ANESTHESIOLOGY

Countering Opioid-induced Respiratory Depression in Male Rats with Nicotinic Acetylcholine Receptor Partial Agonists Varenicline and ABT 594

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Activation of α4β2 nicotinic acetylcholine receptors by the full agonist A83580 markedly reduced opioid-induced respiratory depression in rats without compromising analgesia
- Varenicline, which is used to treat smoking addiction, and ABT 594, which produced analgesia in trials of patients with diabetic peripheral neuropathic pain, are potent, partial agonists of α4β2 nicotinic acetylcholine receptors

What This Article Tells Us That Is New

- Pre- or coadministration of varenicline or ABT 594 with opioids markedly reduced the degree of respiratory depression they caused in rats
- Varenicline and ABT 594 reversed moderate to severe respiratory depression produced by fentanyl without interfering with opioid-induced suppression of pain
- Administration of ABT 594 and varenicline coadministered with a low dose of naloxone reversed respiratory depression and prevented death caused by a bolus lethal dose of fentanyl or the combination of fentanyl and diazepam

ABSTRACT

Background: Opioids can induce significant respiratory depression when administered as analgesics for the treatment of acute, postoperative, and chronic pain. There are currently no pharmacologic means of reversing opioid-induced respiratory depression without interfering with analgesia. Further, there is a growing epidemic of opioid overdose that could benefit from therapeutic advancements. The aim of this study was to test the ability of two partial agonists of α4β2 nicotinic acetylcholine receptors, varenicline (used clinically for smoking cessation) and ABT 594 (tebanicline, developed as an analgesic), to reduce respiratory depression induced by fentanyl, remifentanil, morphine, and a combination of fentanyl and diazepam.

Methods: Whole body plethysmographic recordings, nociception testing, and righting reflex testing were used to examine ventilation, analgesia, and sedation in adult male Sprague–Dawley rats.

Results: Pre-, co-, or postadministration of varenicline or ABT 594 did not alter baseline breathing but markedly reduced opioid-induced respiratory depression. Varenicline had no effect on fentanyl-induced analgesia and ABT 594 potentiated fentanyl-induced analgesia. Specifically, 10-min administration of fentanyl induced a decrease in respiratory rate to 43 ± 32% of control in vehicle group, which was alleviated by preadministration of varenicline (85 ± 14% of control, n = 8, $P < 0.001$) or ABT 594 (81 ± 36% of control, n = 8, $P = 0.001$). ABT 594 or varenicline with a low dose of naloxone (1 μg/kg), but not varenicline alone, partially reversed fentanyl-induced lethal apnea, but neither compound provided the very rapid and complete reversal of apnea achieved with high doses of naloxone (0.03 to 1 mg/kg). Administration of varenicline (n = 4, $P = 0.034$) or ABT 594 (n = 4, $P = 0.034$) prevented lethal apneas induced by the combination of fentanyl and diazepam.

Conclusions: Activation of α4β2 nicotinic acetylcholine receptors by varenicline and ABT 594 counters opioid-induced respiratory depression without interfering with analgesia.

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Opioids provide very effective analgesia for many clinical indications involving moderate to severe levels of pain.[1] However, opioids can cause varying levels of side effects, including significant suppression of respiratory drive.[2] Further, outside of the clinic, there is a widespread epidemic of opioid abuse that is resulting in an alarming incidence of lethal overdose.[3,4] The focus of the research described here is toward developing a novel pharmacologic means of countering opioid-induced respiratory depression. We have recently demonstrated that activation of α4β2 nicotinic acetylcholine receptors can markedly reduce opioid-induced respiratory depression without compromising analgesia.[5] That proof-of-principle study evaluated the α4β2 nicotinic acetylcholine receptor full agonist A83580. A radiolabeled form of A85380 has been administered to humans in an imaging study designed to examine the central nervous system distribution of the α4β2 nicotinic acetylcholine receptors, but A85380 is currently not being pursued for clinical use.[6] Thus, there is a need to extend upon that pilot study to evaluate the efficacy of compounds targeting α4β2 nicotinic acetylcholine receptors that have been developed for clinical use and have passed through various stages of clinical trials. The two agents examined here, varenicline and ABT 594, are potent, partial agonists of α4β2 nicotinic acetylcholine receptors.[7–12] Varenicline is

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currently used as a prescription medication to treat smoking addiction.[11] ABT 594 (tebanicline) was shown to be effective as an analgesic in Phase II trials involving patients with diabetic peripheral neuropathic pain.[13,14]

In vivo rat models were used to evaluate the efficacy of varenicline and ABT 594 in four experimental scenarios that are clinically relevant to opioid-induced respiratory depression. First, we tested whether a pre- or coadministration of these compounds with opioids, primarily focusing on fentanyl, could minimize the occurrence of significant respiratory depression without compromising the desired analgesia. Such a prophylactic intervention could reduce the need for subsequent intervention and potentially reduce the incidence of overdose in those taking opioids. Second, we tested the effectiveness of compounds to reverse moderate to severe respiratory depression caused by a continuous infusion of opioids. This is particularly relevant, for example, to patients who are administered opioids postsurgically and who are particularly prone to opioid-induced respiratory depression.[15] Administration of the opioid-receptor antagonist naloxone would reverse the respiratory depression, but the desired analgesia would be lost. Third, we tested the efficacy of the compounds, relative to that of naloxone, to counter what is normally a lethal concentration of fentanyl. Fourth, we tested the ability of the compounds to counter severe respiratory depression induced by the combination of fentanyl and the benzodiazepine diazepam because there is a significant incidence of overdose caused by the combination of those agents that cannot always be adequately reversed with naloxone.[3,16]

Materials and Methods

All experimental procedures were approved by University of Alberta Faculty of Medicine Animal Welfare Committee (Edmonton, Alberta). Adult Sprague–Dawley male rats were purchased from Charles River Canada (Sherbrooke, Quebec).

Measurements were performed in whole-body, cylindrical transparent plexiglass plethysmographs that had one inflow and two outflow ports for the continuous delivery of fresh room air and removal of expired carbon dioxide.[5] The plethysmograph volumes were 2,000 (inner diameter, 10.1 cm; length, 25 cm) ml for measures of respiratory parameters of adult (400 to 520 g) rats with a flow rate of 700 ml/min. Rats were anesthetized with 3% isoflurane in an induction chamber and maintained with 2% isoflurane anesthesia during tail vein cannulation (P10 size tubing directly inserted in the lateral tail vein, with both tail veins cannulated if needed). The chamber had an additional port to allow exteriorization of the tail (port outlet sealed with Play-Doh to minimize pressure leakage) for intravenous (iv) drug infusion *via* an infusion pump (KD Scientific, USA). Importantly, with this infusion method, all drug deliveries can be performed with continuous monitoring of plethysmographic recordings without physical handling of the animal. Pressure changes were detected with a pressure transducer (model DP 103; Validyne, USA), signal conditioner (CD-15; Validyne), recorded with data acquisition software (Axoscope, Molecular Devices, USA) *via* analog-digital board (Digidata 1322A, Molecular Devices). Signals were high pass filtered (0.01 kHz), with a sampling rate at 1 kHz. Data was integrated (τ = 80 ms) with Labchart 8 (ADInstruments Inc., USA) and exported to Clampfit (Molecular Devices) for further analyses. Baseline and threshold levels for breaths were set using Clampfit software. Bursts were then automatically detected so that frequency and tidal volume (calculated for the region of the burst that was above the baseline) were measured. A pulse oximeter (Norin 8600V, USA) was placed on the tail to monitor oxygen saturation levels. The experiments were conducted between 10:00 AM and 5:00 PM. Animals were euthanized with an overdose of pentobarbital upon completion of experiments.

It should be noted that our plethysmograph is effective for studying respiratory frequency (f_R) and detection of apneas. An apnea is defined as the absence of airflow (pressure changes) for a period equivalent to or greater than two complete respiratory cycles. Our whole-body plethysmographic system provided semiquantitative measurements of tidal volume (V_T, ml/g) and minute ventilation ($\dot{V}_E = f_R \times V_T$: ml • min^{-1} • g^{-1}), from which we report changes relative to the control state.

Nociception Testing and Righting Reflex

Formalin Test. A dilute solution of formalin (50 μl, 1.5% formalin diluted in saline) was injected into the intraplantar region of the right hind paw, followed by assessment of nocifensive behaviors (licking, lifting, or flinching of the injected paw) in the first phase (0 to 5 min, acute nociceptive response) and second phase (20 to 40 min; reflecting inflammation) of the assay.[17] A simple sum of time spent on licking/lifting is a recognized assessment of formalin induced nocifensive behaviors.[18]

Sedation (loss of righting reflex) was defined as the rat's inability to right itself into the prone position after the animal was placed supine.[5] The duration of sedation was defined as the time interval from the beginning of fentanyl administration to the recovery of righting reflex. Figure 1 provides a graphic outline of the experimental protocol for preadministration of varenicline (1 mg/kg, neck subcutaneously) or ABT 594 (30 μg/kg, subcutaneously) with fentanyl (36 μg/kg over 10 min, iv infusion) and measures of respiratory parameters, nociception and righting reflex.

Pharmacologic Agents

Diazepam and opioids including fentanyl citrate, morphine sulfate, and remifentanil were purchased from Sandoz (Canada). Varenicline tartrate, ABT 594 hydrochloride, and naloxone hydrochloride were from Tocris (USA), dissolved in saline (0.9% NaCl), with the doses administered based on extensive

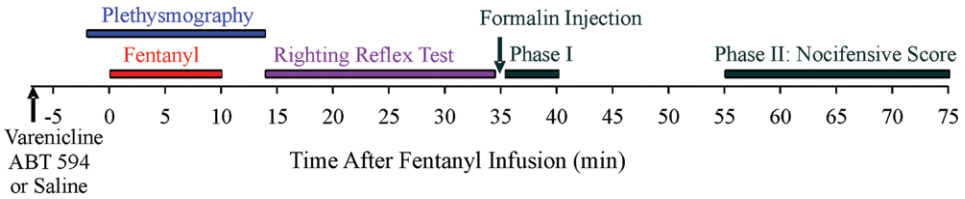

Fig. 1. Graphic outline of the experimental protocols to evaluate the effects of preadministration of varenicline or ABT 594 *versus* vehicle (saline) on fentanyl-induced respiratory depression (plethysmography), analgesia (Formalin test), and sedation (righting reflex test). Varenicline (1 mg/kg, neck subcutaneously, subcutaneously) or ABT 594 (30 μg/kg, subcutaneously) or saline was administrated approximately 7 min before fentanyl (36 μg/kg over 10 min, intravenous infusion). The righting reflex testing started 14 min after fentanyl. The animal was then removed from the chamber for formalin injection (50 μl, 1.5% formalin diluted in saline) into the intraplantar region of the right hind paw at 35 min after fentanyl, followed by assessment of nocifensive behaviors (licking and lifting of the injected paw) in phase I (0 to 5 min after formalin injection, reflecting acute nociceptive response), and phase II (20 to 40 min after formalin injection, reflecting inflammation) of the assay.

literature with these compounds in rodent and human studies.[7,8,12,19–21] The stock solutions used were as follows: varenicline tartrate (5 to 10 mg/ml), ABT 594 hydrochloride (0.1 to 0.3 mg/ml), naloxone hydrochloride (0.01 to 10 mg/ml) and the injection volume was approximately 0.1 ml/kg.

Statistics

Data are expressed as mean ± SD for parametric analysis and median and interquartile ranges for nonparametric analysis (Sigmaplot 13 Systat Software Inc., USA). The nature of the hypothesis testing is two-tailed. We first ran the normality test (Shapiro-Wilk) and equal variance test (Brown-Forsythe). For those data that failed either the normality test or equal variance test, nonparametric statistics were applied. For those data that passed both tests, parametric statistics were used. $P < 0.05$ is taken as a statistically significant difference; n refers to the number of animals, with animal as the unit of analysis for statistical tests. No statistical power calculation was conducted before the study. The sample size was based on our previous experience with these experimental protocols. All the animals were opioid-naïve and only tested once. Rats were randomly assigned to groups using block randomization sequence and tested in sequential order. Specifically, for two group (one dose drug *vs.* vehicle) comparisons, one of two rats (housed in the same cage) was randomly assigned to the vehicle group, with the other being assigned to the drug treatment group. For comparison of multiple groups, each animal was randomly assigned to each group. There were no missing data for analysis. We reported and analyzed all the data including outliers. Blind testing was used whereby one person administered the drug and the second person ran nociception testing, rated behavior, and analyzed the data without knowledge of drug administration. Respiratory parameters were calculated over an average of 1 min of continuous recordings. The respiratory parameters respiratory frequency, V_T, and $\dot{V}_E$ were reported as means relative to control values (*i.e.*, before opioid administration). For figure 2, the significance of changes in respiratory frequency, $\dot{V}_E$, and oxygen saturation after fentanyl administration was assessed with two-way repeated-measures ANOVA (factors: between-subjects; drug × time; Holm–Sidak method). For figure 3, the significance of changes in baseline respiratory frequency and $\dot{V}_E$ after administration of varenicline or ABT 594 was assessed with Kruskal–Wallis one-way ANOVA on ranks (Tukey method), or one-way ANOVA, respectively. For figure 4, the significance of changes in respiratory frequency, and $\dot{V}_E$ after fentanyl administration was assessed with two-way repeated measures ANOVA (Holm–Sidak method), and the significance of changes in oxygen saturation after fentanyl administration was assessed with Mann–Whitney rank sum test. For figure 5, the significance of changes in duration of apneas induced by fentanyl or remifentanil was assessed with Mann–Whitney rank sum test, or independent *t* test, respectively. For figure 6, the significance of changes in respiratory frequency, $\dot{V}_E$, and oxygen saturation after morphine administration was assessed with two-way repeated measures ANOVA (Holm–Sidak method). For figure 7, the significance of changes in respiratory frequency, $\dot{V}_E$, and oxygen saturation after fentanyl administration was assessed with one-way ANOVA (Holm–Sidak method), Kruskal–Wallis one-way ANOVA on ranks (Dunn's Method), and two-way repeated measures ANOVA (Holm–Sidak method), respectively. For figure 8, the significance of changes in fentanyl-induced analgesia was assessed with one-way ANOVA (Holm–Sidak method). For figure 9, the significance of changes in $\dot{V}_E$ after lethal dose fentanyl was assessed with Kruskal–Wallis one-way ANOVA on ranks (Tukey test), or Mann–Whitney Rank Sum test. For figure 10, the significance of changes in $\dot{V}_E$ after fentanyl in combination with diazepam was assessed with Kruskal–Wallis one-way ANOVA on ranks (Tukey test). *Z* test was used for analysis of survival rate in response

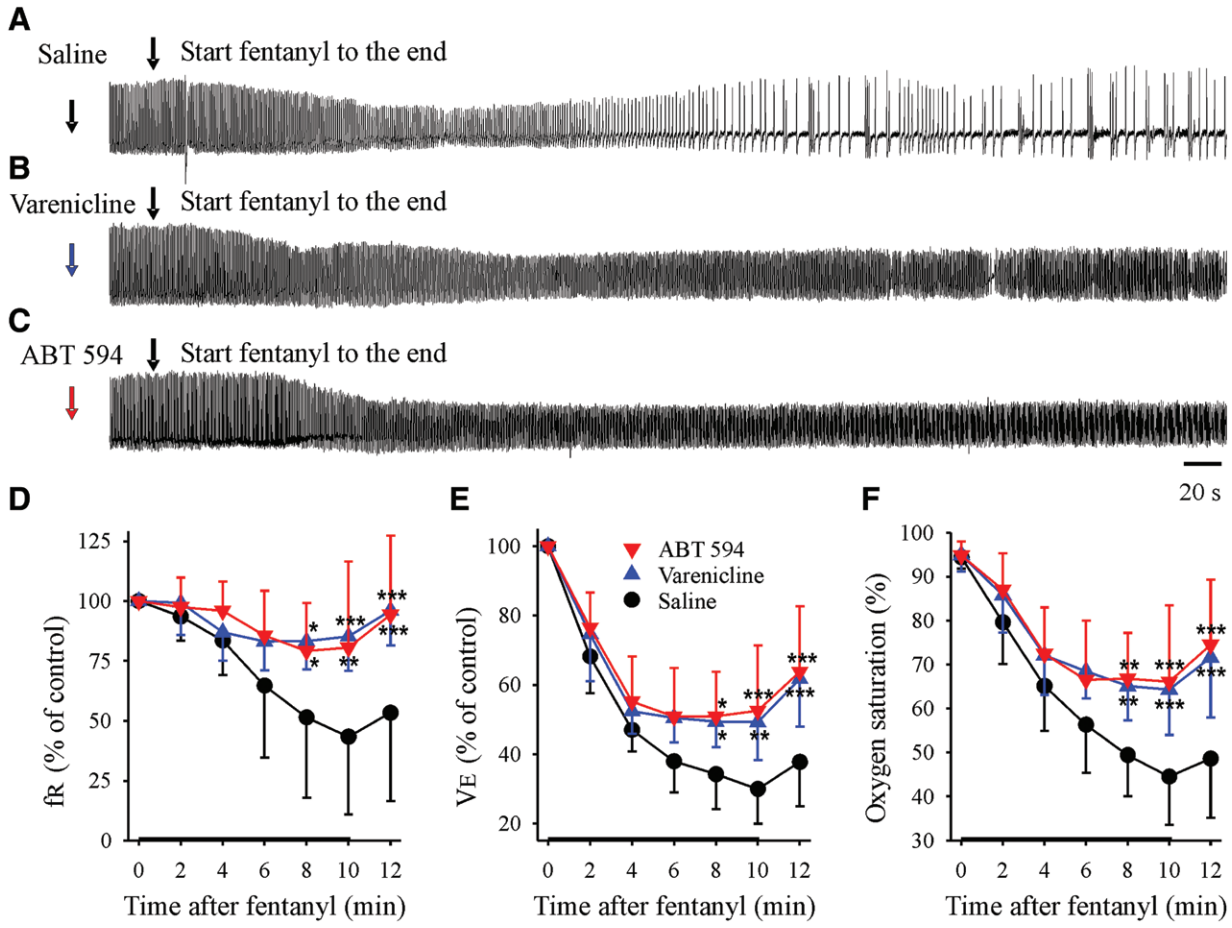

Fig. 2. Preadministration of varenicline or ABT 594 alleviated fentanyl-induced respiratory depression. (*A* and *B*) Representative whole-body plethysmographic recordings from three adult rats. (*A*) Seven minutes after saline (neck subcutaneously), administration of fentanyl (36 μg/kg over 10 min, iv) caused a marked depression of respiratory frequency (f_R) and minute ventilation ($\dot{V}_E$). (*B*) Preadministration of varenicline (1 mg/kg, subcutaneously) approximately 7 min before fentanyl reduced the fentanyl-induced decrease of f_R, and $\dot{V}_E$. (*C*) Preadministration of ABT 594 (30 μg/kg, subcutaneously) approximately 7 min before fentanyl reduced the fentanyl-induced decrease of f_R, and $\dot{V}_E$. (*D–F*) Population data (mean and SD) showing f_R (*D*), $\dot{V}_E$ (*E*) relative to control before fentanyl administration, and oxygen saturation (*F*) with preadministration of varenicline (1 mg/kg, subcutaneously), ABT 594 (30 μg/kg, subcutaneously), or saline approximately 7 min before fentanyl. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, statistically significant difference compared with saline group, using two-way repeated-measures ANOVA (Holm–Sidak method). n = 8 animals each data point.

to administration of lethal dose fentanyl alone (fig. 9), or in combination with diazepam (fig. 10).

Results

Preadministration of Varenicline or ABT 594 Markedly Reduced Fentanyl-induced Respiratory Depression

Vehicle (saline), varenicline (1 mg/kg, neck subcutaneously), or ABT 594 (30 μg/kg, subcutaneously) was injected 7 min before fentanyl administration (36 μg/kg, 10 min iv infusion, fig. 2). Fentanyl caused a marked respiratory frequency decrease in most vehicle-treated animals (fig. 2A). Preadministration of varenicline (fig. 2B) or ABT 594 (fig. 2C) reduced fentanyl-induced decrease in respiratory frequency, without effects on V_T. Population data (fig. 2, D–F) showed preadministration of varenicline or ABT 594 reduced fentanyl-induced decrease in respiratory frequency, $\dot{V}_E$, and arterial oxygen saturation for the whole duration of fentanyl administration. Specifically, 10-min administration of fentanyl induced a decrease in respiratory rate to 43 ± 32% of control in vehicle group, which was alleviated by preadministration of varenicline (85 ± 14% of control, n = 8, $P < 0.001$) or ABT 594 (81 ± 36% of control, n = 8, $P = 0.001$). Note that neither varenicline (1 mg/kg, subcutaneously) nor ABT 594 (30 μg/kg, subcutaneously) affected baseline breathing parameters (respiratory frequency or $\dot{V}_E$) when administered on its own (fig. 3).

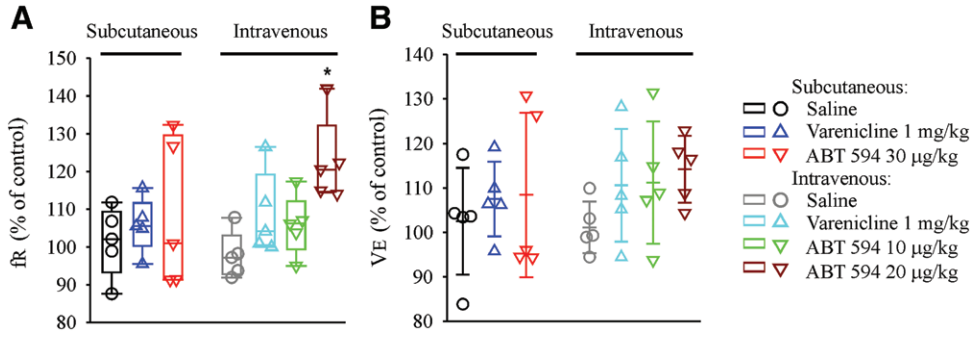

Fig. 3. The effects of varenicline and ABT 594 on the baseline breathing. Population data (median and interquartile ranges for *A*; mean and SD for *B*) showing the effects of administration of varenicline (1 mg/kg neck subcutaneous injection, intravenous injection) or ABT 594 (30 μg/kg, subcutaneous; 10, or 20 μg/kg, intravenous) or saline on respiratory parameters (*A*: respiratory frequency, f_R; *B*: minute ventilation, $\dot{V}_E$; relative to control before drug administration). *$P < 0.05$, statistically significant difference compared with saline group. Kruskal–Wallis one-way ANOVA on ranks (Tukey method) for *A*; one-way ANOVA for *B*. n = 5 animals for each group.

Coadministration of Varenicline or ABT 594 Reduced Respiratory Depression Caused by Fentanyl, Remifentanil, or Morphine

First, we tested whether coadministration of varenicline reduced marked respiratory depression caused by steady infusion of fentanyl over a 45-min period. Vehicle (saline) or varenicline (1 mg/kg, iv for 2 min) was injected at the beginning of fentanyl administration (90 μg/kg, 45 min iv infusion, fig. 4). Fentanyl caused a progressive respiratory frequency decrease in most vehicle-treated animals (fig. 4A). Coadministration of varenicline reduced the fentanyl-induced decrease in respiratory frequency, without marked effect on reducing V_T (fig. 4B). Population data (fig. 4, C–E) showed varenicline reduced fentanyl-induced decrease in respiratory frequency, $\dot{V}_E$, and oxygen saturation for the whole duration of fentanyl administration (45 min).

Second, we tested whether coadministration of ABT 594 reduced respiratory depression and apneas caused by a shorter application of fentanyl or remifentanil. Coadministration of fentanyl (12 μg/kg, 1 min iv) and vehicle caused progressive decrease of respiratory frequency and V_T, leading to profound apneas in some rats (fig. 5A) or in another cohort of rats, initial apneas followed by a rapid recovery of respiratory frequency (fig. 5B), whereas, coadministration of ABT 594 (20 μg/kg, 1 min iv) and fentanyl (12 μg/kg, 1 min iv) decreased fentanyl-induced respiratory depression and apnea (fig. 5C). Coadministration of remifentanil (5 μg/kg, iv, 20 s) and saline caused marked apneas (fig. 5D), whereas coadministration of ABT 594 (20 μg/kg, iv, 20 s) decreased remifentanil-induced apneas (fig. 5E). Population data showed ABT 594 reduced apneas caused by fentanyl (fig. 5F) and remifentanil (fig. 5G).

Third, we tested whether coadministration of varenicline or ABT 594 reduced morphine-induced respiratory depression. Vehicle (saline), varenicline (1 mg/kg, iv over 1 to 2 min), or ABT 594 (20 μg/kg, iv over 1 to 2 min) was injected at the beginning of morphine administration (18 mg/kg, 4 min iv infusion, fig. 6). Coadministration of vehicle with morphine caused an approximately 15-min respiratory depression in respiratory frequency and V_T (fig. 6A). Coadministration of varenicline (fig. 6B) or ABT 594 (fig. 6C) drastically reduced the morphine induced decrease in respiratory frequency, without effects on V_T. Population data (fig. 6D–F) showed coadministration of varenicline or ABT 594 reduced morphine induced decrease in respiratory frequency, $\dot{V}_E$, and oxygen saturation for the period measured after the administration of morphine.

Note on Tidal Volume Changes with Opioids, Varenicline, and ABT 594

Opioids, in addition to suppressing respiratory frequency and causing apneas, also caused a decrease in V_T. There is typically a compensatory rebound in V_T during the period of severe depression of respiratory frequency. We have determined in past studies[22] that the reduced V_T is most likely in part attributable to decreased drive to respiratory motoneurons and a larger component results from opioid-induced muscle rigidity and rib cage stiffness. The rigidity that is particularly pronounced in rats is a well-documented phenomenon, possibly involving striatal μ-opioid receptors.[23] Neither varenicline nor ABT 594 appeared to reduce the visible muscle rigidity, and thus the reduction in V_T caused by opioids persisted.

Reversal of Marked Fentanyl-induced Respiratory Depression by Varenicline or ABT 594

A 10-min iv infusion of 30 μg/kg fentanyl caused significant respiratory depression in respiratory frequency in most rats within 7 min after fentanyl administration. Subsequent injection of saline vehicle (fig. 7A) did not change the

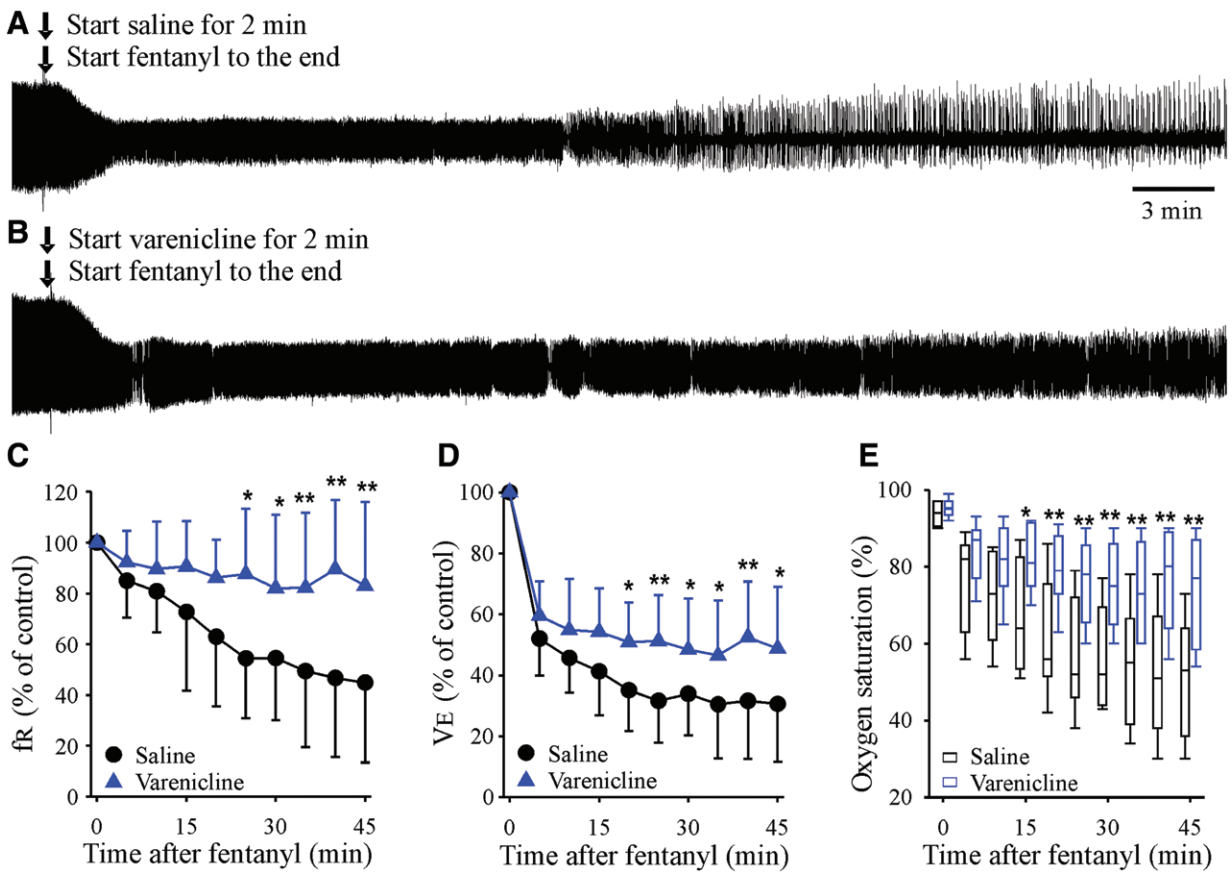

Fig. 4. Coadministration of varenicline alleviated fentanyl-induced respiratory depression. (*A* and *B*) Representative whole-body plethysmographic recordings from two adult rats. Fentanyl was administered at a dose of 90 μg/kg over 45 min (iv infusion), and saline or varenicline was administered (1 mg/kg, iv, 2 min) at the beginning of fentanyl administration. (*A*) Coadministered with saline, fentanyl caused a marked depression of respiratory frequency (f_R) and minute ventilation ($\dot{V}_E$). (*B*) Coadministration with varenicline reduced the fentanyl-induced decrease of f_R, and $\dot{V}_E$. (*C–E*) Population data showing f_R (*C*), $\dot{V}_E$ (*D*) relative to control before fentanyl administration (mean and SD), and oxygen saturation (*E*, median and interquartile ranges). **P* < 0.05, ***P* < 0.01, statistically significant difference compared with saline group, using two-way repeated-measures ANOVA (Holm–Sidak method) for *C* and *D*, or Mann–Whitney rank sum test for *E*. n = 9 animals each data point.

course of fentanyl action. In contrast, subsequent iv injection of varenicline (1 mg/kg, fig. 7B) or ABT 594 (20 μg/kg, fig. 7C) reversed the fentanyl-induced decrease in respiratory frequency, without effects on V_T. Population data (fig. 7, D–F) showed varenicline (0.5 to 1 mg/kg) or ABT 594 (10 to 20 μg/kg) dose-dependently reversed fentanyl-induced decrease in respiratory frequency, $\dot{V}_E$, and arterial oxygen saturation. The onset of the reversal of fentanyl-induced decrease of respiratory frequency by varenicline (1 mg/kg) or ABT 594 (20 μg/kg) was rapid on average although with considerable variability between animals (varenicline: 23 ± 18 s, n = 5; ABT 594: 23 ± 13 s, n = 5). The reversal with naloxone was rapid and less variable (0.3 mg/kg, 11 ± 5 s, n = 4, *P* = 0.378, one-way ANOVA). The reversal of the opioid-induced decrease of respiratory frequency persisted for the complete duration of fentanyl administration (20 min) in all animals tested with varenicline (1 mg/kg, n = 4) or ABT 594 (20 μg/kg, n = 4) and all animals remained sedated. In contrast, all animals became alert within 1 to 3 min after naloxone administration (0.3 mg/kg, n = 4), in an apparent agitated state, disrupting the continuous infusion of fentanyl. Note that neither varenicline (1 mg/kg, iv) nor low-dose ABT 594 (10 μg/kg, iv) affected baseline breathing parameters (respiratory frequency or $\dot{V}_E$) when administered on its own. However, administration of a high dose of ABT 594 (20 μg/kg, iv) increased baseline respiratory frequency without an effect on $\dot{V}_E$ on its own (fig. 3, C and D). Consistent with previous studies of varenicline[12] and ABT 594,[7,8] we did not observe behavioral side effects.

Fentanyl-induced Sedation Was Slightly Alleviated by Varenicline and ABT 594

In addition to respiratory depression, unintended sedation is another serious opioid-induced adverse event which

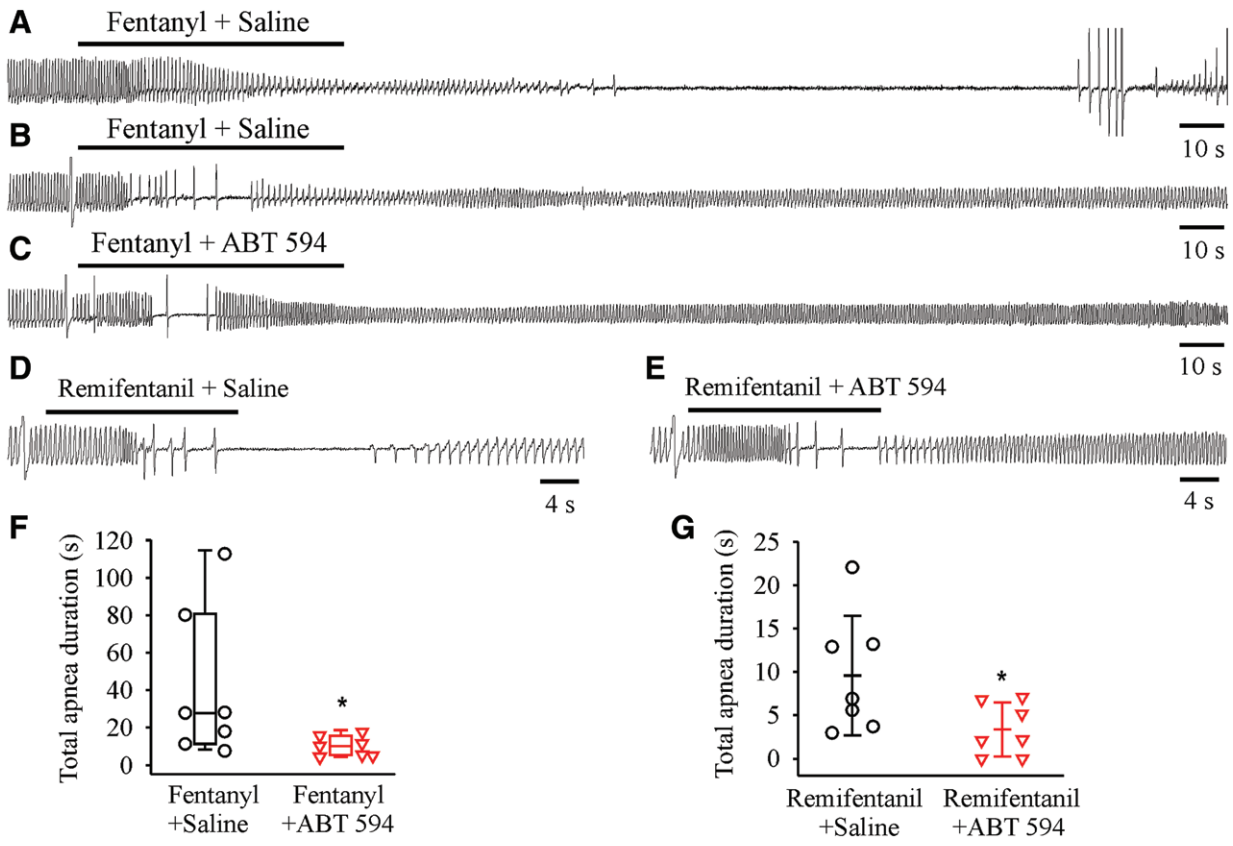

Fig. 5. Coadministration of ABT 594 reduced fentanyl- or remifentanil-induced respiratory depression and apneas. (*A*) Coadministration of fentanyl (12 μg/kg over 1 min, iv infusion) with saline vehicle (1 min iv infusion) caused a marked progressive respiratory depression in respiratory frequency and tidal volume, leading to profound apneas in a rat. (*B*) In another cohort, coadministration of fentanyl (12 μg/kg over 1 min, iv infusion) with saline vehicle (1 min iv infusion) caused marked initial apneas, with a quick recovery in respiratory frequency in a rat. (*C*) Coadministration of fentanyl (12 μg/kg over 1 min, iv infusion) with ABT 594 (20 μg/kg over 1 min, iv infusion) alleviated fentanyl-induced decrease in respiratory frequency, with fewer effects on fentanyl-induced initial apneas and no effect on the reduction of tidal volume in a rat. (*D*) Coadministration of remifentanil (5 μg/kg over 20 s, iv) and saline (iv) caused a marked respiratory depression and apneas. (*E*) Coadministration of remifentanil (5 μg/kg over 20 s, iv) with ABT 594 (20 μg/kg over 20 s, iv) alleviated remifentanil-induced respiratory depression and apneas. Population data (median and interquartile ranges for *F*; mean and SD for *G*) showing coadministration of ABT 594 (20 μg/kg, iv) decreases duration of apneas induced by fentanyl (*F*) or remifentanil (*G*). $^*P < 0.05$, statistically significant difference compared with saline group, using Mann–Whitney rank sum Test for fentanyl (*F*), and independent *t* test for remifentanil (*G*). n = 7 animals for each group.

contributes to patient morbidity and increased length of hospitalization.[24] Although the underlying mechanisms are not fully understood, opioid-induced sedation is thought to involve the anticholinergic activity of opioids.[25] Thus, we tested the hypothesis that varenicline (1 mg/kg, subcutaneously, 7 min before fentanyl) or ABT 594 (30 μg/kg, subcutaneously, 7 min before fentanyl) would reduce the sedation induced by fentanyl (36 μg/kg over 10 min iv infusion). The sedation (loss of righting reflex) was modestly, but statistically significantly, shortened in the varenicline- or ABT 594–treated group compared with saline group (varenicline: 20 ± 3 min after fentanyl, $P = 0.018$; ABT 594: 21 ± 4 min after fentanyl, $P = 0.047$ *vs.* saline: 25 ± 4 min after fentanyl, n = 8 each, one-way ANOVA, Holm–Sidak method).

Fentanyl-induced Analgesia in Adult Rats Was Unaffected by Varenicline but Mildly Enhanced by ABT 594

We assessed the effects of varenicline (1 mg/kg, subcutaneously, 7 min before fentanyl), or ABT 594 (30 μg/kg, subcutaneously, 7 min before fentanyl) on fentanyl- (36 μg/kg over 10 min iv infusion) induced analgesia. Fentanyl administration (pretreatment with saline) induced marked analgesia as measured by the formalin test, whereas pretreatment of varenicline did not affect fentanyl-induced analgesia (fig. 8). However, pretreatment of ABT 594 mildly enhanced fentanyl-induced analgesia in both phases of formalin test (fig. 8). We did not repeat previous work examining the effects of varenicline or ABT 594 on basal

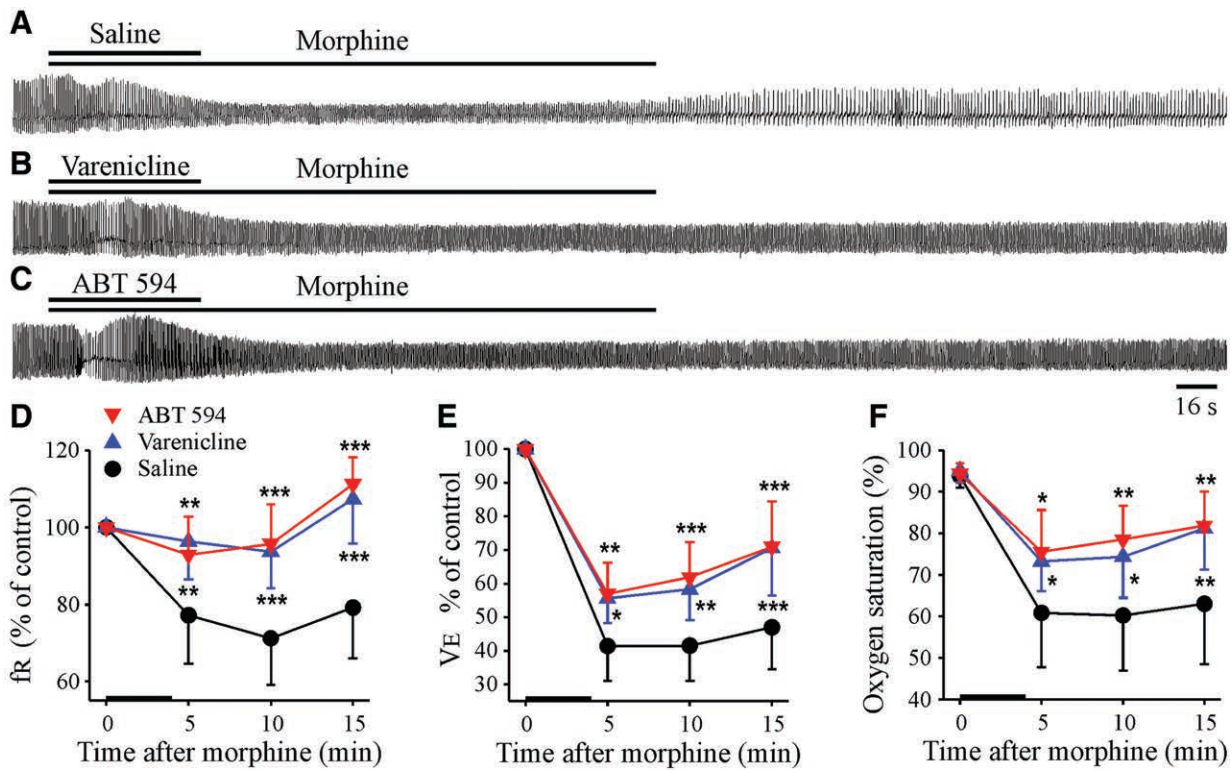

Fig. 6. Coadministration of varenicline or ABT 594 reduces morphine induced respiratory depression. (*A*) Coadministration of saline vehicle (iv over 1 min) with morphine (18 mg/kg over 4 min, iv infusion) caused a marked respiratory depression in frequency (f_R) and tidal volume (V_T). (*B*) Coadministration of varenicline (1 mg/kg, iv over 1 min) with morphine (18 mg/kg over 4 min, iv infusion) alleviated morphine-induced decrease in f_R, without effect on V_T. (*C*) Coadministration of ABT 594 (20 μg/kg iv over 1 min) with morphine (18 mg/kg over 4 min, iv infusion) alleviated morphine induced decrease in f_R, without effect on V_T. (*D–F*) Population data (mean and SD) showing f_R (*D*), $\dot{V}_E$ (*E*) relative to control before morphine administration, and oxygen saturation (*F*). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, statistically significant difference compared with saline group, using two-way repeated-measures ANOVA (Holm-Sidak method). n = 10, 6, 6 animals each data point for saline, varenicline, or ABT 594 treatment, respectively.

nociceptive response to formalin injection. A previous study has shown that varenicline (2 to 3 mg/kg, subcutaneously) dose-dependently decreased nocifensive response in mouse formalin test, without effects at the low dose of 1 mg/kg.[12] Previous studies have shown that ABT 594 (10 to 100 μg/kg intraperitoneal) produced statistically significant dose-dependent antinociceptive effects in rat hot box and formalin test.[7,8]

Effects of Varenicline and ABT 594 to Counter a Lethal Dose of Fentanyl

First, we tested the effects of varenicline, ABT 594, or naloxone alone on fentanyl lethality. Administration of a high dose of fentanyl (120 μg/kg over 1 min, iv) caused immediate apneas. Subsequent saline injection had no effect on fentanyl-induced apneas (fig. 9A) and all four rats died. Apnea also persisted after subsequent administration of varenicline (1 mg/kg, iv) and all four rats died. However, subsequent administration of ABT 594 (30 μg/kg, iv) decreased the apneas (fig. 9B), and all four rats treated survived, without any sign of seizure or other behavioral dysfunctions. Administration of high doses of naloxone (0.03 mg/kg, n = 2; or 1 mg/kg, iv, n = 2) reliably reversed the apneas (fig. 9C) and all four rats treated survived and became alert within 10 min after naloxone administration, in an apparent agitated state. Note that the effect of ABT 594 (onset: 125 ± 48 s, $P = 0.036$) was slower relative to naloxone (onset: 58 ± 14 s). A lower dose of naloxone (1 μg/kg, iv) did not reliably rescue animals, and two of four died of lethal apnea (fig. 9D). We further tested whether a low dose of naloxone in conjunction with varenicline could reliably rescue from lethal apnea. Data in figure 9E show the results of administration of a low dose of naloxone (1 μg/kg, iv) followed by administration of varenicline (1 mg/kg, iv). The fentanyl-induced apneas were reversed and all four rats survived. Overall, ABT 594 alone, high doses of naloxone alone, and varenicline in combination with low dose of naloxone were reliable for rescuing from

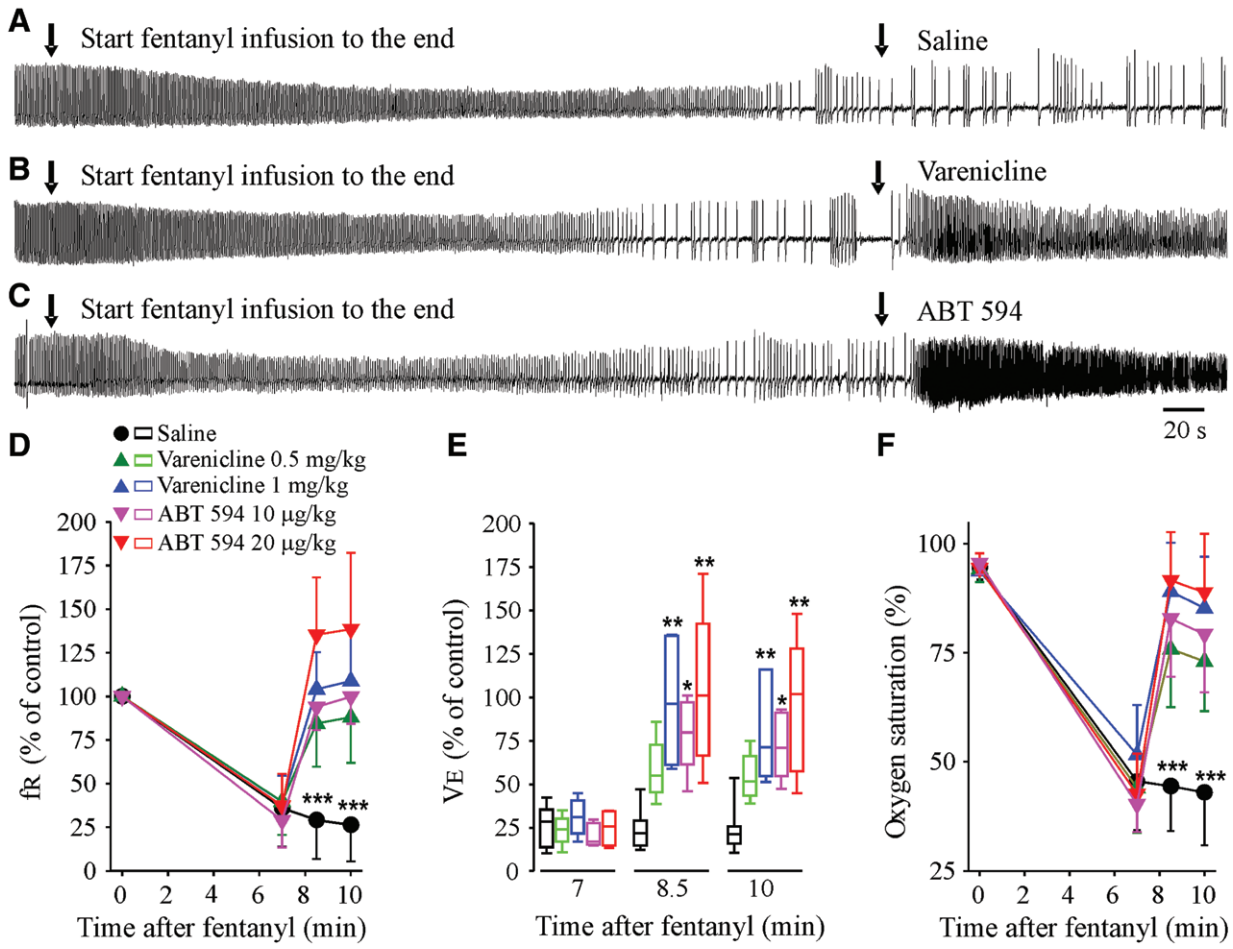

Fig. 7. Administration of varenicline or ABT 594 dose-dependently reversed fentanyl-induced respiratory depression. Administration of fentanyl (30 μg/kg over 10 min, iv infusion) caused a marked decrease in respiratory frequency (f_R) and minute ventilation ($\dot{V}_E$) in whole body plethysmographic recordings from 3 rats. (*A*) Subsequent administration of saline vehicle (iv) had no effect on fentanyl-induced respiratory depression. (*B*) Subsequent administration of varenicline (1 mg/kg, iv bolus, approximately 7 min after fentanyl) reversed fentanyl-induced respiratory depression. (*C*) Subsequent administration of ABT 594 (20 μg/kg, iv bolus, approximately 7 min after fentanyl) reversed fentanyl-induced respiratory depression. (*D–F*) Population data (mean and SD for *D* and *F*; median and interquartile ranges for *E*) showing changes of respiratory parameters (*D*: f_R; *E*: $\dot{V}_E$; relative to control prior to fentanyl administration) or arterial oxygen saturation (*F*) after administration of saline, varenicline (0.5, 1 mg/kg, iv), or ABT 594 (10, 20 μg/kg, iv). $^{**}P < 0.01$, $^{***}P < 0.001$, statistically significant difference between saline-treated group and other four drug-treated groups, using one-way ANOVA (Holm–Sidak method) for *D*, or two-way repeated-measures ANOVA (Holm–Sidak method) for *F*. $^{*}P < 0.05$, statistically significant difference compared with saline treated group using Kruskal–Wallis one-way ANOVA on Ranks (Dunn's method) for *E*. n = 10 animals each data point for saline treatment, n = 5 animals each data point for other treatments.

lethal apneas (fig. 9F–H). Neither varenicline nor ABT 594 provided the very rapid and complete reversal of apnea after a lethal bolus of fentanyl that was achieved with high doses of naloxone (0.03 to 1 mg/kg). Note that the duration of sedation is drastically shorter in the high doses of naloxone group (0.03 to 1 mg/kg, 6 ± 3 min, n = 4, one-way ANOVA, Holm–Sidak method) compared with the ABT 594 treatment (30 μg/kg: 33 ± 12 min, n = 4, $P = 0.006$) or 1 μg/kg naloxone plus varenicline group (32 ± 9 min, n = 4, $P = 0.005$).

Varenicline and ABT 594 Counter Lethal Apnea Caused by Combination of Fentanyl and Diazepam

Coadministration of fentanyl (50 μg/kg, iv) with diazepam (9 mg/kg, iv) over a short period (1 min) induced lethal apnea. Subsequent administration of saline vehicle had no effect on the severe respiratory depression and lethal apnea; all four rats tested died (fig. 10A). Administration of varenicline (1 mg/kg, iv) reduced the period of apneas and the animals were capable of generating a complex pattern of gasping and breathing that eventually normalized; all four

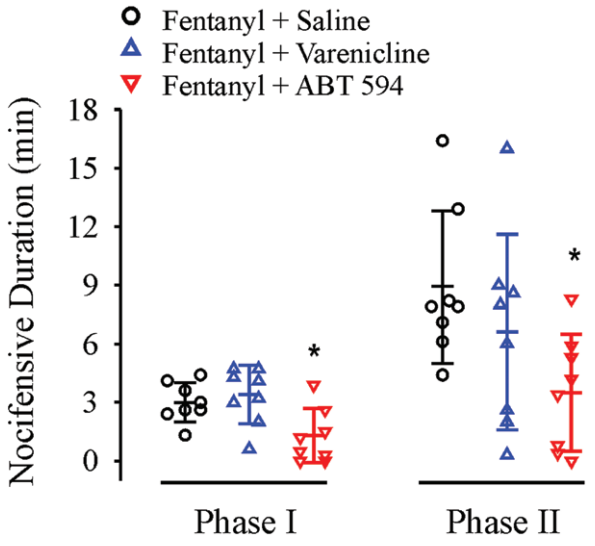

Fig. 8. The effects of varenicline and ABT 594 on fentanyl-induced analgesia. After preadministration (neck subcutaneously, at 7 min before fentanyl) of saline, varenicline (1 mg/kg), ABT 594 (30 μg/kg), the effects of fentanyl administration (36 μg/kg over 10 min iv infusion) on the time (mean and SD) spent engaging in nociceptive behaviors (licking and lifting response of the injured paw to formalin injection) in the phase I and phase II (0 to 5 min, 20 to 40 minutes after formalin). Formalin (50 μl, 1.5% formalin diluted in saline) was injected into the intraplantar region of the right hind paw at 35 min after fentanyl. *$P < 0.05$, statistically significant difference compared with saline group, using one-way ANOVA (Holm–Sidak method). n = 8 animals for each group.

rats tested survived (fig. 10B). Administration of ABT 594 (30 μg/kg, iv) also reduced the period of apnea and a similar complex pattern of breathing leading to a normalized pattern and the survival of all four rats tested (fig. 10C). Population data show that both varenicline and ABT 594 rescue lethal apneas caused by coadministration of fentanyl and diazepam (fig. 10, D and E). Note all rats survived with administration of fentanyl (50 μg/kg, 1 min iv, n = 4) alone or diazepam (9 mg/kg, 1 min iv, n = 2) alone. The median lethal dose for diazepam is 32 mg/kg (http://www.pfizer.com/files/products/material_safety_data/PZ00145.pdf; accessed July 5, 2019).

Discussion

The primary goal of this study was to build on the previous proof-of-principle study showing that activation of α4β2 nicotinic acetylcholine receptors was effective for countering opioid-induced respiratory depression.[5] Specifically, we sought to identify effective compounds that have been advanced to clinical trials for other indications and thus would be amenable to early stage clinical trials for the novel use of countering respiratory depression. Varenicline is currently used clinically for smoking cessation. It is relevant in the context of pain control that varenicline has been demonstrated to have analgesic properties of its own in rodent models[12] and thus may also work to enhance the effects of opioids. ABT 594 has broad spectrum analgesic activity in rodent models of acute, persistent, and neuropathic pain.[7–9] Thus, it too may provide the added advantage of allowing for opioid sparing to achieve a desired level of analgesia. ABT 594 passed through Phase I clinical trials and was shown to be effective in Phase II trials in patients with diabetic peripheral neuropathic pain, but with dose-dependent side effects (nausea, dizziness, vomiting, abnormal dreams, and asthenia) and discontinuation rates with use over multiple days.[13,14] ABT 594 may be more suitable for acute rather than chronic administration. Pre- or coadministration of varenicline or ABT 594 with opioids markedly reduced the degree of respiratory depression caused by fentanyl, remifentanil, and morphine at concentrations that did not alter baseline minute ventilation on their own. Whereas varenicline had no effect on fentanyl-induced analgesia, ABT 594 potentiated fentanyl-induced analgesia. Thus, the strategy of administering one of the α4β2 nicotinic acetylcholine receptor–activating agents along with an opioid could increase the safety margin against the onset of severe respiratory depression that would enhance or at least not interfere with analgesia. Currently there are no agents that could serve that purpose in the clinic.

We demonstrated that varenicline and ABT 594 were effective at reversing moderate to severe respiratory depression induced by the administration of fentanyl. Importantly, neither compound interfered with opioid-induced suppression of pain or altered baseline breathing at the effective concentrations. Further, ABT 594 moderately enhanced the fentanyl-induced analgesia. Thus, they show the potential to address the unmet clinical need of providing a drug therapy that would reverse opioid-induced respiratory depression without interfering with, or in some cases enhancing, analgesia. This would clearly be advantageous in situations in which a patient is demonstrating suppression of breathing attributable to opioids but where pain management is a priority.

We then examined whether ABT 594 and varenicline would be effective against a rapid infusion of a high dose of fentanyl that is typically lethal. Naloxone is very effective at reversing opioid-induced respiratory depression in such a scenario, but there are some indications that may warrant the development of supplementary approaches.[21,26,27] The half-life of a naloxone in humans is approximately 15 to 30 min, and thus it is necessary in some instances to retain the subject for additional administrations of naloxone to counter the opioids that are cleared from the system over a longer time frame.[21,26,27] A drug, as is the case for varenicline and ABT 594, with a longer half-life of over hours[28,29] could eliminate that problem. Further, after naloxone administration the subject will rapidly become alert, often in a very agitated

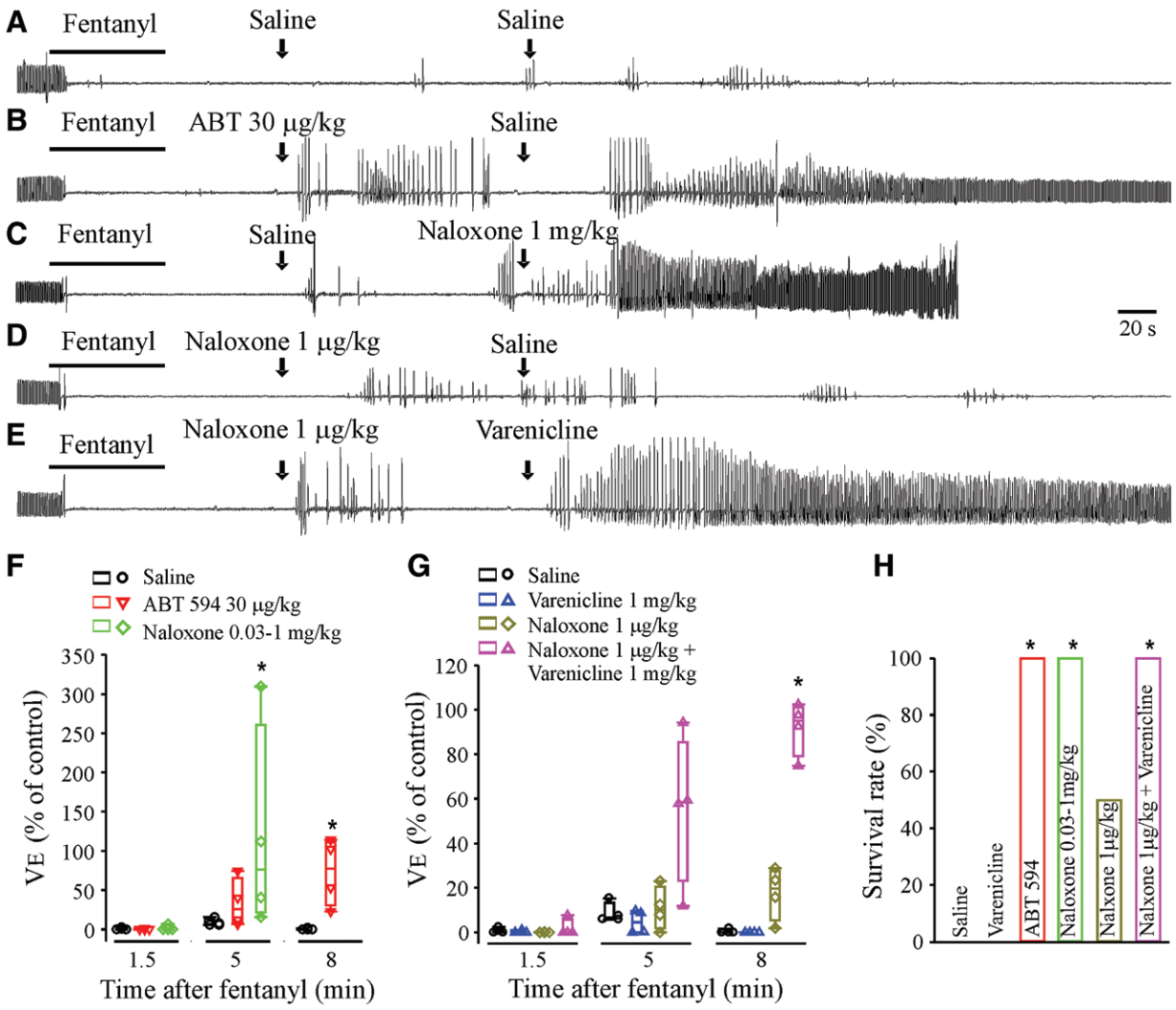

Fig. 9. Administration of a low dose of naloxone followed by varenicline reversed fentanyl-induced lethal apnea. All drugs were administrated bolus iv. Administration of a high dose of fentanyl (120 μg/kg) over a short period (1 min) induced lethal apnea. (*A*) Saline vehicle injection post fentanyl administration had no effects on profound, severe respiratory depression, apnea, and lethality. (*B*) Administration of a high dose of ABT 594 (30 μg/kg) reduced apneas and resulted in the recovery of respiratory rhythm and rat survival. (*C*) Administration of a high dose of naloxone (1 mg/kg) reduced apneas and resulted in a rapid recovery of respiratory rhythm and rat survival; the rat woke up within 4 min after naloxone administration, agitated. (*D*) Administration of a low dose of naloxone (1 μg/kg) had no marked effects on apneas and lethality. (*E*) Administration of a low dose of naloxone (1 μg/kg), followed by varenicline (1 mg/kg), reduced the period of apnea and induced the quick recovery of respiratory rhythm and survival. (*F–H*) Population data showing the time course of changes of minute ventilation ($\dot{V}_E$, relative to before fentanyl, *F*, *G*, median and interquartile ranges), and survival rate (*H*) in response to administration of lethal dose fentanyl (120 μg/kg) with postadministration of bolus saline, varenicline (1 mg/kg), ABT 594 (30 μg/kg), high doses of naloxone (0.03 to 1 mg/kg), low dose of naloxone (1 μg/kg), low dose of naloxone (1 μg/kg) followed by varenicline (1 mg/kg). *$P < 0.05$, statistically significant difference compared with saline group (*F*), compared with both saline and varenicline groups (*G* and *H*). Kruskal–Wallis one-way ANOVA on ranks (Tukey test), or Mann–Whitney rank sum test for analysis of $\dot{V}_E$ in *F*; Kruskal–Wallis one-way ANOVA on ranks (Tukey test) for analysis of $\dot{V}_E$ in *G*, *z* test for analysis of survival rate (*H*). n = 4 animals for each group.

state. An agent that could be administered to counter respiratory depression but not result in the immediate loss of sedation would provide improved management for emergency medical staff. We observed that administration of varenicline did not effectively reverse respiratory depression and death caused by a bolus lethal dose of fentanyl. ABT 594 (30 μg/kg) did prevent lethality, but the reversal of respiratory depression was much slower relative to that achieved with naloxone. High doses of naloxone (0.03 to 1 mg/kg) induced a rapid and complete reversal of respiratory depression (often with overshoot of $\dot{V}_E$) and rescued all animals.

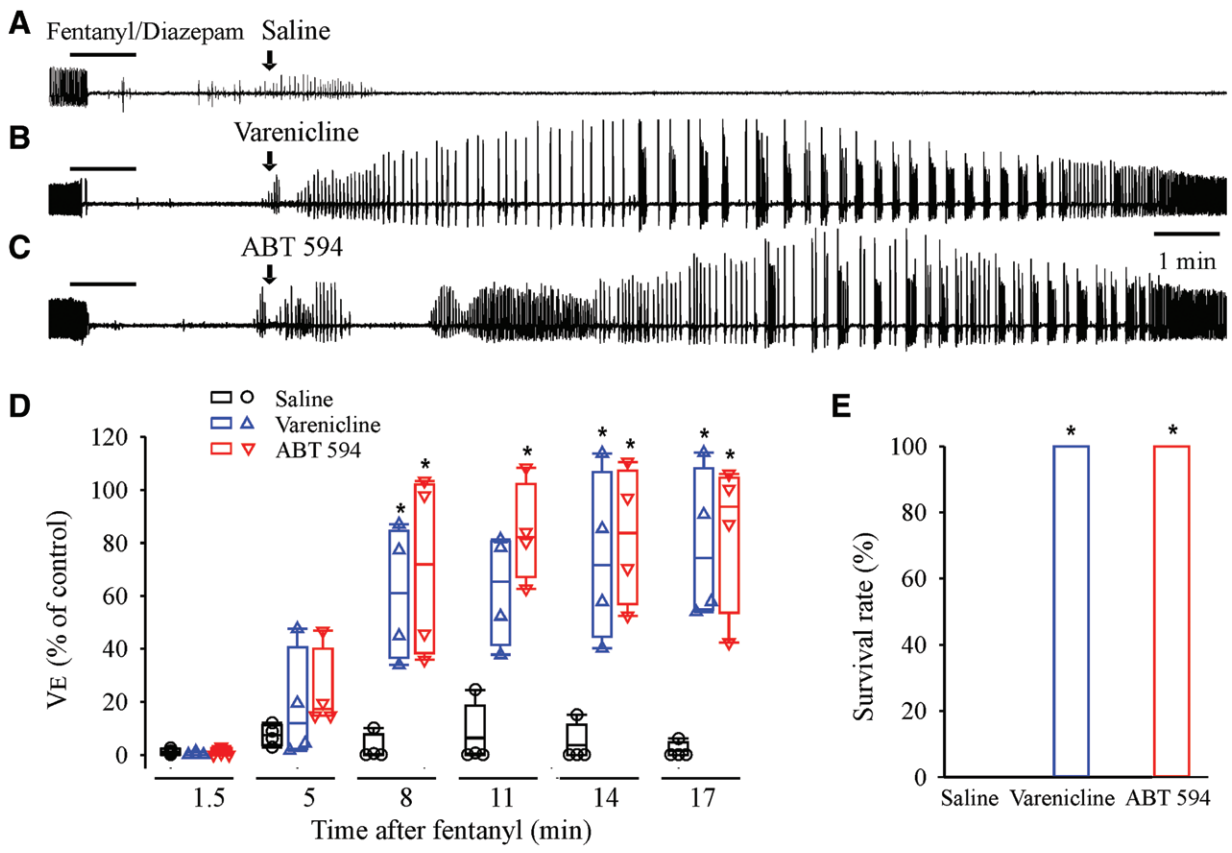

Fig. 10. Subsequent administration of varenicline or ABT 594 partially reverses lethal apnea induced by coadministration of fentanyl and diazepam. All drugs were administrated intravenously. Coadministration of fentanyl (50 μg/kg) with diazepam (9 mg/kg) over a short period (1 min) induced lethal apnea. Saline vehicle or drug was administered at 2 to 4 min after fentanyl and diazepam administration. (*A*) Administration of saline did not alter the respiratory depression and the rat died. (*B*) Administration of varenicline (1 mg/kg) reduced the period of apnea and degree of respiratory depression and the rat survived. (*C*) Administration of ABT 594 (30 μg/kg) reduced the period of apnea and suppression of respiratory rhythm and the rat survived. (*D*) Population data (median and interquartile ranges) showing the time course of changes of minute ventilation ($\dot{V}_E$ relative to that prior to fentanyl/diazepam). *$P < 0.05$, statistically significant difference compared with saline group, using Kruskal–Wallis one-way ANOVA on ranks (Tukey test). (*E*) Survival rate: *$P < 0.05$, statistically significant difference compared with saline group, using *z* test. n = 4 animals for each group.

We then pursued experiments to determine whether varenicline would accentuate the ability of a low dose of naloxone (1 μg/kg) to counter fentanyl-induced respiratory depression. There was a clear synergistic effect of varenicline with the low dose of naloxone. These data suggest that it is worth further investigating whether varenicline could be used in conjunction with low-dose naloxone to provide a strategy that would provide a longer half-life of protection and a less pronounced reversal of opioid-induced sedation and onset of an agitated state of withdrawal.

There is an additional problem with opioids and overdose attributable to the associated use with benzodiazepines such as diazepam, alprazolam, and clonazepam. Benzodiazepines are commonly prescribed for anxiety or to treat insomnia. The following data highlight the significance of the problem. Among 70,237 drug overdose deaths in the United States in 2017, 47,600 (67.8%) involved opioids and 11,537 (16.4%) involved benzodiazepines.[3,30] More than 30% of overdoses involving opioids also involve benzodiazepines.[31] The overdose death rate among patients receiving both drugs was 10 times higher than among those only receiving opioids.[16] Drugs such as benzodiazepines and barbiturates can suppress breathing *via* facilitation of inhibitory GABAergic transmission in respiratory networks.[32] Naloxone can counter the opioid-induced suppression of breathing by antagonizing opioid receptor signaling but will not act on the benzodiazepine-induced depression *via* enhanced GABAergic transmission. We hypothesized that varenicline and ABT 594 would be effective. We observed a reversal of respiratory depression and increased survival in the presence of lethal doses of coadministered fentanyl and diazepam. Thus, this is an additional impetus to further

investigate a role for varenicline and ABT594 as an adjunct therapy to naloxone to the treatment of severe drug-induced apnea outside the clinic.

Experiments were performed on male rats, and although we are not aware of any marked sex differences for opioid-induced respiratory depression or response to nicotinic receptor agonists, the possibility of sex-related differences in the combinatorial actions of compounds should be considered.

Summary

Collectively, these data further support the fact that activation of α4β2 nicotinic acetylcholine receptors is a potential means of countering opioid-induced respiratory depression without interfering with analgesia. Varenicline and ABT 594, based on past preclinical and clinical work for other indications, have the potential to be tested in early-stage clinical trials for opioid-induced respiratory depression. This will clearly be important to evaluate the relevance of these rodent data to use in humans. However, there have been previous findings in rodent preclinical studies toward countering opioid-induced respiratory depression that showed similar promise that have not yet translated to clinical use. This could in part be attributable to unrecognized differences in the susceptibility of rodents and humans to opioid-induced respiratory depression. This includes serotonergic agents, calcium-activated potassium channel blockers, ketamine, and ampakines.[22,33–41] Ampakines, for example, work well to counter opioid-induced respiratory depression in rodents,[22,36] but there were mixed results from Phase IIa clinical trials.[37,38] There was efficacy against doses of fentanyl that caused moderate levels of respiratory depression[37] but lack of efficacy against administration of remifentanil that caused pronounced respiratory depression.[38] Further, there has yet to be the development of injectable formulation of ampakines. The nicotinic acetylcholine receptor–targeting agents have the distinct advantage of being amenable for delivery in multiple formulations, including as an injectable. Given the unmet clinical need of identifying an agent that can counter opioid-induced respiratory depression while maintaining analgesia and the need for additional means of countering the alarming incidence of overdose, we propose that these preclinical data warrant the consideration of varenicline and ABT 594 for early stage human trials.

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Competing Interests

A patent application for the use of nicotinic receptor agonists for treating multiple causes of respiratory depression, including opioids, has been submitted by the University of Alberta (Edmonton, Alberta, Canada).

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