an appropriate treatment to address respiratory depression, animal models studying the laryngeal effects of high-dose fentanyl fail to demonstrate a significant impact on acute vocal cord closure in dose ranges relevant to humans (Willette et al., 1982, 1987; Janssen Pharmaceutica, 2017). In an in situ model of the larynx, Willette et al. (1987) demonstrated that upper airway effects of opioids are mediated by peripheral and central mechanisms and can be blocked by 100 mcg/kg doses of naloxone for morphine, but require doses of 0.8 mg to

1.6 mg/kg of naloxone to effectively inhibit the centrally mediated airway effects of fentanyl (Willette et al., 1982, 1987). Given that fentanyl and morphine have similar binding affinities at μ-opioid receptors, this discrepancy suggests that other receptor targets modulate differing effects and emphasizes the lesser role that μ-opioid receptors play in centrally mediated F/FA-induced laryngospasm (Willette et al., 1982, 1987; Volpe et al., 2011). The differing effects may also indicate initial mediation of effects by μ-opioid receptors and interdependency of the circuitry controlling laryngospasm, emphasizing that naloxone may be ineffective as a single treatment agent. Additionally, rapid injection of doses of naloxone greater than 0.4 mg (0.005 mg/kg for a 70 kg adult) in opioid-dependent individuals is consistently associated with pulmonary edema and significant catecholamine release (Rzasa Lynn and Galinkin, 2018). However, there is some evidence that naloxone (20–40 mcg/kg) may be effective in reversal of laryngospasm for low-dose fentanyl (3–5 mcg/kg) in severely ill, premature neonates (n = 2), but there are currently no significant studies in adult humans to demonstrate this same effect and there has been no consideration of the role that brain development plays in centrally mediated respiratory mechanics (Fahnenstich et al., 2000). In fact, studies of sudden infant death syndrome, which involves the sudden onset of respiratory failure induced by vocal cord closure in human neonates, have demonstrated the significant role of the maturation of medullary cholinergic circuitry, specifically muscarinic receptors, in maintaining airway patency. The incidence of sudden infant death syndrome rapidly decreases as brain maturation and muscarinic receptor development occur (Richardson et al., 1997; Thach, 2001). It is noteworthy that fentanyl has significant activity at muscarinic receptors, selectively isolates these receptors in vagal nuclei, and causes differential effects on opposing laryngeal muscle contractility (Yamanoue et al., 1993; Lalley, 2003). These mechanisms will be discussed in greater detail in *Human Physiology*, in the context of potential underlying mechanisms of action for F/FAs in overdose death and the development of effective pharmacologic therapies that combine μ-receptor antagonists with drugs that block laryngospasm.

In summary, naloxone pharmacology has been reviewed extensively (Skolnick, 2018), but its effectiveness for blocking laryngospasm has not been well studied in humans and will need to be reevaluated in the context of the laryngospasm component of WCS in F/FA overdose. This has critical importance, since current evidence from public health and human autopsy data strongly suggests that muscle rigidity and laryngospasm may be an under-recognized cause of death in F/FA overdose, while animal data indicate that the underlying mechanism of F/FA-induced laryngospasm is only minimally modulated by μ-opioid receptors (Willette et al., 1982, 1987; Yamanoue et al., 1993; Burns et al., 2016; Somerville et al., 2017). Taken together, these factors may partially explain the continued rise in F/FA-related overdose deaths in spite of the availability and awareness of μ-opioid receptor antagonist therapy (naloxone).

Naloxone

Naloxone (Narcan) is a μ-opioid receptor antagonist that was developed in the early 1960s by the chemist Jacob Fishman for treatment of opioid-induced constipation (Yardley, 2013). Naloxone's true clinical utility was not fully realized until the early 1970s when it became a Food and Drug Administration–approved treatment of the reversal of opioid overdose in emergencies or inpatient settings (Rzasa Lynn and Galinkin, 2018). It is currently approved by the Food and Drug Administration for administration by several routes, including intravenous, intramuscular, subcutaneous, and intranasal, but can also be administered via endotracheal tube in patients who are intubated (Rzasa Lynn and Galinkin, 2018).

Since the late 1970s, efforts have been made to make naloxone more available to the community for use by first responders (Somerville et al., 2017). Since the 1990s, naloxone has been effectively employed by the lay public to dramatically decrease the incidence of death from heroin and morphine overdose. The importance of naloxone's availability to the public has been demonstrated several times in the wake of the heroin epidemic of the 1970s, through its resurgence in the early 1990s (Somerville et al., 2017). More recently and prior to the era of fentanyl, naloxone has proven exceedingly effective in preventing unintended overdoses from prescription opioids. Prior to the synthetic opioid era, a number of community programs reported a near 100% post-naloxone administration survival rate (Clark et al., 2014). Currently, in spite of community awareness and increased access to naloxone, deaths from synthetic opioids have reached record highs and do not seem to be slowing (Baumann et al., 2018; Skolnick, 2018). This fact is coupled with increasing reports of unsuccessful resuscitation attempts with naloxone following F/FA exposure, or successful resuscitation attempts only via multiple and escalating doses (Schumann et al., 2008; Fairbairn et al., 2017; Baumann et al., 2018; Moss and Carlo, 2019). These reports are further confounded by the fact that F/FAs are being combined with multiple illicit drugs (e.g., heroin, fentanyl, carfentanil, and Xanax) of unknown quantity, quality, synthesis, and combination, and the fact that these illicit drugs are not easily assayed. Although it is difficult to ascertain the effectiveness of naloxone under these conditions, these clinical observations indicate the urgency for the development of more effective treatments and better understanding of the unique properties of fentanyl and its analogs.

As described in the previous section, naloxone failed to demonstrate efficacy in an animal model of fentanyl-induced laryngospasm at concentrations relevant to humans, and there are no clear studies in humans that have shown the reversal of F/FA-induced laryngospasm and/or WCS with naloxone. It is important to note that the dose level of fentanyl used for the experiment (7.5 mcg/kg) by Willette et al. (1987) is in a range of dosing that can cause WCS in humans. Furthermore, naloxone has not demonstrated consistent efficacy in animal models of F/FA-induced muscle rigidity (upper airway effects or laryngospasm were not concurrently tested in these models) due to limitations or confounding variables in study design, and more importantly because naloxone has no intrinsic activity at either α-adrenergic or cholinergic receptors, as described subsequently in *Preclinical Research: Central Role of the Locus Coeruleus and α-Adrenergic Receptors in FIMR/WCS* and *Human Physiology* (LaBella et al., 1982; Lui et al., 1989; Hustveit and Setekleiv, 1993; Yamanoue et al., 1993; Hustveit, 1994). This is significant in that α-adrenergic and cholinergic receptors in the CNS may be the most significant factors in WCS and the rapid respiratory failure in F/FA overdose. One study in particular noted that naloxone was an effective

treatment of muscle rigidity induced by fentanyl in lower extremities and torso (Jerussi et al., 1987). The effect of naloxone on laryngospasm was not evaluated in this model. In addition, there was no discussion of the confounding use of the α-adrenergic receptor antagonist droperidol (Inapsine) as part of the anesthetic administered to the study animals. Other α-adrenergic antagonists, such as prazosin, inhibited F/FA-induced rigidity in animal studies, which possibly contradicts the proposed effect of naloxone against rigidity in the earlier study (Jerussi et al., 1987; Lui et al., 1989; Fu et al., 1997). Similarly, in humans droperidol decreased both chest wall rigidity and upper airway pressure with high-dose fentanyl (Stoelting et al., 1975; Yasuda et al., 1978). Yasuda et al. (1978) demonstrated that increases in tracheal pressure from fentanyl could be inhibited with droperidol, which they attributed to its α-adrenergic antagonist effects, but it failed to suppress a similar effect from morphine and N_2O (Stoelting et al., 1975; Yasuda et al., 1978), and the study by Stoelting et al. (1975) compared fentanyl directly against a formulation of droperidol and fentanyl (fentanyl 10 mcg/kg and droperidol 100 mcg/kg) in human subjects, which demonstrated clear decreases in chest wall rigidity and intrathoracic pressure with droperidol, but upper airway effects were not noted.

The significance of naloxone's ineffectiveness at antagonizing F/FA-induced muscle rigidity impacting respiratory mechanics in WCS is of critical importance if the laryngospasm component of WCS is the mechanism of rapid death from F/FAs, as suggested from autopsy and public health data involving F/FA overdose (Burns et al., 2016; Somerville et al., 2017). Mechanical failure of respiration with F/FA overdose usually develops less than 2 minutes after administration and presents prior to respiratory depression (Grell et al., 1970; Burns et al., 2016; Somerville et al., 2017). Naloxone can readily treat respiratory depression by antagonizing both $\mu1$- and $\mu2$-opioid receptors, the principle sites of fentanyl- and morphine-induced respiratory depression (Chen et al., 1996). However, a study by Yang et al. (1992) used high-dose alfentanil and electromyography (EMG) in spontaneously ventilating rats to demonstrate that the CNS sites of respiratory depression and muscle rigidity are clearly distinct in the rat model and that rapid failure of respiratory mechanics is correlated with the onset of muscle rigidity. Yang et al., 1992 concluded that: "alfentanil-induced muscle rigidity is mediated by a receptor population different from that mediating respiratory depression." Thus, naloxone may be ineffective in treating WCS (laryngospasm and respiratory muscle rigidity).

Naloxone, fentanyl, and morphine have overlapping binding affinities at μ-opioid receptors with K_i values of 1–3 nM, 0.7–1.9 nM, and 1–4 nM, respectively, although it is a common misconception that the potency of F/FAs is due to greater binding affinity at μ-opioid receptors (https://www.pharmacytimes.com). Fentanyl is more selective than morphine or naloxone for μ-opioid receptors over δ- or κ-opiate receptor subtypes, possibly explaining the reduced effectiveness of naloxone in antagonizing F/FA overdose (Chen et al., 1996). For example, naloxone has K_i values of 16 and 12 nM at the δ- and κ-opioid receptors, respectively, compared with almost no binding affinity of fentanyl at either subtype (Tam, 1985; Raynor et al., 1994). In addition, naloxone is less lipophilic than fentanyl (Maguire et al., 1992).

Naloxone has no apparent effects in opioid-naive or nonopioid-dependent patients in doses up to 1 mg/kg (Posner and Burke, 1985). However, significant changes in blood pressure and respiratory rate without a significant change in pulse are observed in healthy volunteers when given naloxone at doses of 2–4 mg/kg (Cohen et al., 1982; Ackerman et al., 1990). In active opioid users, rapidly administered naloxone causes laryngospasm, pulmonary edema, hemodynamic instability, and cardiac arrhythmias from significant increases in catecholamine release at doses as low as 0.4 mg (Ackerman et al., 1990; Clarke et al., 2005; Schumann et al., 2008; Horng et al., 2010; Fairbairn et al., 2017; Baumann et al., 2018). Therefore, high-dose naloxone for the treatment of WCS rigidity in F/FA overdose should be contraindicated.

To summarize, high-dose naloxone is unlikely to reverse the nonrespiratory depression symptoms of WCS and may instigate further harm to the patient (Schumann et al., 2008; Fairbairn et al., 2017; Baumann et al., 2018). As previously mentioned, high-dose naloxone can potentially increase noradrenergic output from the CNS, which can significantly worsen laryngospasm, induce cardiac arrhythmias, and trigger pulmonary edema (Horng et al., 2010). More importantly, the studies reviewed in *Fentanyl Pharmacology and Pharmacokinetics* and *Wooden Chest Syndrome* indicate that μ-opioid receptors may play a minor role in F/FA-induced WCS and that naloxone may be ineffective as a single treatment agent. The combination of a rapid-acting μ-receptor antagonist along with an antagonist of WCS (laryngospasm and respiratory muscle rigidity) could be a more suitable treatment. Next, we discuss combining α_1-adrenergic receptor antagonists and cholinergic agents with μ antagonists to concurrently treat respiratory depression and F/FA-induced WCS.

Preclinical Research: Central Role of the Locus Coeruleus and α-Adrenergic Receptors in FIMR/WCS

Opioid-induced abdominal wall rigidity with high-dose morphine was first noted by Hamilton and Cullen (1953), and later described by Freund et al. (1973), when morphine was combined with nitrous oxide. However, its underlying mechanism remains poorly understood. By the early 1970s, it was recognized that rigidity occurs even more consistently with fentanyl, and while fentanyl was still confined to the operating room, no formal drug development for the problem was pursued due to the lack of a perceived necessity. However, the need for new pharmacotherapies has become acute since fentanyl has made its way into the domain of illicit drug use, where it is difficult to assess the difference between rigidity and respiratory depression. The perception of need for new drug development to treat WCS is further clouded by the lack of understanding of the underlying mechanism. There has been very little systematic examination of underlying mechanisms for F/FA's nonanalgesic/anesthetic effects in over 20 years.

Rat models for FIMR, first developed in the 1980s, have suggested underlying mechanisms for the phenomenon. Systematic isolation of regions of the brainstem and spinal cord indicate that the LC and noradrenergic neurotransmission are part of the underlying mechanisms for FIMR (Weinger et al., 1988; Lui et al., 1989, 1990, 1993, 1995). Other mechanisms have been examined including cerulospinal glutamatergic pathways in the rat spinal cord, suggesting that N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors may be involved in FIMR and that intrathecal glutamate-inhibiting drugs or receptor antagonists may be effective in treating

FIMR (Fu et al., 1997). Similarly, cholinergic pathways in medullary nuclei were suggested by Hustveit (1994) in studies of underlying mechanisms of F/FA-induced muscle rigidity in centrally mediated anticholinergic syndrome (Hustveit, 1994). However, the most consistent experimental data set for the underlying mechanism of FIMR seems to involve the LC and noradrenergic pathways (Lui et al., 1989, 1990, 1993, 1995).

In a series of physiologic, pharmacologic, histochemical, and immunocytochemical experiments in the rat, fentanyl elicited muscular rigidity by activating high-density μ-opioid receptors in the LC and muscular contraction in the muscles of respiration (muscles of the chest wall, abdomen, and diaphragm) via coeruospinal fibers connected to spinal motor neurons terminating in the chest wall and abdomen. Sprague-Dawley rats were anesthetized with ketamine (120 mg/kg, i.p.), and EMG monitoring of gastrocnemius and rectus abdominus muscles was used to correlate fentanyl dose and muscle rigidity. Intravenous fentanyl (50 or 100 mcg/kg) consistently produced increases in EMG activity in both muscle groups, as did direct injection of fentanyl into the pontine nucleus (2.5 mcg/50 nl). The LC has a topographical fiber distribution, and FIMR was attenuated by bilateral electrolytic lesions of the middle and ventral portions of the LC at the level of the trigeminal motor nucleus. Sham-operated rats demonstrated FIMR (Lui et al., 1989, 1990, 1993, 1995).

The α_1-adrenergic receptor antagonist prazosin (125 mcg/kg, i.v.), administered 10 minutes prior to fentanyl, inhibited FIMR, supporting the role of noradrenergic transmission in FIMR. The prazosin-induced hypotensive effect was controlled for as the cause of rigidity by comparing its effects with the antihypertensive agent hydralazine (25 mg/kg, i.v.). Activation of EMG signaling was not elicited by prazosin alone. The origin of LC coeruospinal efferent fibers is primarily from the ventral-posterior portion of the pontine nuclei (pons) and terminates in noradrenergic varicosities in the ventral horn of the rat spinal cord (Loughlin et al., 1986). Noradrenergic outflow originating from the LC travels via these cerulopsinal fibers to terminate in these ventral horn cells (Lui et al., 1989, 1990, 1993, 1995). Ablation of the LC, ablation of the ventral portion of the pontine nucleus, or severing of these cerulospinal fibers eliminates FIMR (Lui et al., 1989). Antagonism of α_1-adrenergic receptors in the brainstem or spinal cord prevents the activation of noradrenergic neurons at the level LC and results in the inhibition of FIMR. Although fentanyl may induce muscular rigidity by activation of the LC and coeruospinal noradrenergic pathway via μ-opioid receptors in the LC, these findings were an unexpected outcome, which seem inconsistent with other studies that have documented the inhibitory effects of opioids on the sympathetic nervous system, leaving the mechanism in need of further exploration as described previously (Lui et al., 1989).

Sohn et al. (2005) later demonstrated that fentanyl antagonizes α_1-adrenergic receptor subtypes in pulmonary canine artery preparations, with a rank order of potency of $\alpha_{1B} > \alpha_{1A} >>> \alpha_{1D}$, but this does not explain the inhibition of FIMR by prazosin noted by Lui et al. (1989). Fentanyl's selective binding of α_1-adrenergic receptors may facilitate the binding of norepinephrine at α_{1D} subtypes, and the effectiveness of prazosin at inhibiting FIMR in the animal model may be a direct result of its significantly greater binding affinity for α_{1D} receptors than that of either fentanyl or norepinephrine (Sohn et al., 2005).

Chemical denervation was performed on spinal motor neurons innervating peripheral skeletal muscle in the hind limbs of rats (e.g., sacrococcygeus dorsi lateralis muscle) and served as a comparable quantitative index for EMG measures in previous studies using gastrocnemius and rectus abdominus muscles to study FIMR (Lui et al., 1989). Histochemistry and immunohistochemistry identified the sacrococcygeus dorsi lateralis muscle cells innervated by the ventral motor horn of the spinal cord. The noradrenergic nerve terminal neurotoxin DSP4 preferentially destroyed the spinal cord noradrenergic nerve terminals originating from the LC, and microinjection of fentanyl (2.5 mcg/50 nl) into the LC failed to produce the increase in EMG activity recorded in the sacrococcygeus dorsi lateralis muscle of the control group. These results further support the role of the coeruospinal noradrenergic pathway in FIMR. The study also demonstrated that FIMR is a centrally mediated effect and fentanyl has no direct effect on skeletal muscle (Lui et al., 1993), as demonstrated by Willette et al. (1987) on laryngeal muscles. Importantly, additional studies (Lui et al., 1989, 1993) demonstrated that intrathecal administration of the α_1-adrenergic receptor antagonist prazosin at the L3 lumbar level of the spinal cord in rats inhibits FIMR at doses of 5 and 10 nmol/10 μl (e.g., 16.82 and 33.64 pg) (Lui et al., 1995). However, the α_2-adrenergic antagonist yohimbine, given in equimolar amounts intrathecally, is ineffective at antagonizing FIMR, indicating specificity of the α_1-receptor effect. Intrathecal prazosin effectively inhibits both intravenous fentanyl (100 mcg/kg) and LC fentanyl microinjection (2.5 mcg/50 nl), and prazosin dose dependently inhibits EMG activation by 75%–90% over control values (fentanyl) and by ~80% for fentanyl LC microinjection (e.g., 10 nmol prazosin). In each case, the EMG activity was inhibited for 20 minutes postfentanyl dosing.

These results suggest that spinal α_1-adrenergic receptors in the noradrenergic coeruospinal pathway play a key role in FIMR. Additionally, α_2-adrenergic receptor stimulation reduces noradrenergic output from the presynaptic terminals of the LC, and subsequent studies by Weinger et al. (1989) demonstrated that α_2-adrenergic receptor agonism also inhibits FIMR, corroborating the role of noradrenaline in FIMR.

Animal studies do not easily translate to humans and have some significant limitations. The dose of the α_1-adrenergic antagonist in animal studies (e.g., 125–250 mcg/kg) is a lethal hypotensive dose in most humans; therefore, this limits any clinical utility without significant modification. However, the most significant limitation is that in most of these studies animals were anesthetized and had tracheostomies, which bypassed any involvement of the vocal cords and/or any possible demonstration of laryngospasm (Lui et al., 1989, 1990, 1993, 1995; Weinger et al., 1989). The size of the animal is the most obvious limitation to any type of study of the airway in real time. As previously mentioned in *Wooden Chest Syndrome*, in humans vocal cord closure and laryngospasm may be the most significant clinical event in WCS (Scamman, 1983; Bennett et al., 1997). The need for more accurate animal models of WCS and FIMR/vocal cord laryngospasm will be necessary in order to develop more effective prophylaxis and reversal therapeutics for F/FA overdose and poisoning from environmental exposure. The correlation

between the underlying mechanism of FIMR and increased LC noradrenergic activity has significant relevance to the development of effective therapeutics.

Fentanyl Pharmacology in Humans

WCS effect in humans primarily involves the upper airway with more modest contributions from thoraco-abdominal rigidity and can be immediately relieved by succinylcholine or tracheostomy (Scamman, 1983).

Incidence of Fentanyl-Induced Rigidity in the Controlled Surgical Setting. Current anesthesia literature suggests that WCS is an uncommon event (Çoruh et al., 2013). However, earlier clinical experiences with F/FAs in anesthesia were quite the contrary (Grell et al., 1970). When fentanyl was first introduced to the United States market, as previously mentioned, it revolutionized surgical anesthesia, but only after anesthesiologists learned the nuances of its administration. Early, large-scale clinical studies were the first to describe chest wall rigidity and airway compromise/laryngospasm induced by F/FAs and became a guiding principle in the training of anesthesiologists. The end result is that the incidence of WCS today is quite rare or taken for granted because anesthesiologists have become skilled at compensating for this risk, not unlike early experiences with morphine-induced histamine release and hypotension (Grell et al., 1970).

In 1970, Grell et al. (1970) published a landmark study that looked at the use of fentanyl as an anesthetic agent. Fentanyl was administered to a wide range of 500 surgical subjects (age, procedure type, and comorbidity classification) via intravenous infusion at 30 μg/min or a single bolus dose of 3.3 μg/kg. Of 500 subjects, 499 developed some degree of muscle rigidity, which presented within 60–90 seconds at a minimum dose of 250 μg or ∼3 μg/kg and persisted for 8–15 minutes. The conclusion from the Grell et al. (1970) study was that: "The onset and degree of muscle rigidity was directly related to the size of the dose and rapidity of injection." A significant limitation of the study was that the degree of rigidity was not quantified, since later studies by Streisand et al. (1993) and Bennett et al. (1997) demonstrated that muscle rigidity occurs with significantly higher doses of F/FAs (e.g., 15 μg/kg for fentanyl and the equivalent of 30 μg/kg of fentanyl with sufentanil 3 μg/kg). In fact, Streisand et al. (1993) only demonstrated rigidity and upper airway symptoms (e.g., difficulty with mask-assisted ventilation) in ∼50% of study subjects with a total dose of 15 μg/kg, and the latter study by Bennett et al. (1997) visually demonstrated vocal cord closure in 28 of 30 subjects with a sufentanil 3 μg/kg bolus over 2 minutes. While these doses of F/FAs would be considered high for routine cases in healthy individuals having elective surgeries such as hernia repairs and cholecystectomies, they have been commonly and safely used in patients with severe cardiovascular disease for procedures such as heart valve replacement and coronary artery bypass grafting (Streisand et al., 1993; Bennett et al., 1997). However, what should remain clear is that the size of the dose becomes relative to the speed of injection, which remains a significant factor in the incidence of complications from F/FA overdose in the current opioid crisis. In the controlled setting of clinical practice, low doses of F/FAs rarely cause WCS.

Fentanyl-Induced Rigidity and Loss of Consciousness. Grell et al. (1970) demonstrated that muscle rigidity induced by fentanyl has an exceedingly frequent incidence following rapid intravenous injection and occurs at a minimum threshold dose of approximately 250 mcg. However, the study did not measure the concurrent incidence of loss of consciousness with muscle rigidity. In an effort to understand the CNS mechanism underlying WCS, Streisand et al. (1993) looked at the incidence and duration of rigidity and loss of consciousness associated with plasma concentrations. Fentanyl was administered to 12 healthy volunteer subjects at an infusion rate of 150 mcg/min to a total dose of 15 mcg/kg (e.g., ∼1 mg/70 kg). The result was muscle rigidity that occurred in six out of 12 subjects (50%), starting at a plasma fentanyl concentration of 21.5 ± 4.4 ng/ml (range 16–28 ng/ml), which resolved at 6.9 ± 1.5 ng/ml (range 5.2–8.7 ng/ml). Rigidity started at 3 ± 0.9 minutes (range 1–4 minutes) after the peak plasma fentanyl concentration, and lasted for 11.5 ± 5.8 minutes (range 7–23 minutes). All subjects who developed rigidity were apneic, unresponsive, and had no recall of commands to breathe or positive pressure ventilation. Essentially, WCS coincided with unconsciousness of subjects in the experiment. The study concluded, "These findings support the hypothesis that unconsciousness occurs in the unstimulated subject during fentanyl-induced apnea and rigidity" (Streisand et al., 1993).

The results of this study are significant and should be emphasized for several reasons: the study correlates the occurrence of WCS with a loss of consciousness and demonstrates that the plasma concentrations of fentanyl are not significantly different between those subjects with rigidity and those subjects without rigidity. Interestingly, there were no significant changes in recorded hemodynamics among the subjects, indicating that the alteration in level of consciousness in these subjects may have been more of a centrally mediated effect rather than a cerebral perfusion defect or cardiovascular event. A rapid loss of consciousness associated with the FIRMR in this study could have several physiologic explanations. A sudden hemodynamic shift such as a fall in cardiac output or cerebral perfusion pressure can easily cause alterations or loss of consciousness. However, Streisand et al. (1993) found no fundamental differences in hemodynamics between those subjects who became unconscious and those that did not. This suggests a centrally mediated effect resulting from rapid disruption of thalamo-cortical connectivity, as has been previously demonstrated by electroencephalogram monitoring studies in human subjects who lost consciousness after fentanyl administration (Uhrig et al., 2014). The rapid loss of consciousness in the aforementioned studies is consistent with eyewitness and survivor accounts of F/FA overdose and WCS (Somerville et al., 2017).

Respiratory Depression or WCS. Large-scale human trials with fentanyl have noted that while it may take 7–9 minutes for pulmonary/respiratory mechanics to decline by 50% (e.g., respiratory rate, tidal volume, and minute volume), muscle rigidity routinely occurs 60–90 seconds at a minimum dose of 250 mcg in adults (Grell et al., 1970). Other smaller scale studies have clearly demonstrated that severe rigidity occurs prior to and more rapidly than significant respiratory depression in study subjects receiving high-dose fentanyl infusions of 150 mcg/min up to 15 mcg/kg (Grell et al., 1970). Morphine and heroin by comparison are not known to cause airway compromise due to rigidity (Hamilton and Cullen, 1953; Jenkins et al., 1994) and have a significantly

greater time to CNS entry and onset (e.g., 19 minutes for morphine to reach 80% of peak effect) (Owen et al., 1983).

In summary, neither heroin nor morphine induces significant airway compromise in humans due to rigidity as a single agent (Sokoll et al., 1972; Freund et al., 1973). By comparison, F/FAs as single agents produce muscle rigidity and airway compromise rapidly, and these effects are consistently correlated with dose and rate of administration.

Public Health Data

Somerville et al. (2017) examined the characteristics of fentanyl overdose in Massachusetts from 2014 to 2016. The study compiled data consisting of county medical examiner chart reviews of 196 opioid-confirmed deaths from October 2014 to March 2015, summary reviews of death scene evidence, and 64 fentanyl-related overdose survivors and witness interviews. Two-thirds of the 196 decedents were confirmed positive for fentanyl by forensic tissue analysis. Death scene evidence in these cases revealed consistent reports of victims/bodies found with injection needles and tourniquets still in place and syringes found in hand, indicating rapid death after intravenous injection. Witnesses of fentanyl overdose reported seeing a rapid loss of consciousness following intravenous injection, sudden rigidity or seizure-like activity, rapid onset of facial and oral mucosa cyanosis (blue lips), and unusual gurgling sounds, and reported that multiple doses of naloxone were administered in the majority of cases where fentanyl was suspected (e.g., over 80% of respondents used two doses of naloxone if it was available). The conclusion of the study was that evidence from over one-third of medical examiner charts and 75% of interview respondents demonstrated that fentanyl overdose can begin suddenly, progress to death rapidly, and manifest atypical physical symptoms compared with heroin overdose. This study yields public health data from an endemic fentanyl overdose community, and reiterating studies described previously concluded that death from fentanyl is rapid and atypical compared with overdose death seen with heroin. More specifically, this study also yields data supporting the observation that fentanyl overdose is fundamentally different from respiratory depression seen with heroin and morphine-derived alkaloid overdose and may include vascular compromise and laryngospasm (hence, the aforementioned facial cyanosis and gurgling sounds) (Somerville et al., 2017).

Fentanyl Metabolism in Humans

In a study that examined intravenous fentanyl kinetics, McClain and Hug (1980) administered [³H]fentanyl to seven healthy adult male subjects at doses of 3.2 and 6.4 μg/kg by intravenous bolus over a 90-second interval. The metabolite norfentanyl appeared in the plasma at a concentration of ~3 ng/ml, at 90–120 seconds after intravenous bolus, representing 18.9% of the total [³H]fentanyl dose in plasma. This study established the rapid order in which fentanyl is metabolized and the time scale for the appearance of its hepatic metabolite, norfentanyl (McClain and Hug, 1980).

Under normal physiologic conditions, fentanyl is rapidly oxidized through N-dealkylation at the piperidine ring to the inactive metabolite norfentanyl by the CYP3A4 catalytic enzyme in the human liver (Feierman and Lasker, 1996;

Tateishi et al., 1996). As a result, potential drug interactions may occur when fentanyl is given concurrently with other drugs that are substrates or inhibitors of the enzyme (e.g., alfuzosin, alprazolam, carbamazepine, budesonide, cyclosporine, dexamethasone, lovastatin, etc.) (Stanley, 2014). Other minor metabolites are also created through other pathways; however, all metabolites are inactive, and only a small amount of fentanyl (~8%–10%) is renally cleared (McClain and Hug, 1980; Mather, 1983; Poklis, 1995). Fentanyl analogs such as α-methylfentanyl, alfentanil, butyrfentanyl, carfentanil, and sufentanil are also primarily metabolized via the CYP3A4 hepatic pathway, generating N-dealkylated metabolites that are primarily inactive (Guitton et al., 1997; Sato et al., 2010; Feasel et al., 2016).

Autopsy Data

In a study by Burns et al. (2016) in Ohio, 48 deaths from fentanyl from January to September 2015 were identified by the county medical examiner/county coroner's office using quantitative analysis by liquid chromatography and mass spectrometry on tissue from the decedents. The coroner found that of the 48 fentanyl deaths, 42% (20/48) of the decedents had no measurable concentrations of the main metabolite, norfentanyl, in tissue, while 52% (25/48) showed less than 1 ng/ml of detectable norfentanyl. Mean fentanyl concentrations in the decedents were 12.5 ng/ml with a range from 0.5 to >40 ng/ml. Mean norfentanyl concentrations were 1.9 ng/ml (range 0 –8.3 ng/ml); however, elevated fentanyl levels had no correlation to rises in norfentanyl levels. Some of the decedents had levels of fentanyl as high as 22 ng/ml and had no detectable levels of norfentanyl.

McClain and Hug (1980) established that intravenous fentanyl is rapidly metabolized by the liver to norfentanyl, is detectable in serum in approximately 120 seconds after intravenous administration, and serum levels of fentanyl for surgical analgesia range from ~1 to 15 ng/ml. It should be emphasized that the lack of detectable or significant metabolite levels of norfentanyl in the decedents studied by Burns et al. (2016) suggests that these individuals expired rapidly (e.g., in approximately 2 to 3 minutes) and had a rapid decline in the cardiac output necessary to maintain hepatic perfusion and metabolism (Burns et al., 2016). The presentation of WCS in F/FA overdose, with its combination of rapid onset of respiratory failure, decreased cardiac output, and perfusion is a very different physical presentation than the gradual onset of respiratory depression and cardiac arrest seen with heroin and seems clinically consistent with death scene evidence in this study.

Neurochemistry and Neuroanatomy in WCS

GABA Interneurons. GABA interneurons are part of an inhibitory network throughout the CNS and are particularly abundant in the LC, where norepinephrine release from the presynaptic terminal is inhibited by GABA efferent signaling (Aston-Jones et al., 2004; Jin et al., 2016; Breton-Provencher and Sur, 2019). Importantly, the LC maintains basal skeletal muscle tone in the axial skeleton and torso via noradrenergic activation of spinal motor neurons. Inhibition of GABA interneurons allows for increases in skeletal muscle tone through increased noradrenergic activity in the LC (Nakamura et al., 2002). Conversely, activation of GABA interneurons can cause

a decrease in noradrenergic tone in the adjacent subcoeruleus, as occurs in rapid eye movement sleep or cataplexy, demonstrating that the loss of noradrenergic tone inhibits contractility of all major muscle groups except muscles of respiration and those involved in rapid eye movement (Fraigne et al., 2015). At the level of the GABA interneuron, fentanyl and morphine stimulate μ-opioid receptors and inhibit GABAergic signaling and neurotransmission in several places in the brain, including the periaqueductal gray (Vaughan and Christie, 1997), hippocampus (Alreja et al., 2000), and dorsal raphe nucleus (Jolas and Aghajanian, 1997). Although, there is no description of fentanyl-induced GABA interneuron inhibition in the LC itself, reports demonstrate fentanyl binding to GABA interneuron μ-opioid receptors and inhibition of neurotransmitter output (Nakamura et al., 2002; Griffioen et al., 2004). Recent evidence also indicates the presence of these GABA interneurons in the LC (Breton-Provencher and Sur, 2019). The binding of fentanyl or morphine to μ-opioid receptors located on GABA interneurons in the LC should inhibit interneuron activation and paradoxically increase the release of norepinephrine at the presynaptic terminal, activating spinal motor neurons in the ventral horn of the spinal cord, and followed by postganglionic fibers terminating in skeletal muscle (Lui et al., 1989). What has not been clearly established is the degree to which μ-opioid receptor binding of GABA interneurons supercede or affect the hyperpolarization of LC neuronal cells by μ-opioid receptor agonists (Chiu et al., 1993). However, what is known from electron microscopy and immunocytochemical studies of the LC is that a significant percentage of cells within the dense body of the LC stain positive for GABA, indicating the presence of parasynaptic GABA interneurons and the potential for integrating LC function (Aston-Jones et al., 2004). In addition, electron microscopy studies suggest that μ-opioid ligands act mainly parasynaptically on LC neurons, with approximately 5% of labeled μ receptors associated with axoglial interfaces, indicating that a minor action of μ opioids in the locus may be presynaptic (Moyse et al., 1997).

In a series of experiments using a spinal cord ischemia model in rats, Nakamura et al. (2002) used intrathecal morphine to cause spastic paraparesis. For a reversible injury model, anesthetized rats had intrathecal catheters placed along with an arterial occlusion catheter used to generate spinal cord ischemia on inflation. Motor function was quantified by ambulation after reperfusion with a motor deficit index scale. The presence of spasticity or flaccidity was determined by the presence of an exaggerated flexion response to a pinch of the hind paw. The study used the GABA$_A$ agonist muscimol (intrathecal dose of 0.3, 1, or 3 μg per 10 μl) and the GABA$_B$ agonist baclofen (intrathecal dose of 1, 3, or 10 μg per 10 μl) to demonstrate that GABA agonists attenuate the spastic paraparesis induced by intrathecal morphine (intrathecal dose of 30 mcg) after a noninjurious interval of spinal cord ischemia. The agonists proved ineffective. However, the GABA antagonists bicuculline (intrathecal dose of 4 mcg) and 5-AA (intrathecal dose of 70 mcg) produced a dose-dependent increase in the motor deficit index similar to morphine (intrathecal dose of 10 mcg), and the combination of morphine and GABA antagonists (bicuculline or 5-AA) also produced a further increase in the motor deficit index. In addition, morphine and GABA antagonists had a synergistic, not an additive, interaction in producing spastic paraparesis. Thus, there are μ-opioid

receptors on GABA interneurons in the spinal cord; and their stimulation inhibits GABA interneuron signaling, resulting in increased muscle contractility (Nakamura et al., 2002).

Similarly, fentanyl binds μ-opioid receptors on GABA interneurons and inhibits GABA interneuron afferents (Griffioen et al., 2004). In an experiment using fluorescent tracers, infrared cameras, and whole-cell patch-clamp electrophysiology, parasympathetic cardiac vagal neurons were identified and stimulated with fentanyl. This demonstrated the inhibition of GABA neurotransmission to cardiac vagal neurons, resulting in increased parasympathetic output to the heart via the vagus nerve, which may be the underlying mechanism of opioid-induced bradycardia (Griffioen et al., 2004). Fentanyl was tested at concentrations of 0.1, 0.5, 1 and 5 μM, and the amplitude was significantly reduced by 25%–48% with maximal inhibition at 0.1 μM or ~50 mcg/kg. The results indicate that fentanyl reduces the frequency and amplitude of GABAergic inhibitory postsynaptic currents at pre- and postsynaptic sites in cardiac vagal neurons and the effects are abolished by the μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (Chieng et al., 1996; Griffioen et al., 2004). Thus, fentanyl inhibits GABA interneurons in the nucleus ambiguus by binding μ-opioid receptors on GABA interneurons innervating cardiac vagal neurons (Griffioen et al., 2004).

Fentanyl Effects on Catecholamines In Vitro and In Vivo. As previously described, fentanyl differs from morphine since the former has considerably increased lipid solubility, greater selectivity for μ-opioid receptors over κ and δ, but has similar binding affinity as morphine on μ-opioid receptors. In addition, fentanyl, but not morphine, is capable of acting as a norepinephrine reuptake inhibitor (Mather, 1983; Atcheson et al., 1993). High-dose fentanyl (e.g., 1–100 mM) blocks reuptake of [^{3}H]norepinephrine into human neuroblastoma and rat pheochromocytoma cells (Atcheson et al., 1993). Morphine exhibited no such effect at any concentration tested. It is also important to point out that the dose range of fentanyl used in these experiments may have physiologic relevance in humans based on calculated brain lipid opioid concentrations (fentanyl) calculated from plasma opiate concentrations, where 6.9 ng/ml of plasma fentanyl corresponds to a concentration of 2.96×10^{-3} M in brain lipid (Freund et al., 1973; Stone and DiFazio, 1988; Hustveit and Setekleiv, 1993; Yamanoue et al., 1993; Sohn et al., 2005).

Notably, this effect on human neuroblastoma and rat pheochromocytoma cells was not antagonized by naloxone, suggesting the site of action for fentanyl's effect does not involve μ receptors (Atcheson et al., 1993). Subsequent studies with similar models of human SH-SY5Y neuroblastoma cells have demonstrated the presence of endogenous muscarinic (M) 3 receptor sites on these cells, where fentanyl might act as a potential antagonist (Yamanoue et al., 1993; Bundey and Nahorski, 2001). Animal studies have also demonstrated evidence of both α_2- and α_1-adrenergic receptors (specifically, the α_{1D} subtype) on neuroblastoma cells where fentanyl has α_1-subtype antagonist effects (Baron and Siegel, 1989; Wenner et al., 2016). This study was performed to better understand the relationship between neuronal amine uptake and analgesia and was not analyzed or performed in the context of FIMR or WCS. However, the study demonstrates a key difference between fentanyl and morphine, implicating

a mechanism for fentanyl-enhanced noradrenergic activity outside of μ-opioid receptor binding. This appears to be consistent with fentanyl-induced noradrenergic activity in the LC leading to FIMR in the animal model and has been an ongoing unexplained phenomenon, which contradicts the well-established concept from in vitro rat brain slice voltammetry studies that opiates directly inhibit norepinephrine release from LC neurons. In this in vitro voltammetry model, opioids bind μ receptors on LC neurons, hyperpolarize neuronal cell membranes, and result in a subsequent decrease in cell firing and norepinephrine release (Aghajanian, 1982; North and Williams, 1985; Lui et al., 1989; Christie, 1991; Chiu et al., 1993).

Conversely, in vivo animal (sheep) and human studies using intravenous morphine (1 mg/kg), fentanyl (10 mcg/kg), and indwelling intrathecal spinal catheters for serial cerebrospinal fluid (CSF) sampling, have shown increases in CSF levels of norepinephrine and acetylcholine three to four times above baseline levels within 10 minutes of injection with morphine (1 mg/kg), suggesting that fentanyl (e.g., ~680 times more lipophilic than morphine) or a more lipophilic analog would have a similar centralized effect in a fraction of this time (Bouaziz et al., 1996). Additionally, the rate of infusion in this series of experiments, particularly for fentanyl (e.g., 10 mcg/kg, i.v., infused over 10 minutes), could explain the slow onset of catecholamine rise and is juxtaposed to real-world rapid injection practices commonly seen in F/FA-related overdose. Also noteworthy in this study (Fig. 1) is that the first measurement occurred only 10 minutes after injection and showed a >200% increase in norepinephrine levels, suggesting the possibility that a significant increase may have gone unmeasured prior to the first lumbar CSF sample obtained, and where travel time from brain to distal spinal cord and CSF production must be considered to accurately assess neurotransmitter concentration (Bouaziz et al., 1996). Similar considerations must be made regarding lipophilicity and CSF travel time for the single human subject in the study where drug enters the ventricles of the brain from the vascular space and must travel distally to the lumbar region (e.g., CSF volume in adult human of 1.5–2 ml/kg, production rate of 25 ml/h, and flow rate of 0.3 ml/min) (Bouaziz et al., 1996; Wright et al., 2012; Brinker et al., 2014). In humans, the clinical onset time for F/FA CNS effects is well documented for occurring in a single circulation time with intravenous injection (e.g., 60–90 seconds), suggesting rapid distribution in CSF with CNS entry and transport beginning in ventricles after systemic arterial delivery (McClain and Hug, 1980; Mather, 1983; Bouaziz et al., 1996). More specifically and related to the significance of increased norepinephrine and acetylcholine with opioids, iontophoretic studies of the LC demonstrate that both muscarinic and nicotinic receptors are present in rat and human LC, acetylcholine (ACh) muscarinic receptor (M2 subtype) binding increases norepinephrine release in LC, and the LC itself is rich in acetylcholinesterase (Albanese and Butcher, 1980; Egan et al., 1983; Egan and North, 1985, 1986). The LC receives cholinergic input from cells in the neighboring pontine reticular formation where ACh release may be stimulated by opioids and may involve GABA interneurons or M3 subtype binding in the case of fentanyl (McNaughton and Mason, 1980; Yamanoue et al., 1993).

The contradictory results between the in vitro voltammetry studies and in vivo studies are explained to some degree by this existing literature and help to fulfill some of the essential prerequisites for the activation of skeletal muscle (e.g., chest wall and laryngeal muscles) in WCS via the release of ACh and subsequent release of norepinephrine in the LC by F/FAs (Rasmussen and Jacobs, 1985; Gaumann et al., 1988; Bouaziz et al., 1996). What should be evident at this point is that F/FAs have off-target binding sites (muscarinic and α_1-adrenergic receptors) that may function independently of μ-opioid receptor activation, or alternatively are minimally affected by μ-opioid receptor antagonism, once these muscarinic or α-adrenergic receptors are activated in a secondary capacity from μ-opioid receptor agonism. In the former case, we would expect there to be no effects of naloxone since it has no direct binding capacity at muscarinic or α-adrenergic receptors (Jarrott et al., 1979; Huchet et al., 1986; Hustveit and Setekleiv, 1993); in the latter case, only a partial effect with high-dose naloxone would be expected, as demonstrated by Willette et al. (1982, 1987). In the latter case, where naloxone has a partial effect, the efficacy of naloxone would depend on the balance of these offsite receptors with respect to their dominance, or lack thereof, for μ-opioid receptor effects.

To summarize the case of catecholamine release with F/FAs, there are several mechanisms that could have synergistic effects and increase the plausibility of both cholinergic and noradrenergic mechanisms underlying WCS. Although fentanyl and F/FAs are clearly not agonists at sympathetic receptors, they may increase the release of norepinephrine at the LC via GABA interneuron inhibition, increase the release of ACh for LC cholinergic receptor stimulation, and act as norepinephrine reuptake inhibitors in the LC at the presynaptic terminal. These combined mechanisms would allow for significant sympathetic effects for F/FAs without being direct agonists of α-adrenergic receptors or directly increasing the firing of LC neurons (Sriraman et al., 2007). Additionally, as mentioned previously, Sohn et al. (2005) demonstrated that fentanyl binds α_1-adrenergic receptor subtypes with a rank order of B > A >>> D, which may facilitate selective subtype binding of α_1-adrenergic receptors by norepinephrine and serve to concentrate its effects on the α_{1D}-receptor subtype. F/FA facilitation of subtype binding and its potential for significant effects on cardiovascular and hepatic systems will be discussed in *Human Physiology*.

Fentanyl Effects on Catecholaminergic Activity in Human Subjects. Several clinical studies further support the hypothesis that WCS is mediated by noradrenergic function and that fentanyl increases norepinephrine release in the CNS and periphery. Hicks et al. (1981) studied 10 adult patients with ischemic heart disease who were induced with a range of high-dose fentanyl for cardiac surgery. Plasma samples of catecholamines were assayed radio-enzymatically for norepinephrine, epinephrine, and dopamine. Norepinephrine levels were significantly elevated after 15 mcg/kg of fentanyl, remained elevated after 30 mcg/kg of fentanyl, but declined toward baseline values at the 50 mcg/kg dose. Also notable was a dose-dependent decrease in left ventricular function at doses greater than 30 mcg/kg of fentanyl. The catecholamine response in the study was interpreted as either a dose- or time-related effect of fentanyl or the stress of induction of anesthesia, rather than the alternative that high-dose fentanyl elevates norepinephrine levels. The study did not monitor or correlate FIRMR or laryngospasm, did not

control for the stress of induction, and also did not offer an explanation for the seemingly biphasic response of norepinephrine levels to increasing doses of fentanyl (Hicks et al., 1981).

In a study of 50 healthy adult females having total abdominal hysterectomy under general anesthesia, patients received either 2 or 4 mcg/kg of fentanyl 5 minutes before or 5 minutes after surgical incision with serial catecholamines measured 1 minute prior to opioid dose and serially thereafter for 20 minutes. Serum epinephrine levels decreased significantly at several time points and more so with the higher dose; however, plasma norepinephrine was elevated after fentanyl administration irrespective of time to induction or dose levels and was not attenuated at higher doses (Hori and Nagasaka, 2002). Unlike the study described previously (Hicks et al., 1981), this latter study controlled for the stress of anesthesia induction and incision and similarly yielded a dose-dependent increase in norepinephrine levels to fentanyl at lower doses. Although these two studies were not monitoring for WCS, they each demonstrated that norepinephrine levels were elevated by fentanyl, consistent with the results of in vivo experiments demonstrating that fentanyl facilitates Ach-mediated release of norepinephrine and reinforces a plausible underlying mechanism for WCS (Payne et al., 1986; Bouaziz et al., 1996).

Catecholamine Release and Laryngospasm. Animal studies suggest that FIMR muscle rigidity in the chest wall and limbs is mediated by noradrenergic receptors in the locus coeruleus, while vocal cord closure/laryngospasm in the intrinsic muscles of the larynx associated with high-dose F/FAs is cholinergically driven and involves the balance of sympathetic and parasympathetic innervation to the intrinsic muscles of the larynx (Willette et al., 1982, 1987; Lui et al., 1989; Lalley, 2003). Literature from human studies indirectly supports the possibility that similar mechanisms control vocal cord closure/laryngospasm (Yasuda et al., 1978; Scamman, 1983; Streisand et al., 1993; Bouaziz et al., 1996; Bennett et al., 1997; Lin et al., 2004; Fodale et al., 2005; Varney et al., 2009; Horng et al., 2010; El Baissari et al., 2014). However, to date there has been no direct demonstration in humans or animals that fentanyl facilitates noradrenergic or cholinergic signaling in the laryngospasm component of WCS. The data presented subsequently examine the existing support for that hypothesis.

The most suggestive clinical study to date was performed by Yasuda et al. (1978), in which the authors measured and compared tracheal pressure increases induced by either morphine (0.5 mg/kg) or fentanyl (0.006 mg/kg) in healthy, adult human subjects (Yasuda et al., 1978). The study involved endotracheal intubation of the patients with a fluid-filled pilot balloon at the level of the vocal cords, which could be used to accurately measure tracheal pressure in real time as equianalgesic doses of the opioids were administered. Both opioids increased tracheal pressure significantly from baseline (morphine increased by 27% and fentanyl increased by 44% above control). Interestingly, morphine demonstrated an initial 8% decrease in tracheal pressure that lasted ~2 minutes, whereas fentanyl had an ~14% increase in tracheal pressure within 30 seconds of administration. Also of significance, the tracheal constrictive effect of fentanyl was blocked by pretreatment with the neuroleptic agent droperidol (0.25 mg/kg) and contrasted with the tracheal effects of morphine, which were blocked by pretreatment with the anticholinergic muscarinic

antagonist atropine (0.5 mg). Droperidol has α_1-adrenergic antagonist effects and significant anticholinergic effects that are less defined (Stoelting et al., 1975; Boros et al., 1984; Nashan et al., 1984). Atropine had no effect on fentanyl action, indicating that each of these opioids has a unique mechanism of action on the intrinsic laryngeal muscles and smooth muscle of the trachea (Yasuda et al., 1978). A possible confounding variable in the study is that 70% N_2O was used as part of the anesthetic, which is known to cause severe muscle rigidity and airway compromise when combined with morphine (Sokoll et al., 1972; Freund et al., 1973). This confounder for morphine may indicate a further distinction from the underlying mechanisms of F/FAs.

Freund et al. (1973) demonstrated in adult human subjects that moderate- to high-dose morphine (e.g., 2 mg/kg, i.v.) can cause some degree of abdominal wall rigidity and a decrease in thoracic wall compliance as measured by EMG and airway pressures, but the addition of 70% N_2O/30% O_2 in spontaneously ventilating patients caused severe board-like rigidity with immediate airway compromise (could not be mask ventilated) (Freund et al., 1973). Six of the nine adult subjects in the study required endotracheal intubation and all subjects had an 8-fold increase in abdominal wall EMG activity within 3 minutes of N_2O administration (Freund et al., 1973). The muscle rigidity was completely reversed in six of the six intubated patients with 3 mg/kg of intravenous thiopental. It is notable that thiopental is a barbiturate sedative hypnotic agent that is well-known for its supraspinal effects, indicating a central origin for the rigidity. Additionally, spinal monosynaptic reflexes in the study subjects showed significant decrease in amplitude, indicating that the abdominal rigidity and airway compromise induced by morphine and N_2O was unrelated to spinal motoneuron excitability (Freund et al., 1973). Regarding N_2O effects, although the authors could offer no explanation at the time for the dramatic increase in muscle rigidity and airway effects, subsequent research on the molecular mechanisms of N_2O in a rat model have shown significant increases in norepinephrine release (>50% from baseline) from the LC triggered by 60%–70% N_2O (Fukuhara et al., 1998). In the rat model of FIMR demonstrated by Lui et al. (1989), the presumed increase in norepinephrine release from the LC induced by fentanyl caused similar rigidity effects as when N_2O was combined with morphine in humans (Freund, 1974). Although norepinephrine levels were not measured directly by Lui et al. (1989), they did not use N_2O as part of the anesthetic for these experiments. The inhibition of the muscle rigidity induced by F/FAs (fentanyl and alfentanil) with the α_1-adrenergic antagonist prazosin and the α_2-adrenergic agonist clonidine strongly suggests the involvement of elevated levels of norepinephrine in the LC as a significant component of muscle rigidity induced by F/FAs.

Perhaps the most significant observation to be made from this study in the context of F/FAs is that it helps to distinguish the effects of fentanyl and morphine from each other. The study clearly demonstrates that morphine itself has no significant impact on upper airway function. Specifically, it does not cause either laryngospasm or vocal cord closure on its own, but requires N_2O for the upper airway to become compromised. Conversely, fentanyl and its analogs can cause laryngospasm/vocal cord closure as single agents and seem to be significantly impacted by α-adrenergic receptor activity and norepinephrine levels in the LC (Scamman, 1983; Lui

et al., 1989; Bennett et al., 1997). The work by Freund et al. (1973) establishes supportive evidence that the laryngospasm component of WCS appears to be unique to F/FAs, which was later supported by Scamman (1983).

A more recent case study examined the occurrence of severe vocal cord closure/laryngospasm with the use of tramadol, a relatively weak μ-opioid receptor agonist that also acts as a monoamine reuptake inhibitor. The case report (Fodale et al., 2005) described an elderly male with mild age-related compromise of hepatic metabolism, who following an intravenous tramadol dose (e.g., 2 mg/kg bolus followed by 0.4 mg/kg per hour) experienced a severe episode of vocal cord laryngospasm. Fiberoptic bronchoscopy of the glottis documented vocal cord closure without edema, indicating laryngospasm presumably induced by the opioid since he was not taking any other medications known to induce laryngospasm. The episode gradually resolved over ~25 minutes after 1 mg of intravenous naloxone was administered (Fodale et al., 2005). This study is significant in demonstrating that tramadol, a weak μ agonist (e.g., morphine is 6000 times more potent), produced fentanyl-like effects, possibly indicating that both drugs act as monoamine reuptake inhibitors in the LC (Lui et al., 1989; Atcheson et al., 1993; Halfpenny et al., 1999; Fodale et al., 2005).

Supporting this hypothesis, in rat brain slice real-time voltammetry of norepinephrine efflux and reuptake, the (−) tramadol enantiomer induced significant efflux and blocked norepinephrine reuptake in the LC (Halfpenny et al., 1999). It is also significant that the short half-life of naloxone (e.g., 30–60 minutes vs. tramadol half-life of 360 minutes) and its rapid egress from CNS after entry due to its high lipophilicity (e.g., 15-fold greater than morphine), indicates that it is usually removed from the site of action in the CNS within 10 minutes (Dean et al., 2009). In fact, 1 mg of naloxone (15 mcg/kg dose) in the case subject (~65 kg) would be expected to produce a ~50% occupancy of μ receptors, which makes it unlikely that the reversal of the laryngospasm was due to naloxone (Clarke et al., 2005).

Vocal cord dysfunction (VCD) is a pulmonary condition that presents with airway compromise similar to asthma, but is unrelated and unresponsive to asthma therapeutics (e.g., bronchodilators, nebulizers, epinephrine, and inhaled steroids). VCD is an uncommon condition characterized by paradoxical vocal fold motion during inspiration, which leads to stridor, tachypnea, chest tightness, and a feeling of suffocation or air hunger (Mikita and Mikita, 2006; Morris and Christopher, 2010; see "It Is Hard to Breathe when Your Vocal Cords Don't Work!" (https://www.emrap.org/episode/february2015/airwaycorner)]. VCD is most commonly observed among conditioned athletes, and is thought to emerge as a symptom of autonomic dysfunction that imbalances vagal innervation at intrinsic muscles of the larynx during high aerobic stress. VCD has responded inconsistently to various therapeutics including cholinergic, anticholinergic, and noradrenergic agents (Morris et al., 1999). Success with therapeutic strategies for reducing sympathetic or adrenergic innervation to the intrinsic muscles of the larynx for treatment of VCD reinforces the syndrome as a sympathetic phenomenon. Varney et al. (2009) used low-dose amitriptyline (e.g., 20 mg), the sedating tricyclic reuptake inhibitor, to gradually treat VCD over several weeks. As the sedative effects increased over time for the study participants, sleep improved and symptoms of VCD improved in ~88% of the 62 patients studied (Varney et al., 2009; Ramakrishna and Subhash, 2012). It is quite possible that the treatment mechanism involves amitriptyline's downregulation of α_1-adrenergic receptors (Ramakrishna and Subhash, 2012).

The relationship between catecholamine release and laryngospasm is readily observed in the routine securing of the airway with an endotracheal tube during the induction of general anesthesia. Laryngospasm, hypertension, and cardiac arrhythmias commonly result from inadequate blockade of the sympathetic nervous system during instrumentation of the airway and point to the influence of adrenergic innervation in addition to the cholinergically mediated innervation of laryngeal musculature. Specifically, noradrenergic nerve fibers directly innervate the intrinsic muscles of the larynx via laryngeal nerves in animals and humans and are contributed to vagal nerves via the superior cervical and middle cervical ganglia (Hisa, 1982; Hisa et al., 1982; Basterra et al., 1989; Yoshida et al., 1992). It is also important to note that prior to intubation or laryngeal mask placement anesthesiologists will administer opioids along with sedative hypnotics (such as propofol) to blunt the sympathetic activation that occurs with instrumentation of the airway. If opioids are not concurrently administered, a severe adrenergic response is likely to occur. The dose of propofol, an alkylphenol derivative that acts at the GABA$_A$ receptor, provides sedation and amnesia but no analgesia. The necessary dose of propofol as a single agent to blunt an adverse sympathetic response is two to three times greater than when used with an opioid and a paralytic, and likely to cause severe hypotension and inadequate blockade of laryngospasm (Koenig et al., 2015). Paradoxically, the blunting of sympathetic airway responses with opioids is balanced against the clinical phenomenon of fentanyl-induced cough, which is thought to result from centrally mediated sympathetic inhibition and vagal predominance in the laryngeal muscle innervation (El Baissari et al., 2014). The effect can be benign or produce life-threatening laryngospasm. It occurs immediately after injection of fentanyl and can occur at low doses, but seems to share a similar underlying mechanism with the severe laryngospasm seen in WCS since both seem to be managed successfully with α-adrenergic and anticholinergic agents (Basterra et al., 1989; Yoshida et al., 1992; Lin et al., 2004; Horng et al., 2010; El Baissari et al., 2014).

Finally, when discussing muscle rigidity induced by F/FAs, it is important to note that centrally mediated skeletal muscle rigidity has a number of possible neurotransmitter-based etiologies including endogenous deficiencies, pharmacological induction (e.g., selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, antipsychotics, and anticholinergics), and neurologic conditions (e.g., Parkinson's disease and stiff person syndrome) (Shanthanna, 2010). In fact, there have been reports in the anesthesia literature of serotonin and neuroleptic malignant syndromes occurring when selective serotonin reuptake inhibitors and neuroleptics/antipsychotics are used with anesthetic drugs such as fentanyl, which has been shown to have some proserotonergic effects (Gollapudy et al., 2012). However, this may be due to multiple concurrent neurotransmitter disruptions resulting from polypharmacy, since fentanyl, as a single agent, has never been documented to cause either serotonin or neuroleptic malignant syndromes (Pilgrim et al., 2011). In addition to

proserotonergic effects, fentanyl has demonstrated anticholinergic effects (muscarinic receptor antagonism) at high doses in postoperative anesthesia patients with clinical symptoms similar to central anticholinergic syndrome (confusion, agitation, delirium, vigorous shivering, hot dry skin, purposeless movements, severe tachycardia, hypertension, and changes in vocal cord function) (Hustveit, 1994). Subsequent studies by Hustveit (1994) in rat brain demonstrated that fentanyl, but not morphine, had significant muscarinic receptor binding with K_i values in the micromolar range, in which it was concluded that muscarinic receptor binding of fentanyl is likely to occur during high-dose fentanyl anesthesia (50–150 mcg/kg) (Stone and DiFazio, 1988; Hustveit and Setekleiv, 1993; Yamanoue et al., 1993). In fact, the prerequisite for central anticholinergic syndrome is the antagonism of muscarinic receptors in the CNS (Hustveit, 1994).

As illustrated from the previous review, there is significant evidence that GABAergic, cholinergic, and noradrenergic pathways play a significant role in F/FA-induced WCS and will guide the development of effective therapeutic compounds.

Human Physiology

Although the animal (rat) model is exceedingly informative in certain aspects, it is incomplete in examining the involvement of the larynx and respiratory system in WCS and the effects of F/FAs on the cardiovascular and hepatic systems. This section examines the physiology in each of these systems in the context of their contribution to the clinical symptoms of WCS.

Fentanyl's Effects on Larynx and Vocal Cords: The Vagus Nerve. The complexity of the innervation of vocal cords and the innervation of the airway is significant and a full review is outside the focus of this article. The reader is referred to the following studies for detailed reviews: Rex (1970), Basterra et al. (1989), and Jette and Thiebeault (2011).

Among humans, the clinical model of WCS demonstrates the significant involvement of the larynx and vocal cords, in which laryngospasm is the key feature of WCS (Scamman, 1983; Bennett et al., 1997). Laryngospasm is defined as the involuntary closure or occlusion of the glottic opening controlled by intrinsic muscles of the larynx. Although the underlying molecular mechanisms of WCS have not been definitively established, the combined synthesis of the animal and human data in this review may provide a plausible path to explain how fentanyl induces laryngospasm as a component of WCS in humans. The path begins with increased norepinephrine in the LC by fentanyl (indirectly), followed by activation and/or inhibition of noradrenergic and cholinergic signal paths through the CNS, and terminates on laryngeal muscles, resulting in laryngospasm. In this model, the predominant autonomic tone of the system will determine the mechanism of vocal cord closure. Pharmacologic points of therapeutic intervention will be suggested by the mechanism described.

Vagus nerve, laryngeal nerves, muscles, and fibers. The vagus nerve originating in the nucleus ambiguus of the medulla exits the brain to supply the intrinsic striated muscles of the larynx, providing bilateral motor innervation via the external branch of the superior laryngeal and the recurrent laryngeal nerves. The cricothyroid muscle is the sole tensor of the vocal cords and is innervated via the external

branch of the superior laryngeal nerve. All other muscles of the larynx are innervated by the recurrent laryngeal nerve and include the lateral cricoarytenoid, posterior cricoarytenoid (PCA) and thyroarytenoid (TA) muscles (Rex, 1970). Importantly, the PCA muscles are the sole abductors of the vocal cords and vocal folds, and the lateral cricoarytenoid and TA muscles adduct and increase medial compression of the vocal cords (Rex, 1970). The vagus nerve normally provides dominant parasympathetic tone to the intrinsic muscles of the larynx and allows the abduction of the vocal cords via the PCA, keeping the glottis/airway open for relaxed inspiration and expiration (Rex, 1970).

F/FA activation of the LC. Following systemic administration, F/FAs enter the CNS via the carotid and vertebral arteries, cross the blood-brain barrier, and bind to μ-opioid receptors on GABA interneurons in various regions of the brain, including the LC, which has a significant density of these interneurons (Aston-Jones et al., 2004; Jin et al., 2016; Breton-Provencher and Sur, 2019). These interneurons serve an inhibitory function throughout the brain, and when activated inhibit the release of the principal neurotransmitter of that brain region (Nakamura et al., 2002). Stimulation of μ-opioid receptors on the interneurons inhibits GABA cell firing and prevents GABA release (Nakamura et al., 2002; Griffioen et al., 2004). In the LC, where norepinephrine is the principal neurotransmitter, this would result in the loss of GABA-mediated inhibition of LC firing and increase the release of norepinephrine from presynaptic terminals of the LC (Yoshida et al., 1992; Moyse et al., 1997; Breton-Provencher and Sur, 2019). In addition, F/FAs use similar mechanisms (GABA interneuron mediated) to release ACh from various regions in the CNS (Weigel and Luksch, 2014). In the case of the LC, the neighboring pontine reticular formation (pedunculopontine tegmental nucleus and lateral-dorsal tegemental nucleus) releases ACh that enters the LC, where it acts to excite muscarinic receptors (e.g., M1, M2, and M3) in the LC to release norepinephrine (McNaughton and Mason, 1980; Egan et al., 1983; Egan and North, 1985, 1986). Additionally, fentanyl is known to antagonize M3 receptors and may facilitate the binding of ACh to M2 receptors in the LC, which are likely to be the most prevalent muscarinic subtype in the LC (Cortes et al., 1984; Cortés and Palacios, 1986; Hustveit and Setekleiv, 1993; Yamanoue et al., 1993; Hustveit, 1994). As norepinephrine levels increase in the synapse from these combined mechanisms, norepinephrine binds α_1-adrenergic receptors on the postsynaptic terminal and increases the noradrenergic outflow of the LC to several sites that ultimately impact muscle tone in the chest wall, abdomen, diaphragm, and laryngeal muscles (Willette et al., 1982, 1987; Scamman, 1983; Lui et al., 1989, 1990, 1993, 1995; Bennett et al., 1997). In addition to selectively binding muscarinic receptors, fentanyl selectively binds the α_1-adrenergic receptor subtypes on the postsynaptic terminal in the rank order of B >A >>> D and potentially facilitates the binding of norepinephrine to the α_{1D} subtype, where fentanyl has its least affinity and norepinephrine its greatest (rank order of 1D >>> 1A > 1B) (Morrow and Creese, 1986; Sohn et al., 2005). The combination of these mechanisms would facilitate a robust noradrenergic signal from the LC and rapid increase in muscle contractility via coerulospinal sympathetic fibers to spinal motor neurons and superior cervical and other

sympathetic ganglia (T1–L2) that terminate on skeletal muscle (e.g., chest wall, diaphragm, and laryngeal muscles), or via noradrenergic fibers to respiratory motor centers in the medulla [dorsal respiratory group (DRG) and ventral respiratory group (VRG)] and motor efferents in the nucleus ambiguus vagal nuclei controlling innervation of the intrinsic laryngeal muscles (Lui et al., 1989; Barutell et al., 2004; Griffioen et al., 2004; Kouvaras et al., 2008; Bentzen and Grunnet, 2011; Tian et al., 2015).

Noradrenergic pathway: sympathetic fibers in the vagus nerve. As noted previously, sympathetic nerve fibers originating from the LC ultimately contribute innervation directly and indirectly to the intrinsic muscles of the larynx through several pathways. In the most direct path, LC coerulospinal fibers terminate in spinal motor neurons that then relay noradrenergic efferent signals from the CNS to sympathetic ganglia (Hisa, 1982; Hisa et al., 1982; Basterra et al., 1989; Yoshida et al., 1992). The superior cervical sympathetic ganglion is particularly significant regarding WCS, since it supplies the head and neck with sympathetic innervation. Specifically, the superior cervical ganglion provides sympathetic fibers to the terminal branches of the vagus nerve (superior laryngeal and recurrent laryngeal nerves) that serve as the sole innervation of the intrinsic muscles of the larynx (Yasuda et al., 1978; Hisa, 1982; Hisa et al., 1982; Morrow and Creese, 1986; Basterra et al., 1989; Yoshida et al., 1992; Allen and Murcek, 2018).

Human anatomic and histologic studies have clearly demonstrated the presence of sympathetic fibers in vocal cord tissue and surrounding muscle, suggesting the viability of laryngeal muscle control and/or activation by sympathetic innervation (Basterra et al., 1989; Allen and Murcek, 2018). In addition, histochemical studies have elucidated the contractile properties of the striated skeletal muscle fibers in the intrinsic laryngeal muscles in great detail (Yokoyama et al., 1995). These studies have demonstrated a significant differential in the ratio of type I (slow twitch) to type II (fast twitch) fibers in vocal cord adductor compared with abductor muscles (Yokoyama et al., 1995). Fast twitch fibers are generally considered more susceptible and responsive to sympathetic innervation (Yokoyama et al., 1995). Vocal cord abductors have predominately type I fibers (60%) compared with predominately type II fast twitch fibers (>90%) in adductor muscles (Ohyama et al., 1972; Diamond et al., 1992; Yokoyama et al., 1995; Hoh, 2005). This has significant implications for the effects of increased sympathetic outflow from the LC and offers a plausible mechanism whereby norepinephrine can rapidly activate vocal cord adductors to cause severe laryngospasm, particularly in a system where cholinergic/parasympathetic tone is diminished or compromised (e.g., anticholinergic drug effects).

ACh/cholinergic innervation of the vocal cord/larynx. The locus coeruleus, as the major noradrenergic nucleus of the brain, gives rise to sympathetic fibers that innervate extensively throughout the neural axis and plays a central role in system arousal level and autonomic regulation (Samuels and Szabadi, 2008b). The LC controls autonomic nuclei in the spinal cord as well as autonomic nuclei in the medulla, specifically the rostral ventrolateral medulla, dorsal motor nucleus of the vagus, and nucleus ambiguus, which gives rise to the efferent motor fibers of the vagus nerve (Samuels and Szabadi, 2008b). Activation of the LC increases sympathetic

activity and signaling in noradrenergic fiber projections to the medulla and decreases parasympathetic activity via these projections into the nuclei (Samuels and Szabadi, 2008b). Significant changes in LC noradrenergic activation, as described with fentanyl, can decrease baseline parasympathetic tone and vagal innervation to vocal cords, allowing for sympathetic effects on adductor muscles to predominate, resulting in vocal cord closure (Yasuda et al., 1978; Ozawa et al., 2003; Samuels and Szabadi, 2008b). This appears to be a plausible path to F/FA-induced laryngospasm via sympathetic innervation of the medulla; however, there are several other alternatives to consider that directly involve cholinergic nuclei in the medulla and nucleus ambiguus.

ACh agonism, particularly at muscarinic (M1–M5) M1 receptors in the pontine reticular formation can increase the relaxation of PCA (sole laryngeal abductor) muscles, as noted by Lydic (1989), despite the fact that the nucleus ambiguus contains the motor neurons controlling the PCA and laryngeal adductors (Baghdoyan et al., 1989; Lydic and Baghdoyan, 1989; Lydic et al., 1989). This must be considered in the initial CNS release of ACh by fentanyl and may create the basis for a cholinergically and sympathetically mediated imbalance resulting in vocal cord closure (Bouaziz et al., 1996; El Baissari et al., 2014). In this case, fentanyl, which acts as a selective M3 antagonist and appears to be located in medullary motor nuclei, could facilitate selective isolation of M1 and M2 receptors for ACh binding, resulting in increased relaxation of laryngeal abductor muscles and diminished opposition to sympathetically mediated laryngeal adductor contraction (Cortes et al., 1984; Cortés et al., 1986; Cortés and Palacios, 1986; Lydic et al., 1989; Levey et al., 1994; Richardson et al., 1997). Immunoprecipitation studies of muscarinic receptor distribution have consistently shown low levels of M3 receptors throughout the brain (~10%); however, there is a significant concentration of M3 in brainstem, pontine, and medullary motor nuclei (Nattie and Li, 1990; Levey et al., 1994; Richardson et al., 1997). In contrast, the M1 cholinergic receptor subtype is localized primarily in the brain and autonomic ganglia (Yamanoue et al., 1993).

In addition to LC noradrenergic innervation of medullary nuclei, the ability of fentanyl to act as an anticholinergic via M3 receptors gives it the ability to act directly on these nuclei and may have significant consequences in the underlying mechanism of WCS (Yasuda et al., 1978; Willette et al., 1982, 1987; Hustveit and Setekleiv, 1993; Yamanoue et al., 1993; Hustveit, 1994; Richardson et al., 1997; Lalley, 2003). The selective specificity of fentanyl for M3 receptors has greater significance when we consider that selective antagonist studies in the ventral medulla (nucleus ambiguus and arcuate nucleus) have demonstrated that M3 is the most likely subtype involved in centrally mediated respiratory control of motor outflow through the nucleus ambiguus and spinal cord neurons innervating the major muscles of respiration (Ehlert and Tran, 1990; Nattie and Li, 1990; Richardson et al., 1997; Abe et al., 2003). In any case, in sympathetically mediated adductor contraction, cholinergically mediated abductor relaxation, or combined cholinergic and sympathetically mediated effects, laryngospasm is likely to be the end result.

Finally, the direct effects of fentanyl on vagal nuclei were demonstrated by Lalley (2003) while measuring the dose-related effects of intravenous fentanyl on membrane and discharge properties of medullary respiratory neurons in adult cats and

by mapping axonal projections of medullary respiratory neurons to the respective muscles implicated in the control of tidal volume, chest wall compliance, and upper airway flow resistance (Lalley, 2003). Intravenous fentanyl prolonged neuronal discharges leading to persistent tonic firing in vagal postinspiratory motor neurons controlling laryngeal adductors and conversely caused depression of depolarizations in vagal laryngeal abductor motor neurons. Lalley (2003) concluded: "the actions of fentanyl on vagal Aug-I and laryngeal post-inspiratory neurons are complementary in their potential to promote vocal fold closure." Although Lalley (2003) did not make direct measurement of mechanical disturbances of ventilatory function or directly identify the receptors (μ, M3, or α_1 subtypes) bound by fentanyl in these experiments, the link between opioid-mediated changes in neuronal activity and ventilatory disturbances of respiratory mechanics are implied from the known anatomic and functional properties of the neurons examined. More importantly, although the receptors bound by fentanyl were not identified, the depolarization differential and selectivity of fentanyl for vagal neurons controlling laryngeal adductors and abductors was clearly demonstrated, but has several possibilities of control (Egan and North, 1985; Stone and DiFazio, 1988; Baron and Siegel, 1989; Nattie and Li, 1990; Hustveit and Setekleiv, 1993; Yamanoue et al., 1993; Hustveit, 1994; Levey et al., 1994; Richardson et al., 1997; Lalley, 2003; Ge et al., 2015; Burns et al., 2016). Activation of α_1-adrenergic receptors in the LC or medulla send excitatory signals via adrenergic/noradrenergic projections directly to vagal preganglioninc neurons in the ventrolateral medulla that maintain neural control of airway smooth muscle and motor efferent control of laryngeal muscles, as demonstrated in the rat model (Nattie and Li, 1990; Samuels and Szabadi, 2008b; Ge et al., 2015). Based on the previous detailed descriptions of M receptor subtype distribution in these motor nuclei, these noradrenergic signals appear to be translated in the motor nuclei by muscarinic receptors (Cortes et al., 1984; Cortés et al., 1986; Cortés and Palacios, 1986; Lydic et al., 1989; Levey et al., 1994; Richardson et al., 1997). This suggests that the receptors involved in the excitation of vagal postinspiratory motor neurons controlling laryngeal adductors, and conversely causing depression of depolarizations in vagal laryngeal abductor motor neurons in the Lalley cat model, are likely to be muscarinic (M1, M2, and/or M3) in a distribution differential on the abductors and adductors that would allow for opposing muscle effects, as suggested by Lalley (2003). An additional level of control lies with fentanyl's ability to act as an M3 antagonist at plasma concentrations readily found in high-dose anesthesia or F/FA overdose in humans (10–7 M or ~22 ng/ml), allowing fentanyl to directly bind M3 receptors in these same vagal motor neurons and the possibility of several scenarios of M receptor subtype modulation ending in laryngospasm (Stone and DiFazio, 1988; Yamanoue et al., 1993; Hustveit, 1994; Burns et al., 2016). In one scenario, assuming M3 receptors are located on PCA and TA muscles (with M1 on PCA and M2 on TA), fentanyl, acting as a selective M3 antagonist, could facilitate isolation of M1 and M2 receptors for ACh binding and result in decreased depolarization (relaxation) of laryngeal abductor muscles and increased depolarization (contraction) of laryngeal adductors.

The roles of M1, M2, and M3 receptors will need to be further clarified and the specific distribution of these muscarinic subtypes on PCA (adductors) and TA (adductors) more thoroughly identified in humans. Similar selective activation of vagal nerve fibers controlling adduction and abduction of laryngeal muscles in humans offers another plausible mechanism, whereby F/FAs via sequential or coordinated activation of centrally mediated motor activity could trigger the laryngospasm component of WCS. A plausible therapeutic solution would be either an M3-specific agonist to directly reverse fentanyl's selective effects on M3 receptors or to use an anticholinergic such as atropine to antagonize facilitated ACh binding effects on M1 or M2 (e.g., M1, M2, M4, and M5) receptors that fentanyl may cause. These agents could ultimately be combined with μ-receptor and α-adrenergic antagonists for the reversal of F/FA overdose or used as prophylaxis agents in chemical exposures.

The mechanisms described in this section are a complex interplay of fentanyl's activity in the LC and vagal medullary respiratory motor nuclei, with significant effects on autonomic balance. In the case where two autonomic inputs have opposing effects, the basic effect of LC activation of sympathetic innervation is to inhibit parasympathetic output (Samuels and Szabadi, 2008a). Aspects of this model will require further examination, but provide a fundamental basis for the development of more effective therapeutics for the treatment of WCS induced by F/FAs in humans.

Diaphragm, Abdomen, and Thoracic Wall Innervation. Along with the glottic muscles, the main muscles of respiration include the diaphragm, intercostals, and abdominal wall muscles. The diaphragm receives its motor nerve impulses from the medullary centers via the phrenic nerve, which carries motor fibers from C3, C4, and C5 and also receives sympathetic nerve fibers from the cervical sympathetic chain. The intercostal muscles are stimulated by way of cervical, thoracic, and lumbar motor nerves and spinal motor neurons that terminate on skeletal muscles of the thorax and abdomen (De Troyer et al., 2005). The control of these muscle groups is complex, receiving multiple control signals from higher brain centers for voluntary control and subcortical regions for autoregulatory and reflexive controls (De Troyer et al., 2005).

Respiration and the Feedback Loop of Respiratory Mechanics with Medulla DRG and VRG. The regulation and rhythmicity of breathing is based on a feedback loop of acid/base balance and blood chemistry (e.g., blood pH and $PaCO_2$ levels) that reflexively changes with metabolic and respiratory function. The dynamics of respiration increase or decrease based on afferent signals from the vagus and glossopharyngeal nerves (cranial nerves X and IX, respectively) that include peripheral chemoreceptors and mechanical changes in the lung parenchyma, airway, and joint proprio-receptors (Ikeda et al., 2017). These monitoring signals return to respiratory centers or respiratory-related neurons in the pons (i.e., apneustic and pneumotaxic centers), medulla (i.e., the DRG), central chemoreceptors, and VRG. The DRG is composed mostly of inspiratory neurons and subsequently controls the rhythmic initiation of inspiration impulses by sending signals to the motor nerves governing the diaphragm and external intercostal muscles. The VRG contains inspiratory and expiratory neurons and sends signals via the nucleus ambiguus to laryngeal and pharyngeal muscles and to the diaphragm, abdominal, and intercostal muscles. The DRG and VRG receive input from the pons and LC, which essentially stimulates or inhibits respiratory drive reflexively

and can override the rhythmicity of the vagus and medullary feedback loop (Ikeda et al., 2017). The FIRMR component of WCS involves the innervation of the diaphragm, chest, and abdominal wall via the LC and spinal motor neurons, while the vocal cord/laryngeal component involves innervation of all intrinsic muscles of the larynx via the LC, superior cervical ganglia, vagus nerve, and vagal nuclei. In this scenario, LC activation by fentanyl could allow for increased medullary DRG- and VRG-driven muscle contraction, resulting in discoordination of medullary rhythmic respiratory drive signals and feedback loops. The subsequent triggering of diaphragm contraction and external intercostal muscle contraction against closed vocal cords is activated via the aberrant sympathetic signaling effects of fentanyl on the LC, vagal, and medullary nuclei. Additionally, the vagus nerve fibers contain μ-opioid receptors, innervate stretch receptors in lung parenchema, and can send afferent signals to vagal nuclei when activated by opioid binding (Willette and Sapru, 1982). This activity can trigger the Hering-Breur reflex discussed next (Chávez et al., 1998).

Hering-Breuer Reflex. The scenario described previously is possibly complicated by the activation of another reflex arc involving the pulmonary stretch receptors located in the visceral pleura of the lung and the walls of the bronchi and bronchioles that then activate the Hering-Breur reflex. The Hering-Breur reflex arc was first noted in 1868 when Josef Breur and Ewald Hering observed that a maintained distension of the lungs during mechanical ventilation of anesthetized animals caused a cessation of inspiratory drive (Dutschmann et al., 2014). The reflex acts to prevent overinflation of the lung by sending inhibitory signals to the DRG to cease further inspiration.

The reflex may also include VRG-mediated closure of the vocal cords. In anesthesia with human subjects, overdistention of the lungs by manual ventilation can result in severe laryngospasm and may also be evident in the clinical phenomenon of the exercise-induced VCD (Dutschmann et al., 2014). Further complicating matters, and as mentioned previoulsy, μ-opioid receptors are located on the vagal fibers that innervate the actual juxtacapillary stretch receptors surrounding the alveoli. Activation of these fibers sends afferent signals to medullary respiratory centers and retrograde signals via the vagus nerve to the heart and vocal cords in an animal model where morphine and peptide opioids were delivered into the right atrium (Willette and Sapru, 1982). Delivery of these opioids into the right atrium caused significant electrical pulse activity, measured by EMG, to the cardiac vagal fibers and vagal fibers leading to the vocal cords. The peak pulse occurred within 1 to 2 seconds with rapidly decreasing amplitude over ~10–15 seconds (Willette and Sapru, 1982). Vocal cord closure was not noted, since this had the limitation of being a tracheostomy rat model; however, pulmonary peak pressures were elevated during the initial EMG peak.

Cardiac Function in WCS. Cardiac output can be significantly inhibited by vagus nerve activation in the nucleus ambiguus. Fentanyl binds μ-opioid receptors in the vagal nuclei and GABA interneurons of the nucleus ambiguus and can cause severe bradycardia and decreased cardiac output, with direct consequences for cerebral and hepatic perfusion pressures. As noted previously, fentanyl also antagonizes

α_1-adrenergic receptor subtypes in a rank order of 1B > 1A >> 1D. This may have significant consequences on the function of cardiac myocytes and coronary arteries, which have selective distributions of these receptor subtypes. With regard to the heart, α_{1A}- and α_{1B}-adrenergic subtypes are concentrated almost exclusively on cardiac myocytes, while the coronary arteries exclusively use α_{1D} subtypes as noradrenergic inputs to control myocardial contractility/ventricular function and coronary artery perfusion of the mycocardium, respectively (Jensen et al., 2009). Selective binding and competitive antagonism of the cardiac α_{1A}- and α_{1B}-adrenergic subtypes by F/FAs can prevent the binding of catecholamines to the myocardial cells, resulting in depressed contractility and ventricular function (Hicks et al., 1981).

Similarly, fentanyl can antagonize α_{1D} receptors in coronary vessels, but has significantly weaker binding than at 1A and 1B receptors and competes poorly with norepinephrine at the 1D subtype (Sohn et al., 2005). The competitive binding of F/FAs to the 1A and 1B subtypes may simply help to concentrate norepinephrine and facilitate norepinephrine binding to coronary vessel 1D receptors, resulting in coronary artery vasoconstriction and a rapid decrease in coronary perfusion pressure and myocardial function.

Although the exact mechanisms in F/FA overdose and toxicity will need further attention, significant F/FA effects on the cardiovascular system cannot be discounted. A study by Wang et al. (2006) in chamber-isolated mouse myocardium demonstrated that each respective ventricle has different inotropic responses with the administration of the α_{1A}-adrenergic receptor agonist phenylephrine. Right ventricle tissue stimulation results in measurable negative inotropic effects in isolated myocardial fibers, while left ventricle stimulation results in positive inotropic effects. Myocardial α_1-adrenergic receptor subtype distribution in the mouse model is relevant in the human cardiovascular system (O'Connell et al., 2013). F/FA-selective antagonism of α receptors in human ventricular myocardium may result in paradoxical, inotropic isolation of the ventricles. The reversal of these demonstrated effects could promote left ventricle negative inotropy, while simultaneously promoting right ventricle positive inotropy in the presence of norepinephrine. The physiologic result would likely be severe pulmonary edema due to high forward pressure (right ventricle positive inotropy) against high back pressure (left ventricle negative inotropy), meeting in the pulmonary capillary beds and resulting in plasma ultrafiltrate or exudate in the alveoli (Wang et al., 2006).

Muscarinic receptor subtypes must also be considered in coronary artery vasoconstriction, since fentanyl has been shown to act as an M3 antagonist and the M3 receptor has been demonstrated to be the most prevalent subtype in porcine coronary arteries. Ach stimulation of M3 receptors appears to act as a coronary vasoconstrictor and could be potentially antagonized by fentanyl. The impact of these experimentally isolated effects as demonstrated by Yamanoue et al. (1993) remains unclear in the face of the α-adrenergic effects described, but would seem worthy of further exploration to determine the dominance of each effect (Yamanoue et al., 1993; Sohn et al., 2005).

These proposed cardiac mechanisms may explain the rapid onset of vascular, hepatic, and CNS effects described in the public health and autopsy data findings as mentioned

previously, including: 1) rapid onset of cyanosis, 2) immediate loss of consciousness (central thalamocortical inhibition and/or decreased cerebral perfusion decrease), and 3) decreased hepatic metabolism (Somerville et al., 2017). Additionally, the selective effects of fentanyl on α_1-adrenergic receptors and their specific cardiovascular distribution may offer at least a partial explanation for the vascular component of F/FA-induced WCS.

Hepatic Function in WCS. By decreasing cardiac output, high-dose fentanyl can secondarily cause decreased hepatic artery perfusion and/or potentially decreased cerebral perfusion, resulting in significant malfunction in fentanyl metabolism and rapid loss of consciousness, respectively (Griffioen et al., 2004). Hepatic perfusion normally constitutes approximately 25% of cardiac output, and hepatic metabolic function can be significantly compromised with decreases in blood flow of as little as 10% (Tuñón et al., 1999). The liver has a dual blood supply: 70% of hepatic blood flow is supplied by the portal vein and the rest by the hepatic artery. Portal vein flow is not tightly regulated, but is directly affected by systemic hypotension or decreases in cardiac output. In hepatic perfusion, studies up to a 50% decline in portal flow can occur if hepatic artery tone is compromised (Lautt, 1996; Møller and Henriksen, 2008; Schuppan and Afdhal, 2008).

Burns et al. (2016) noted that F/FA overdose decedents showed no detectable fentanyl metabolites in 20 of 48 cases (42%) and low levels (<1 ng/ml) in 25 of 48 cases (52%). Given the rapidity of normal fentanyl hepatic metabolism, this study indicates that hepatic metabolism becomes compromised within the first 2 minutes of F/FA overdose (McClain and Hug, 1980). Hypothetically, if a baseline of cardiac output continued, a compromise in hepatic metabolism could result in recirculation of high plasma/serum levels of F/FA back to the CNS with little to no decrease from the first-pass redistribution into the brain from the initial intravenous injection. If hepatic metabolism did not return, the effects of WCS would continue inevitably to death.

In summary, WCS is a syndrome that includes laryngospasm, respiratory muscle rigidity/contraction, cardiovascular compromise, and a concurrent decline in hepatic metabolism. Each component can be explained as a consequence of α_1-adrenergic, noradrenergic, and cholinergic receptor activity in addition to μ-opioid receptor activation.

Proactive Strategies

Drug Development. Based on the data presented here, there should be little doubt that laryngospasm, as part of WCS, plays a critical role in overdose death from F/FAs and that noradrenergic systems, cholinergic systems, and μ-opioid receptors are significant and interdependent components of this syndrome. This suggests that the most effective antagonist (immediate reversal therapy) and prophylaxis treatment strategies would consist of agents that can concurrently treat respiratory depression (μ-opioid receptor mediated) and the rapid respiratory failure of WCS (α-adrenergic, μ-opioid receptor, and muscarinic receptor mediated). Several points are worth clarifying here. The significant lethality that is occurring with F/FAs is partly due to their combination with other opiates such as heroin, which can have synergistic effects on respiratory drive when combined with F/FAs (Kiyatkin, 2019). All opioids cause some degree of dose-dependent respiratory

depression and it is likely to emerge in a subject that has taken a large enough dose of F/FAs to cause WCS, that is, provided that the subject survives the acute mechanical failure of respiration. Therefore, naloxone and longer-acting μ-opioid antagonists such as nalmafene or naltrexone should be included in all opioid overdose treatment regimens. However, based on the mechanisms described, naloxone is clearly not effective as a sole therapeutic agent in F/FA overdose and appears to have a particularly minimal effect on the laryngospasm component of WCS as demonstrated in the animal model described by Willette et al. (1982, 1987).

Effective therapies in the future for F/FA overdose or toxic exposure prophylaxis will consist of α_1-adrenergic antagonists, possibly α_2 agonists, anticholinergic agents, and μ-opioid receptor antagonists. In the case of the α antagonists, it should be pointed out that although prazosin demonstrated effective inhibition of FIMR in the animal experiments noted, it is a nonselective agent that can cause profound hypotension in humans as it was used in these experiments. However, the effect of hypotension may be ameliorated to some degree by either using selective agents that have high affinity for α_{1D} subtypes (tamsulosin and terazosin) or combining smaller doses of prazosin with selective agents (Jerussi et al., 1987; Lui et al., 1990, 1993, 1995; Fu et al., 1997; Burns et al., 2016). It is also important to note that while both fentanyl and prazosin antagonize α_1-adrenergic receptors, fentanyl is a very weak antagonist (K_i = ~1000 nM) at these receptors compared with prazosin (K_i = ~0.1 nM) (Sohn et al., 2005). This is a critical distinction, and as a result prazosin can completely block norepinephrine activity at all α_1-adrenergic subtypes, while fentanyl binds weakly at α_{1D} subtypes (rank order of 1B > 1A >> 1D) compared with norepinephrine, which has its greatest affinity for the 1D subtype (Morrow and Creese, 1986; Sohn et al., 2005) Although the significance of this fact in the context of WCS was not pointed out by Sohn et al. (2005), this suggests that any available norepinephrine activity may be focused on interaction at the α_{1D} subtype (Yamanoue et al., 1993).

It should also be pointed out that α_2 agonists (clonidine) have been noted to be effective for treatment of FIMR in animal models and may be effective if used in combination with the other agents listed here, with the added benefit of decreasing the norepinephrine release seen in acute withdrawal and rapid reversal of opioid overdose in opioid-tolerant individuals (Jerussi et al., 1987; Weinger et al., 1989). Finally, the effects of selective anticholinergic agents (M1 and M2) or nonselective anticholinergic agents, with greater affinity for M1–M5 receptors than fentanyl, may also be effective agents to use in combination with α-adrenergic and μ-opioid receptor antagonist formulations. In this case, droperidol may be an effective agent, since it combines both α_1-adrenergic antagonist properties with anticholinergic effects that appear to inhibit the upper airway effects and decreased thoracic wall compliance that has been demonstrated with fentanyl (Stoelting et al., 1975; Yasuda et al., 1978; Parmentier and Dagnelie, 1979).

Education and Treatment Protocol Development for Medical Practitioners. Clear guidelines are needed regarding administration of antagonist therapies. Benefits of using non-μ-opioid receptor antagonist therapy prophylaxis agents to prevent death from WCS in active heroin/fentanyl

users should be explored as part of education programs. EMS and paramedic protocols for managing F/FA overdose should promote intubation and use of rapid-acting muscle paralytics such as succinylcholine as opposed to administering excessive doses of naloxone. Emergency rooms may establish notification protocols to request anesthesia call teams once they are alerted that an overdose involving fentanyl or synthetic opioids is en route.

Education/Protocol Development: Vulnerable Populations (Drug Users). Recommendations should be made to active opioid users to modify injection practices with drug testing kits, test shots (small test dose preceding a larger dose), safe injection sites, and the use of chaperones or trained observer support. Prophylaxis agents that can be adapted to the population at risk should be made available for vulnerable populations that are still actively using opiates. These populations would be well suited for prophylactic muscle rigidity antagonist therapy that would strictly isolate prophylaxis against chest wall rigidity and laryngospasm and would exclude opioid antagonist therapy. Here, the rational is that if patients are exposed to fentanyl while using heroin, they would be less likely to suffer the rapid failure of respiratory mechanics associated with WCS. This would create the temporal window to use a reversal drug to treat respiratory depression and/or get expert medical attention.

Environmental and First-Responder Protective Strategies. First responders including paramedics, emergency medical providers, firefighters, law enforcement, military personnel, and service animals are at risk of environmental exposure if F/FAs are an environmental contaminant. The task of managing and maintaining safety can be compromised if prophylaxis agents are unavailable. Prophylaxis agents can consist of antagonists for both muscle rigidity (chest wall and laryngeal muscles) and respiratory depression, treating both side effects concurrently in the case of large-scale exposure to F/FAs. This can allow for a therapeutic window for the administration of more concentrated reversal agents and treatments as needed. F/FAs have been weaponized previously on the battlefield and in covert military operations around the world, creating an imminent danger that needs addressing (Armenian et al., 2018). The threat of aerosolized forms of the potent fentanyl analog carfentanil was actualized in the Moscow theater incident of 2003, in which ~1800 civilians were held hostage. Military responders piped carfentanil into the ventilation system of the theater to end the standoff. Civilian casualties exceeded 100, despite the reported availability and administration of naloxone for treatment (Pilch and Dolnik, 2003). F/FAs are readily transported through mail systems and traditional ports of entry (Armenian et al., 2018), which should instigate the concentrated development of effective strategies for protection.

Conclusions

Fentanyl is at the epicenter of the current United States opioid crisis and is appropriately considered the deadliest drug in America, with approximately 30,000 deaths per year since 2017, an increase from 3000 deaths in 2013 (Armenian et al., 2018). F/FAs vary in potency, with some being many times more potent than fentanyl. In addition to individuals suffering from opioid use disorder, potent F/FA's represent a significant risk to civilian populations. Public health and

autopsy data indicate that the deaths occurring from F/FAs are rapid, involve profound cardiovascular compromise, are atypical compared with the respiratory depression seen with heroin, and suggest WCS as a significant factor in rapid airway compromise and death. Current EMS field and emergency room data and eyewitness first responders indicate that escalating doses of naloxone are common and often ineffective in the presence of F/FAs. Longstanding clinical evidence from anesthesiology has identified the unique side effect profile of F/FAs and indicates that rapid infusion consistently causes FIRMR and vocal cord laryngospasm.

Anesthesiologists in the United States have been safely administering F/FAs to surgical patients for over 50 years and routinely treat the symptoms of rigidity and airway compromise with rapid-acting muscle paralytics such as succinylcholine and endotracheal intubation. Clinical studies in surgical patients receiving high-dose fentanyl or sufentanil have identified the fundamental component of WCS as acute airway compromise from laryngospasm induced by the opioid itself. Physiologic studies in animals indicate that FIMR and vocal cord closure involves noradrenergic pathways in the LC and spinal cord and the selective modulation of medullary vagal nuclei controlling intrinsic muscles of the larynx and respiration.

Preliminary neurochemical and neuroanatomic data support the possibility of paradoxical activation of the LC with increased noradrenergic activity and selective activation of vagal motor nuclei by fentanyl. These data reiterate that in spite of the current widespread awareness and availability of the opioid reversal drug naloxone, deaths caused by fentanyl are not decreasing, possibly due to naloxone's minimal effectiveness in the treatment of the fentanyl-induced vocal cord/upper airway compromise seen in WCS. The key point of this article is to share with the medical and scientific community the existing evidence to support that F/FAs are a unique class of molecules with pharmacologic properties that clinically differentiate them from morphine-derived alkaloids, particularly in their rapidity of onset and the severity of effects on respiratory muscle mechanics (WCS). There should be little doubt that this is a key factor in the dramatic rise in opioid overdose deaths that involve F/FAs. The urgent need for the expedient identification of effective therapeutics for F/FA overdose will only occur if we first recognize that the current treatment of naloxone has significant limitations. This article serves as an effort to create this awareness and as a call to action.

In conclusion, the complex nature of WCS speaks to the necessity of using several drugs (α_1-adrenergic antagonists, μ-opioid receptor antagonists, and selective muscarinic/cholinergic agents) in formulations that will decrease or inhibit the severity of laryngospasm, FIRMR, respiratory depression, and the cardiovascular effects of F/FA overdose and/or toxic exposure.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Torralva, Janowsky.

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