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Intravenous and intratracheal thyrotropin releasing hormone and its analog taltirelin reverse opioid-induced respiratory depression in isoflurane anesthetized rats.

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Running Title Page

TRH reversal of opioid-induced respiratory depression

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Abbreviations: TRH, thyrotropin releasing hormone; OIRD, opioid-induced respiratory depression

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Abstract

Thyrotropin releasing hormone (TRH) is a tripeptide hormone and a neurotransmitter widely-expressed in the CNS that regulates thyroid function and that maintains physiologic homeostasis. Following injection in rodents, TRH has multiple effects including increased blood pressure and breathing. We tested the hypothesis that TRH and its long-acting analog, taltirelin, will reverse morphine-induced respiratory depression in anesthetized rats following intravenous(IV) or intratracheal(IT) administration. TRH (1 mg/kg plus 5 mg/kg/hour IV) and talitrelin (1 mg/kg IV), when administered to rats pretreated with morphine (5 mg/kg IV), increased ventilation from 50±6 to 131±7% and 45±6 to 168±13% (percent baseline; n=4±SEM), primarily through increased breathing rates (from 76+/-9 to 260±14% and 66±8 to 318±37%). By arterial blood gas analysis, morphine caused a hypoxemic respiratory acidosis with decreased oxygen and increased carbon dioxide pressures. TRH decreased morphine effects on arterial carbon dioxide pressure, but failed to impact oxygenation; taltirelin reversed morphine effects on both arterial carbon dioxide and oxygen. Both TRH and talirelin increased mean arterial blood pressure in morphine-treated rats (from 68±5 to 126±12% and 64±7 to 116±8%, respectively; n=3 to 4). TRH, when initiated prior to morphine (15 mg/kg IV), prevented morphine-induced changes in ventilation; and TRH (2 mg/kg IV) rescued all four rats treated with a lethal dose of morphine (5 mg/kg/min until apnea). Similar to IV administration, both TRH (5 mg/kg IT) and taltirelin (2 mg/kg IT) reversed morphine effects on ventilation. TRH or taltirelin may have clinical utility as an IV or inhaled agents to antagonize opioid-induced cardiorespiratory depression.

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Introduction

Thyrotropin releasing hormone (TRH; Protirelin) is a processed, carboxyamidated tripeptide (L-pyro-glutamyl-L-histidyl-L-prolinamide) originally isolated from the hypothalamus, where it regulates pituitary release of thyroid-stimulating hormone and prolactin. However, TRH is expressed widely in the central nervous system and may provide neurostimulatory, regulatory, and trophic effects. In rodents, TRH mediates physiologic effects through two homologous G-protein coupled receptors, TRH1 and TRH2, however, only a single human TRH receptor has been identified(Matre et al., 1993; Straub et al., 1990). Other additional receptors may exist and remain to be identified(Hinkle et al., 2002; Hogan et al., 2008; Kelly et al., 2015).

TRH is also an FDA approved drug with numerous non-endocrine effects upon exogenous administration (e.g., increases in locomoter activity, body temperature/oxygen consumption, heart rate/blood pressure, breathing, anorexia, and neural regeneration). TRH has been studied as a therapy for multiple human conditions including neurodegenerative diseases such amyotropic lateral sclerosis (ALS) and spinal cord injury as well as epilepsy, depressed mood, fatigue, and shock(Khomane et al., 2011). However, TRH development as a therapeutic is limited by its poor and inconsistent bioavailability; and, for most applications, its neuroendocrine effects are undesired. TRH is unstable in blood with a half-life of 5 mins or less. Due to its instability and high aqueous solubility (cLogP -2.8), only a small fraction of TRH administered intravenously/systemically crosses the blood brain barrier, and its oral bioavailabilty is poor(Khomane et al., 2011). Though most studies administer TRH by direct injection (e.g., by intracerebroventricular, intravenous, subcutaneous, or intraperitoneal routes), interestingly, TRH may have bioavailability when administered by nasal or intratracheal routes, as well(Kubek et al., 2009; Lehrer, 2014; Morimoto et al., 2000; Morimoto et al., 1994).

To address the therapeutic limitations of TRH, many analogs have been developed with improved bioavailability/stability and diminished neuroendocrine effects (e.g., azetirelin, montirelin, posatirelin, or taltirelin), and analogs that inhibit endogenous TRH degradation have also been identified(Khomane et al., 2011). Taltirelin, the only TRH analog approved as a human therapeutic, is administered orally to treat spinal cerebellar degeneration patients in Japan. Taltirelin, relative to TRH, has five- to ten-fold less neuroendocrine effects (i.e., TSH and prolactin release); and, as a neurostimulant, is ten- to one hundred-fold more potent with a duration of action eight-fold longer(Kinoshita, 1998).

TRH and its analogs activate breathing in a number of species including humans and non-human primates(Kraemer et al., 1976; Nink et al., 1991). Breathing effects are likely central in origin, since small TRH doses injected into cerebral ventricles or near the respiratory rhythm-generating pre-Bötzinger complex provoke tachypnea(Hedner et al., 1983; Inyushkin et al., 1999). Additionally, TRH directly activates medullary chemosensing, nucleus tractus solitarius, and hypoglossal motor neurons, and taltirelin restores central carbon dioxide chemosensing in Brown Norway rats (Bayliss et al., 1992; Dekin et al., 1985; Mulkey et al., 2007; Puissant et al., 2015).

Opioids are an important class of drugs for treating acute pain. However, they are highly addictive and notorious in causing sometimes-lethal opioid-induced respiratory depression (OIRD). An agent that prevents or reverses OIRD while preserving analgesia would have significant clinical utility. Interestingly, the breathing effects of TRH and its stable analog posatirelin (RGH 2202), after central or peripheral administration, abolish morphine-induced respiratory depression as quantified by integrated diaphragmatic EMG activity in mechanically-ventilated rats(Kharkevich et al., 1991). Similarly, TRH reversed morphine-induced respiratory

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depression in an *in vitro* rat pup brainstem-spinal cord model(Takita et al., 2000). Of note, TRH does not impair opioid analgesia and does not modify the opioid interaction with its receptor (Bhargava et al.). In fact, TRH and taltirelin may both possess antinociceptive properties(Kawamura et al., 1985; Reny-Palasse et al., 1989; Tanabe et al., 2007).

In this study, we tested the hypothesis that intravenously administered TRH or its analog talitrelin would stimulate breathing in intact, anesthetized, spontaneously-breathing rats and reverse or prevent OIRD, even at otherwise lethal doses. Moreover, we also tested the hypothesis that TRH and taltirelin administered intratracheally would be effective as breathing stimulants.

Materials and Methods

Animal Studies. Studies were approved by the Massachusetts General Hospital (MGH) Institutional Animal Care and Use Committee (IACUC). Male Sprague Dawley rats wiuth weights ranging from 324 to 604 g were used and were obtained from Charles River Laboratories (Wilmington, MA) and housed in the MGH Center for Comparative Medicine Animal Facility.

All studies were performed on rats orotracheally intubated with a 14G Braun angiocatheter and spontaneously breathing 1.5% isoflurane in air. Following anesthesia induction in a plexiglass chamber with 3-5% isoflurane, rats were quickly intubated by direct visualization using an illuminated, fiberoptic wire and correct placement was confirmed by exhaled carbon dioxide (Capstar-100 CO2 analyzer; CWE Inc.; Ardmore, PA). A variable bypass vaporizer was used for isoflurane administration, and air flow (1 L/min) through the vaporizer and as provided to the rat was regulated using a mass flow controller valve (Model GE50A, MKS Instruments Inc., Andover, MA). Inspired and expired gas compositions were monitored using a Capnomac Ultima medical gas analyzer (GE Healthcare, Buckinghamshire, U.K.) and the Capstar-100 CO2 analyzer (CWE Inc.) via side stream sampling ports. A custom-built, automated heat lamp and rectal thermistor were used to maintain anesthetized rats at a body temperature of 37°C in all studies.

Intravenous TRH, taltirelin, and morphine (each diluted into a 0.5 ml volume of saline) were administered through a 24G lateral tail vein catheter and flushed in with 0.5 ml saline. Intratracheal TRH (5 mg/kg) and taltirelin (2 mg/kg) were diluted in a 100 ul final volume of sterile normal saline, drawn into a 10 cm length of PE50 tubing connected to a 1 ml syringe via 23G hypodermic needle. After insertion into the trachea through the bore of the 14G orotracheal angiocatheter, the PE50 tubing and syringe contents, in its entirety, were briskly expelled into the

trachea during inhalation. TRH (diluted to 10 mg/ml in saline) or high-dose morphine (15 mg/ml in normal saline) were administered by continuous infusion with a saline vehicle carrier using a syringe pump (Model 200; KD Scientific; Holliston, MA).

LabView 2013 software (National Instruments, Austin, TX) run on an Apple computer interfaced with three USB-6009 data acquisition boards (National Instruments) was used for all data acquisition (128 Hz sampling rate), signal analysis (in 4 sec time epochs), and gas flow control.

Breathing Studies. Rodent breathing (rate, tidal volume, and minute ventilation) were quantified using a heated pneumotachometer (Model 8420; Hans Rudolph Inc., Shawnee, KS) in combination with a differential pressure transducer and a demodulator (Models CD15 and MP45–14-871; Validyne Engineering, Northridge, CA) and a Capstar-100 CO2 analyzer (CWE Inc., Armore, PA). The stable air flow from the mass flow controller was directed through the vaporizer and via tubing into the pneumotachometer. The intubated rat breathed air/gas from a side port created by a Luer T-shaped coupling piece off the main gas flow tubing (Supplemental Figure 1). Oscillations in the primary air/gas flow stream and in the exhaled CO2 signal imparted by rat inhalation and exhalation were used to quantify breathing frequency and minute ventilation. Minute ventilation was quantified by numerically integrating the flow signal after digital subtraction of the baseline 1 L/min gas flow (Supplemental Figure 1). The system was calibrated before each rat study with a rodent ventilator (Model 683; Harvard Apparatus; Holliston, MA) providing a known minute ventilation (150 ml/min). Tidal volume within each 4 s time epoch was calculated by dividing the measured minute ventilation by the frequency.

Blood Pressure and Blood Gas Analysis. After endotracheal intubation and prior to study, rats were instrumented with a femoral artery catheter while spontaneously breathing ~2%

isoflurane in air. The rat femoral artery was cannulated with a 24G Braun angiocatheter under direct visualization through a stereo microscope (Olympus SZ60) and following surgical exposure. Arterial blood pressure was quantified using a pressure transducer (TruWave, Edwards Life Sciences, Irvine, CA) interfaced with a custom-built amplifier (AD620 operational amplifier; Jameco Electronics, Belmont, CA). An arterial blood sample (approximately 0.3 ml, heparinized at ~3 units/ml after withdrawal) was drawn after at least 15 min of stable breathing and equilibration with inhaled isoflurane 1.5%, and 15 and 30 min after first administration of TRH, taltirelin or morphine. A Vetscan iStat 1 (Abaxis, Union City, CA) blood gas analyzer was used for blood gas analysis with CG4+ cartridges (Abbott Laboratories, Princeton, NJ). Rats instrumented with a femoral artery catheter were euthanized upon completion of the study.

Drugs. TRH was purchased from Sigma-Aldrich (St. Louis, MO) and taltirelin from Tocris Bioscience (Minneapolis, MN). Both were solubilized in sterile, normal saline prior to administration. Isoflurane was purchased from Patterson Veterinary (Greeley, CO), and morphine was from McKesson Medical-Surgical (San Francisco, CA).

Statistical Analysis. Data are reported as mean±SEM. Statistical analysis was done using Prism 7.0c for Mac OS X software (GraphPad Software, Inc., La Jolla, CA). Unless indicated, comparisons were performed using a Student's *t*-test or a one-way ANOVA followed by a Dunnett's or Sidak's multiple comparison posttest; a *P*-value less than 0.05 indicates statistical significance.

Results

To determine a relevant TRH dose needed to impact breathing in our rat model, we conducted a TRH dose-breathing response study in orotracheally intubated, anesthetized, spontaneously-breathing rats. IV bolus TRH (from 0.5 to 2 mg/kg) caused a transient stimulation in minute ventilation (Figure 1) through effects on both tidal volume and breathing rate (Supplemental Figure 2).

TRH's effect on morphine-induced respiratory depression was next tested by administering TRH to rats pre-treated with morphine (5 mg/kg IV over 5 minutes; Figure 2). Rats in these studies were instrumented with a femoral artery catheter for hemodynamic and arterial blood gas monitoring. The dose of morphine was chosen as we know from prior experience that it is non-lethal in anesthetized rats spontaneously breathing air and provides an approximately 50% decrease in ventilation. Minute ventilation was 46±3% of baseline in morphine-treated rats due to decreases in both breathing rate and tidal volume, 68±4% and 69±4% of baseline, respectively (Figure 2A; n=5±SEM) with effects lasting over 40 min. Due to its transient effects (Figure 1), TRH was administered to morphine-treated rats by IV bolus (1 mg/kg) followed by continuous IV infusion (5 mg/kg/h) which, by itself, in the absence of morphine, (Figure 2B), caused a sustained increase in minute ventilation and respiratory rate for at least 40 min. A marked change in respiratory rate and tidal volume were observed in morphine-treated rats upon TRH administration (Figure 2C), which reversed and even over-corrected minute ventilation. The combination of morphine and TRH caused a "rapid-shallow" breathing pattern with a rapid breathing rate and small tidal volumes.

Arterial blood gas results were consistent with the breathing data (Figure 3). TRH by itself caused a mild respiratory alkalosis. Morphine by itself, however, caused a respiratory

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acidosis and hypoxia by increasing P_aCO_2 and decreasing P_aO_2 levels, respectively. TRH treatment lessened the respiratory acidosis of morphine at 15 min, only, but had no effect on oxygenation. Results similar to those of breathing were also observed in rat hemodynamics (Figure 3). By itself, TRH caused an increase in mean arterial blood pressure (23 ± 3 mmHg maximal change; Supplemental Figure 3A), but TRH induced a greater change in the presence of morphine (+53 ± 7 mmHg; Supplemental Figure 3C; $P < 0.01$ by Student's t test).

We next examined the effects of taltirelin, a long-acting TRH analog, on rat breathing and observed results similar to those of TRH. By itself (Figure 4A), taltirelin (1 mg/kg IV bolus) caused a modest increase in minute ventilation and breathing rate. However, in morphine-treated animals, taltirelin caused a marked increase in breathing rate and minute ventilation (Figure 4B) with an onset slower than TRH. In arterial blood gas results, taltirelin prevented morphine-induced hypercarbia and hypoxia, but induced a mild metabolic, lactate acidosis that increased with time (Figure 3A and 3D). Like TRH, taltirelin increased mean arterial blood pressure (Supplemental Figure 3D; +54 ±7 mmHg); we did not study hemodynamics in rats treated with taltirelin only.

We also studied the efficacy of TRH in preventing, rather than reversing, morphine-induced depression of breathing. Initiating TRH administration just prior to morphine prevented morphine-induced (15 mg/kg IV over 15 minutes; note, the dose 3-fold higher than used in earlier studies) inhibitory effects on minute ventilation (Supplemental Figure 4; P=0.7 for the baseline breathing data point just prior to morphine (108 ± 7%) relative to that upon completion of morphine dose (102 ± 12%)). Tachypnea and diminished tidal volume were induced by morphine administered to TRH pre-treated rats (Supplemental Figure 4). Upon discontinuation

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of the TRH infusion, its breathing effects faded and morphine-induced respiratory depression emerged (Supplemental Figure 4).

To test the limits of TRH efficacy as a reversal agent for OIRD, we next employed a clearly lethal dose of morphine. Four rats were administered morphine at a high dose and a rapid rate (5 mg/kg/min until 16 s of apnea were observed; on average 21 ± 5 mg/kg in less than 5 min) followed by TRH (2 mg/kg IV bolus). After 30 mins, IV naloxone, a morphine antagonist (Figure 5) was given. TRH restored ventilation in all four rats. The three control animals that received IV saline in lieu of TRH all died following high-dose morphine administration (data not shown). Since we did not use hemodynamic monitoring in these studies, and since we did not wish to disturb the animals by palpation, the exact time of death (i.e., cardiac arrest) for control animals following induction of apnea was not determined.

Finally, we tested the hypothesis that intratracheal TRH will have bioavailability sufficient to reverse morphine-induced respiratory depression. To test this hypothesis, TRH was administered by bolus into the trachea (5 mg/kg dissolved in 100 μl of saline; also, see Methods) of morphine-treated rats and was determined effective and similar to intravenous TRH in reversing morphine-induced respiratory depression for at least 40 min (Figure 6). Similar results were observed with taltirelin (2 mg/kg, intracheally; n = 4; data not shown). Intratracheal saline (100 μl bolus) by itself did not stimulate breathing in morphine-treated (5 mg/kg IV), anesthetized rats (n = 3; data not shown); one of the saline treated rats died approximately 15 mins after saline administration.

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Discussion

In this manuscript we report studies that address the breathing and hemodynamic effects of intravenous and intratracheal TRH and its analog, taltirelin, in intubated, spontaneously breathing rats, before and after intravenous morphine administration. Both TRH and taltirelin reversed morphine-induced respiratory and hemodynamic depression. Importantly, TRH was also effective at reversing otherwise lethal levels of OIRD and at preventing OIRD, and both TRH and taltirelin were effective when administered intratracheally.

There are limitations to our study that should be considered when interpreting our results. All studies were done in anesthetized rats, which itself enhances the breathings effects of TRH and increases the respiratory depressant effects of morphine (Schaefer et al., 1989). We also used only single doses of TRH or taltirelin with morphine. Additionally, rats are intolerant of hypoxia while under anesthesia, which causes hypotension, and would affect the lethality of morphine(Hou et al., 2005). Future studies will be required to sort out the effects of anesthetics and to optimize dosing for both TRH and taltirelin. Taltirelin did cause an increase in plasma lactate levels in arterial blood gas analysis, which is also concerning. However, taltirelin and TRH both cause an increase in oxygen consumption and prevent the hypothermia associated with anesthesia and opioids (Bhargava et al., 1983; Puissant et al., 2015; Schuhler et al., 2007; Sharp et al., 1984). We speculate that an increase in oxygen demand (from metabolic effects and/or the increased work of breathing) combined with the hypotension and the hypoxia present upon taltirelin administration, may have yielded inadequate blood perfusion and oxygen delivery to tissues. Administration of taltirelin at a lower dose, prior to morphine administration, and/or in the absence of anesthetic may minimize lactate production.

The combination of TRH and morphine (Figures 2 and 5, and Supplemental Figure 4) or taltirelin and morphine (Figure 4) caused a “rapid-shallow” breathing pattern. One reason for

this pattern may be the restrictive effects of opioid-induced skeletal muscle rigidity on pulmonary compliance with increased work of breathing(Weinger et al., 1989). Regardless, this mode of ventilation is undesired for at least two reasons. First, it is inefficient in carbon dioxide elimination due to an increase in the ratio of dead space to alveolar ventilation as compared to a slow and deep ventilation pattern(Kreit, 2015). Second, smaller tidal volumes promote atelectasis with hypoxia from intrapulmonary shunting. Arterial blood gas results bear this out (Figure 3). TRH, despite over correction of morphine-induced decreases in minute ventilation, failed to normalize arterial carbon dioxide and oxygen levels. Similarly, marked over correction by talitrelin merely normalized these levels. However, that said, any ventilation is better than none as evidenced by TRH rescue of rats treated with a lethal, apnea-inducing dose of morphine (Figure 5). Future studies will need to address the impact of TRH and taltirelin dosing on arterial blood gas results. For example, would lower doses support a more "sober", slower and deeper ventilatory pattern better suited for reversing hypercarbia and hypoxia? Additionally, an agent such as dexmeditomidine, an $\alpha 2$ agonist known to eliminate opioid-induce muscle rigidity, might be co-administered with TRH or taltirelin to determine if tidal volumes increase(Weinger et al., 1989).

In our studies, the breathing and hemodynamic response to TRH is exaggerated in the presence of opioids, which implies these drugs are influencing each other's mechanism of action. This interaction, of course, could be occurring at one or more levels (e.g., receptor, neuron/cell signaling, neural circuit, and/or the organ levels). For example, cell signaling events downstream of the TRH receptor may perhaps "bias" that downstream of the mu opioid receptor, an active area of investigation in novel opioid drug development(Schmid et al., 2017). Other investigators have also observed an interaction between opioids and TRH(Bhargava et al., 1983).

TRH decreases both tolerance to opioid antinociception as well as symptoms of acute withdrawal in rodents. Again, it should be noted that TRH does not alter opioid-induced antinociception and does not impact opioid binding to its receptor.

The respiratory tract is a viable route for needle-free, systemic drug delivery. For example, nasal naloxone provides rapid reversal of opioids in the setting of overdose(Barton et al., 2002), and inhaled insulin protein provides normalization of blood sugar levels(Mastrandrea, 2010). We determined that intratracheally delivered TRH reversed OIRD, which suggests inhaled TRH may be a feasible method of rapid, needle-free TRH administration. Additionally, because TRH metabolism on the tracheal and alveolar surfaces is minimal and since transepithelial TRH transport is limited, TRH's short plasma half-life may be overcome by depositing a large TRH dose in the lung for stable, prolonged systemic uptake(Lehrer, 2014; Morimoto et al., 2000; Morimoto et al., 1994). Future work, of course, is needed to optimize TRH delivery through the respiratory tract of the rat, an obligate nose breather with a long and complex nasopharyngeal anatomy (e.g., dosing and nebulized particle size optimization), and to determine if pharmacokinetics are in fact improved relative to intravenous delivery. We also need to address the possibility that TRH effects on breathing are mediated in part through receptors on the lung epithelium, which might be tested by studies in vagotomized rats.

TRH is an endogenous neuropeptide, and the results of this study imply that varying TRH or TRH receptor signaling levels due to effects of genetics, disease, pharmacology, and/or other reasons may modify an animal's or a patient's physiologic response to an opioid and risk for OIRD. Of course, endogenous TRH may have a markedly different pattern of distribution and concentration levels relative to that of TRH supplied by exogenous administration. There are several animal models in which these ideas could be tested. For example, TRH and TRH

receptor knockout mice have been developed and are viable and may be prone to OIRD(Rabeler et al., 2004; Sun et al., 2009; Yamada et al., 1997). Notably, the TRH1 knockout mouse has a prolonged sleep time with pentobarbital(Thirunarayanan et al., 2013). Similarly, the obese leptin knockout mouse (ob/ob) and the Brown Norway rat are TRH deficient in certain neuronal populations(Burgueno et al., 2007; Puissant et al., 2015). Of note, the ob/ob mouse has disordered ventilation at baseline, and the Brown Norway rat lacks a hypercarbic ventilatory response(O'Donnell C et al., 1999; Puissant et al., 2015). Alternatively, a transgenic animal overexpressing the TRH precursor protein gene, prepro-TRH, using its native promoter might be prepared for study(Schuman et al., 2014). Additionally, it will be interesting to determine if there are differences in the breathing effects of intravenous TRH relative to agents that increase endogenous TRH levels through blockade of TRH proteolysis(Scalabrino et al., 2007), or following leptin administration, which induces endogenous TRH expression(Burgueno et al., 2007), and which "gates" TRH responsivity(Rogers et al., 2009). Finally, benzodiazepines (e.g., chlordiazepoxide and midazolam) are antagonists/inverse agonists of the TRH receptor and antagonize some physiologic responses to TRH administration (e.g., TSH and prolactin secretion)(Lu et al., 2004; Roussel et al., 1986). By themselves, benzodiazepines cause minimal respiratory depression. However, benzodiazepines augment OIRD when co-administered with an opioid and are a contributing factor in many opioid-related deaths(McCormick et al., 1984). We speculate this may be mediated in part through effects at the TRH receptor.

A number of CNS stimulants have varying levels of efficacy in reversing OIRD while preserving opioid-induced analgesia (i.e., as "physiologic antagonists"). For example, doxapram and Gal-021 are breathing stimulants that act peripherally through potassium channel inhibition to cause carotid body activation (Cotten et al., 2006; Golder et al., 2015; Mitchell and Herbert,

1975). In human studies, however, Gal-021 may have a ceiling in its efficacy reversing only modest levels of OIRD, which will limit utility(Roozekrans et al., 2015). Similarly, doxapram has limited efficacy and may act in part through increased opioid clearance via augmented cardiac output(Roozekrans et al., 2016). In our study, however, TRH distinguished itself in reversing very deep/lethal levels of OIRD, suggesting excellent efficacy in this use. In this way, TRH resembles ampakines (e.g., CX-717), which act through effects on the brainstem respiratory rhythm generating PreBötzinger complex, and which also cause a "rapid-shallow" breathing pattern(Ren et al., 2009). Phase II clinical trials have demonstrated ampakine efficacy in reversing OIRD (www.respirerx.com). Interestingly and also like ampakines, TRH has limited breathing effects in the absence of opioids. However, ampakines are allosteric modulators of excitatory, AMPA-type glutamate receptors, a molecular target very different from the TRH receptor.

In conclusion, TRH is an FDA-approved drug and an endogenous neuropeptide, with myriad physiologic effects that has been studied as a potential therapeutic agent for a wide range of human afflictions. Our current studies suggest TRH, or one of its over twenty analogs such as taltirelin, may also have clinical utility as an opioid drug therapy adjunct to both prevent and/or reverse OIRD without analgesia compromise. Further pre-clinical and clinical studies are warranted to better define TRH dosing, efficacy, safety, route of administration, and tolerability in this clinical use.

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Authorship Contributions

Participated in research design: Boghosian, Luethy, Cotten.

Conducted experiments: Boghosian, Luethy, Cotten.

Contributed new reagents or analytic tools: None.

Performed data analysis: Boghosian, Luethy, Cotten.

Wrote or contributed to the writing of the manuscript: Luethy, Boghosian, Cotten.

References

Barton ED, Ramos J, Colwell C, Benson J, Baily J and Dunn W (2002) Intranasal administration of naloxone by paramedics. *Prehosp Emerg Care* **6**(1): 54-58.

Bayliss DA, Viana F and Berger AJ (1992) Mechanisms underlying excitatory effects of thyrotropin-releasing hormone on rat hypoglossal motoneurons in vitro. *J Neurophysiol* **68**(5): 1733-1745.

Bhargava HN, Yousif DJ and Matwyshyn GA (1983) Interactions of thyrotropin releasing hormone, its metabolites and analogues with endogenous and exogenous opiates. *Gen Pharmacol* **14**(6): 565-570.

Burgueno AL, Landa MS, Schuman ML, Alvarez AL, Carabelli J, Garcia SI and Pirola CJ (2007) Association between diencephalic thyroliberin and arterial blood pressure in agouti-yellow and ob/ob mice may be mediated by leptin. *Metabolism* **56**(10): 1439-1443.

Cotten JF, Keshavaprasad B, Laster MJ, Eger EI, 2nd and Yost CS (2006) The ventilatory stimulant doxapram inhibits TASK tandem pore (K2P) potassium channel function but does not affect minimum alveolar anesthetic concentration. *Anesth Analg* **102**(3): 779-785.

Dekin MS, Richerson GB and Getting PA (1985) Thyrotropin-releasing hormone induces rhythmic bursting in neurons of the nucleus tractus solitarius. *Science* **229**(4708): 67-69.

Golder FJ, Dax S, Baby SM, Gruber R, Hoshi T, Ideo C, Kennedy A, Peng S, Puskovic V, Ritchie D, Woodward R, Wardle RL, Van Scott MR, Mannion JC and MacIntyre DE (2015) Identification and Characterization of GAL-021 as a Novel Breathing Control Modulator. *Anesthesiology* **123**(5): 1093-1104.

Hedner J, Hedner T, Wessberg P, Lundberg D and Jonason J (1983) Effects of TRH and TRH analogues on the central regulation of breathing in the rat. *Acta Physiol Scand* **117**(3): 427-437.

Hinkle PM, Pekary AE, Senanayaki S and Sattin A (2002) Role of TRH receptors as possible mediators of analeptic actions of TRH-like peptides. *Brain Res* **935**(1-2): 59-64.

Hogan N, O'Boyle KM, Hinkle PM and Kelly JA (2008) A novel TRH analog, Glp-Asn-Pro-D-Tyr-D-TrpNH2, binds to [3H][3-Me-His2]TRH-labelled sites in rat hippocampus and cortex but not pituitary or heterologous cells expressing TRHR1 or TRHR2. *Neurosci Lett* **431**(1): 26-30.

Hou H, Grinberg OY, Grinberg SA, Khan N, Dunn JF and Swartz HM (2005) Cerebral PtO2, acute hypoxia, and volatile anesthetics in the rat brain. *Adv Exp Med Biol* **566**: 179-185.

Inyushkin AN, Merkulova NA and Chepurnov SA (1999) The pre-Botzinger complex participates in generating the respiratory effects of thyroliberin. *Neurosci Behav Physiol* **29**(3): 321-326.

Kawamura S, Sakurada S, Sakurada T, Kisara K, Sasaki Y and Suzuki K (1985) The antinociceptive effects of histidyl-proline diketopiperazine and thyrotropin-releasing hormone in the mouse. *Eur J Pharmacol* **112**(3): 287-294.

Kelly JA, Boyle NT, Cole N, Slator GR, Colivicchi MA, Stefanini C, Gobbo OL, Scalabrino GA, Ryan SM, Elamin M, Walsh C, Vajda A, Goggin MM, Campbell M, Mash DC, O'Mara SM, Brayden DJ, Callanan JJ, Tipton KF, Della Corte L, Hunter J, O'Boyle KM, Williams CH and Hardiman O (2015) First-in-class thyrotropin-releasing hormone

(TRH)-based compound binds to a pharmacologically distinct TRH receptor subtype in human brain and is effective in neurodegenerative models. *Neuropharmacology* **89**: 193-203.

Kharkevich DA, Chizh BA and Kasparov SA (1991) Stimulant effect of thyrotropin-releasing hormone and its analog, RGH 2202, on the diaphragm respiratory activity, and their antagonism with morphine: possible involvement of the N-methyl-D-aspartate receptors. *Brain Res* **551**(1-2): 110-115.

Khomane KS, Meena CL, Jain R and Bansal AK (2011) Novel thyrotropin-releasing hormone analogs: a patent review. *Expert Opin Ther Pat* **21**(11): 1673-1691.

Kinoshita K, Yamamura, M., Sugihara, J., Suzuki, M., Matsuoka, Y. (1998) Taltirelin hydrate (TA-0910): An orally active thyrotropin-releasing hormone mimetic agent with multiple actions. *CNS Drug Reviews* **4**(1): 25-41.

Kraemer GW, Mueller R, Breese GR, Prange AJ, Lewis JK, Morrison H and McKinney WT, Jr. (1976) Thyrotropin releasing hormone: antagonism of pentobarbital narcosis in the monkey. *Pharmacol Biochem Behav* **4**(6): 709-712.

Kreit JW (2015) Alterations in gas exchange due to low-tidal volume ventilation. *Ann Am Thorac Soc* **12**(2): 283-286.

Kubek MJ, Domb AJ and Veronesi MC (2009) Attenuation of kindled seizures by intranasal delivery of neuropeptide-loaded nanoparticles. *Neurotherapeutics* **6**(2): 359-371.

Lehrer S (2014) Inhaled thyrotropin-releasing hormone for treatment of neuropsychiatric disorders. *J Clin Psychopharmacol* **34**(2): 288-290.

Lu X, Huang W, Worthington S, Drabik P, Osman R and Gershengorn MC (2004) A model of inverse agonist action at thyrotropin-releasing hormone receptor type 1: role of a conserved tryptophan in helix 6. *Mol Pharmacol* **66**(5): 1192-1200.

Mastrandrea LD (2010) Inhaled insulin: overview of a novel route of insulin administration. *Vasc Health Risk Manag* **6**: 47-58.

Matre V, Karlsen HE, Wright MS, Lundell I, Fjeldheim AK, Gabrielsen OS, Larhammar D and Gautvik KM (1993) Molecular cloning of a functional human thyrotropin-releasing hormone receptor. *Biochem Biophys Res Commun* **195**(1): 179-185.

McCormick GY, White WJ, Zagon IS and Lang CM (1984) Effects of diazepam on arterial blood gas concentrations and pH of adult rats acutely and chronically exposed to methadone. *J Pharmacol Exp Ther* **230**(2): 353-359.

Mitchell RA and Herbert DA (1975) Potencies of doxapram and hypoxia in stimulating carotid-body chemoreceptors and ventilation in anesthetized cats. *Anesthesiology* **42**(5): 559-566.

Morimoto K, Uehara Y, Iwanaga K and Kakemi M (2000) Effects of sodium glycocholate and protease inhibitors on permeability of TRH and insulin across rabbit trachea. *Pharm Acta Helv* **74**(4): 411-415.

Morimoto K, Yamahara H, Lee VH and Kim KJ (1994) Transport of thyrotropin-releasing hormone across rat alveolar epithelial cell monolayers. *Life Sci* **54**(26): 2083-2092.

Mulkey DK, Rosin DL, West G, Takakura AC, Moreira TS, Bayliss DA and Guyenet PG (2007) Serotonergic neurons activate chemosensitive retrotrapezoid nucleus neurons by a pH-independent mechanism. *J Neurosci* **27**(51): 14128-14138.

Nink M, Krause U, Lehnert H, Heuberger W, Huber I, Schulz R, Hommel G and Beyer J (1991) Thyrotropin-releasing hormone has stimulatory effects on ventilation in humans. *Acta Physiol Scand* **141**(3): 309-318.

O'Donnell C P, Schaub CD, Haines AS, Berkowitz DE, Tankersley CG, Schwartz AR and Smith PL (1999) Leptin prevents respiratory depression in obesity. *Am J Respir Crit Care Med* **159**(5 Pt 1): 1477-1484.

Puissant MM, Echert AE, Yang C, Mouradian GC, Jr., Novotny T, Liu P, Liang M and Hodges MR (2015) RNASeq-derived transcriptome comparisons reveal neuromodulatory deficiency in the CO(2) insensitive brown Norway rat. *J Physiol* **593**(2): 415-430.

Rabeler R, Mittag J, Geffers L, Ruther U, Leitges M, Parlow AF, Visser TJ and Bauer K (2004) Generation of thyrotropin-releasing hormone receptor 1-deficient mice as an animal model of central hypothyroidism. *Mol Endocrinol* **18**(6): 1450-1460.

Ren J, Ding X, Funk GD and Greer JJ (2009) Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthesiology* **110**(6): 1364-1370.

Reny-Palasse V, Monier C and Rips R (1989) Opioid involvement in TRH-induced antinociception in the rat following intracerebral administration. *Pain* **38**(2): 193-201.

Rogers RC, Barnes MJ and Hermann GE (2009) Leptin "gates" thermogenic action of thyrotropin-releasing hormone in the hindbrain. *Brain Res* **1295**: 135-141.

Roozekrans M, Olofsen E, Schrier RV, Boom M, Mooren R and Dahan A (2016) Doxapram-mediated Increase in Cardiac Output Reduces Opioid Plasma Concentrations: A Pharmacokinetic/Pharmacodynamic-Pharmacokinetic/Pharmacodynamic Modeling Study in Healthy Volunteers. *Clin Pharmacol Ther*.

Roozekrans M, Olofsen E, van der Schrier R, van Gerven J, Peng S, McLeod J and Dahan A (2015) Reversal of opioid-induced respiratory depression by BK-channel blocker GAL021: A pharmacokinetic-pharmacodynamic modeling study in healthy volunteers. *Clin Pharmacol Ther* **97**(6): 641-649.

Roussel JP, Astier H and Tapia-Arancibia L (1986) Benzodiazepines inhibit thyrotropin (TSH)-releasing hormone-induced TSH and growth hormone release from perifused rat pituitaries. *Endocrinology* **119**(6): 2519-2526.

Scalabrino GA, Hogan N, O'Boyle KM, Slator GR, Gregg DJ, Fitchett CM, Draper SM, Bennett GW, Hinkle PM, Bauer K, Williams CH, Tipton KF and Kelly JA (2007) Discovery of a dual action first-in-class peptide that mimics and enhances CNS-mediated actions of thyrotropin-releasing hormone. *Neuropharmacology* **52**(7): 1472-1481.

Schaefer CF, Brackett DJ, Biber B, Lerner MR, Holaday JW, Wilson MF and Fagraeus L (1989) Respiratory and cardiovascular effects of thyrotropin-releasing hormone as modified by isoflurane, enflurane, pentobarbital and ketamine. *Regul Pept* **24**(3): 269-282.

Schmid CL, Kennedy NM, Ross NC, Lovell KM, Yue Z, Morgenweck J, Cameron MD, Bannister TD and Bohn LM (2017) Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics. *Cell* **171**(5): 1165-1175 e1113.

Schuhler S, Warner A, Finney N, Bennett GW, Ebling FJ and Brameld JM (2007) Thyrotrophin-releasing hormone decreases feeding and increases body temperature, activity and oxygen consumption in Siberian hamsters. *J Neuroendocrinol* **19**(4): 239-249.

Schuman ML, Peres Diaz LS, Landa MS, Toblli JE, Cao G, Alvarez AL, Finkielman S, Pirola CJ and Garcia SI (2014) Thyrotropin-releasing hormone overexpression induces structural changes of the left ventricle in the normal rat heart. *Am J Physiol Heart Circ Physiol* **307**(11): H1667-1674.

Sharp T, Tulloch IF, Bennett GW, Marsden CA, Metcalf G and Dettmar PW (1984) Analeptic effects of centrally injected TRH and analogues of TRH in the pentobarbitone-anaesthetized rat. *Neuropharmacology* **23**(3): 339-348.

Straub RE, Frech GC, Joho RH and Gershengorn MC (1990) Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. *Proc Natl Acad Sci U S A* **87**(24): 9514-9518.

Sun Y, Zupan B, Raaka BM, Toth M and Gershengorn MC (2009) TRH-receptor-type-2-deficient mice are euthyroid and exhibit increased depression and reduced anxiety phenotypes. *Neuropsychopharmacology* **34**(6): 1601-1608.

Takita K, Herlenius E, Yamamoto Y and Lindahl SG (2000) Effects of neuroactive substances on the morphine-induced respiratory depression; an in vitro study. *Brain Res* **884**(1--2): 201-205.

Tanabe M, Tokuda Y, Takasu K, Ono K, Honda M and Ono H (2007) The synthetic TRH analogue taltirelin exerts modality-specific antinociceptive effects via distinct descending monoaminergic systems. *Br J Pharmacol* **150**(4): 403-414.

Thirunarayanan N, Nir EA, Raaka BM and Gershengorn MC (2013) Thyrotropin-releasing hormone receptor type 1 (TRH-R1), not TRH-R2, primarily mediates taltirelin actions in the CNS of mice. *Neuropsychopharmacology* **38**(6): 950-956.

Weinger MB, Segal IS and Maze M (1989) Dexmedetomidine, acting through central alpha-2 adrenoceptors, prevents opiate-induced muscle rigidity in the rat. *Anesthesiology* **71**(2): 242-249.

Yamada M, Saga Y, Shibusawa N, Hirato J, Murakami M, Iwasaki T, Hashimoto K, Satoh T, Wakabayashi K, Taketo MM and Mori M (1997) Tertiary hypothyroidism and hyperglycemia in mice with targeted disruption of the thyrotropin-releasing hormone gene. *Proc Natl Acad Sci U S A* **94**(20): 10862-10867.

Footnotes

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Figure Legends

Figure 1. Rat breathing response to increasing bolus dose of intravenous TRH. Normalized minute ventilation in orotracheally intubated rats spontaneously breathing 1.5% isoflurane in air, before and after intravenous TRH bolus (down arrow). Data were normalized to the averaged 15 min of baseline breathing prior to TRH administration. Each data point is the average of 4 animals, using 1 min of averaged data from each animal (n=4±SEM). Asterisks (*, **, and ***) indicate statistical significance ($P<0.05$, $P<0.01$, and $P<0.001$, respectively; one-way ANOVA with a Dunnett's posttest) relative to the data point just prior to TRH administration. Dotted red line indicates the 100% level for reference only. In A through D, baseline minute ventilation was 23±0.4 ml/min/100 g (n = 16±SEM).

Figure 2. Intravenous TRH reverses morphine-induced respiratory depression. Normalized minute ventilation (white circles; MV), breathing rate (black circles; BR), and tidal volume (red circles; TV) in orotracheally intubated rats spontaneously breathing 1.5% isoflurane in air before and after intravenous morphine administration (A and C; 5 mg/kg over 5 min; black bar) or saline (B; control; black bar) followed by intravenous TRH (B and C; 1 mg/kg bolus followed by 5 mg/kg/h infusion; white bar) or saline (A; control; white bar). Data were normalized as in Figure 1. Each data point is the average of 4 to 5 animals, using 1 min of averaged data from each animal (n=4 to 5±SEM). Asterisks (*, **, and ***) indicate statistical significance ($P<0.05$, $P<0.01$, and $P<0.001$, respectively; one-way ANOVA with Dunnett's posttest) relative to the data point just prior to morphine (in A) and TRH (in B and C). In A through C, baseline minute ventilation, breathing rate, and tidal volume were, respectively, 30±2 ml/min/100 g, 70±1 min^{-1}, 0.4±0.03 ml/100 g (n = 13±SEM).

Figure 3. Effect of IV morphine, TRH, and taltirelin on changes in arterial blood pH, gas, and lactate levels. Data are from the same experiment/animals represented in Figures 2 and 4 and depict the change (Δ) in arterial blood pH, carbon dioxide, oxygen, and lactate levels (A–D) from baseline at 15 and 30 minutes (15' and 30') after first administration of TRH, taltirelin, or saline (control). Saline followed by TRH (gray bars; see Figure 2B), morphine followed by saline (black bars; see Figure 2A), morphine followed by TRH (white bars; see Figure 2C), and morphine followed by taltirelin (blue bars; see Figure 4B). Each column represents the average change in blood gas values from n = 3 to 5 animals±SEM. Asterisks (*, **, and ***) indicate statistical significance ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; ns, not statistically significant; one-way ANOVA with a Sidak's posttest) relative to animals treated with morphine only (black bars). Baseline blood gas values were pH 7.40+/-0.01, P_aO_2 70±3 mmHg, P_aCO_2 45±1 mmHg, and lactate 1±0.1 mM. (n = 18±SEM).

Figure 4. Intravenous taltirelin reverses morphine-induced respiratory depression. Normalized minute ventilation (white circles; MV), breathing rate (black circles; BR), and tidal volume (red circles; TV) in orotracheally intubated rats spontaneously breathing 1.5% isoflurane in air before and after intravenous saline (A; black bar) or intravenous morphine administration (B; 5 mg/kg IV over 1 min; black bar) followed by intravenous taltirelin (1 mg/kg IV bolus; down arrow). Data were normalized as in Figure 1. Each data point is the average of 4 animals, using 1 min of averaged data from each animal (n=4±SEM). Asterisks (*, **, and ***) indicate statistical significance (P<0.05, P<0.01, and P<0.001, respectively; one-way ANOVA with Dunnett's posttest) relative to the data point immediately prior to taltirelin injection. Baseline minute ventilation, breathing rate, and tidal volume were, respectively, 28±2 ml/min/100 g, 71±5 min^{-1}, 0.4±0.04 ml/100 g (n = 8±SEM).

Figure 5. TRH reverses lethal apnea induced by high-dose, rapid intravenous morphine administration. Normalized minute ventilation (white circles; MV), breathing rate (black circles; BR), and tidal volume (red circles; TV) in orotracheally intubated rats spontaneously breathing 1.5% isoflurane in air before and after high-dose, rapid intravenous morphine administration (A; 5 mg/kg/min IV until apnea; first down arrow) followed by intravenous TRH (2 mg/kg IV bolus; second down arrow) and naloxone (0.4 mg/kg IV bolus; third down arrow). Rats received, on average, 21±5 mg/kg IV morphine in less than 5 min. Data were normalized as in Figure 1. Each data point is the average of 4 animals, using 1 min of averaged data from each animal (n=4±SEM). Since each animal incurred apnea at a slightly different time point after start of morphine infusion, time at which TRH and naloxone where administered relative to start of morphine differed slightly between animals. Baseline minute ventilation, breathing rate, and tidal volume were, respectively, 26±1 ml/min/100 g, 66±3 min^{-1}, 0.4±0.04 ml/100 g (n = 4±SEM).

Figure 6. Intratracheally administered TRH reverses morphine-induced respiratory depression. Normalized minute ventilation (white circles; MV), breathing rate (black circles; BR), and tidal volume (red circles; TV) in orotracheally intubated rats spontaneously breathing 1.5% isoflurane in air before and after intravenous morphine administration (5 mg/kg over 5 min; black bar) followed by intratracheal (IT) TRH (5 mg/kg IT bolus; white bar). Data were normalized as in Figure 1. Each data point is the average of 4 animals, using 1 min of averaged data from each animal (n=4±SEM). Asterisks (***) indicate statistical significance (P<0.001,

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respectively; one-way ANOVA with Dunnett's posttest) relative to the data point just prior to TRH administration. Baseline minute ventilation, breathing rate, and tidal volume were, respectively, 26±1 ml/min/100 g, 66±3 min^{-1}, 0.4±0.01 ml/100 g (n = 4±SEM).

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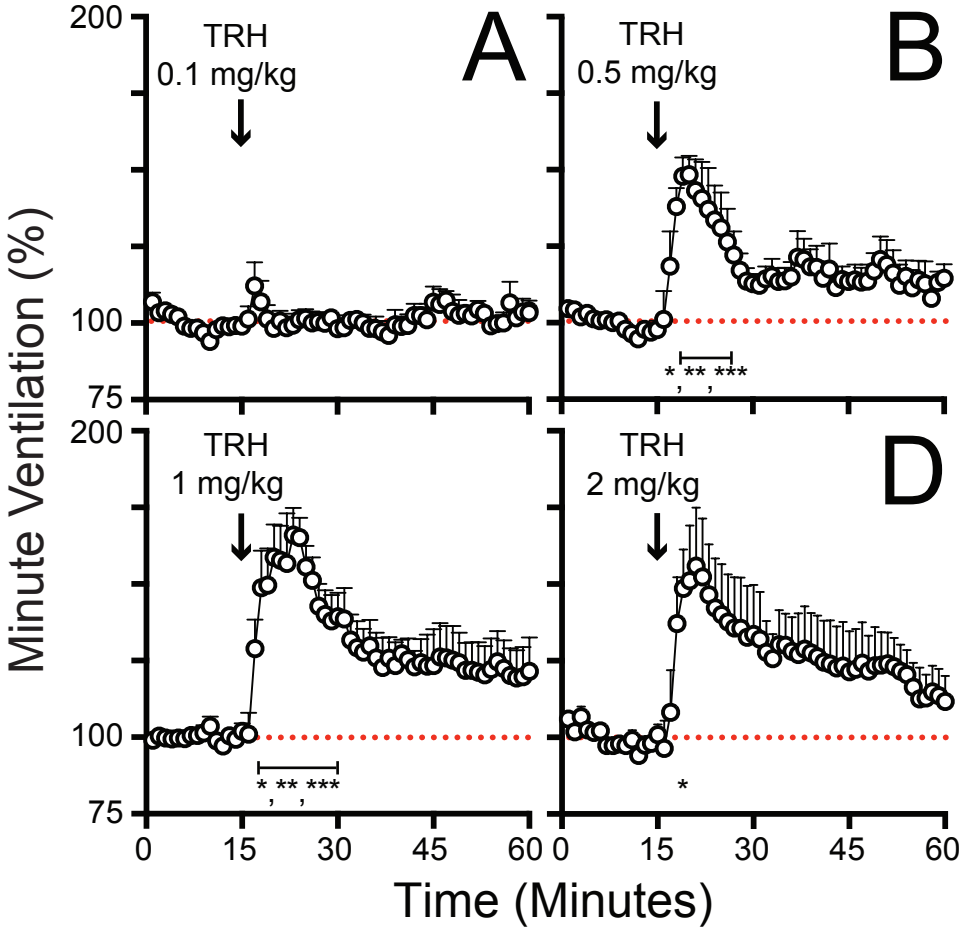

Figure 1

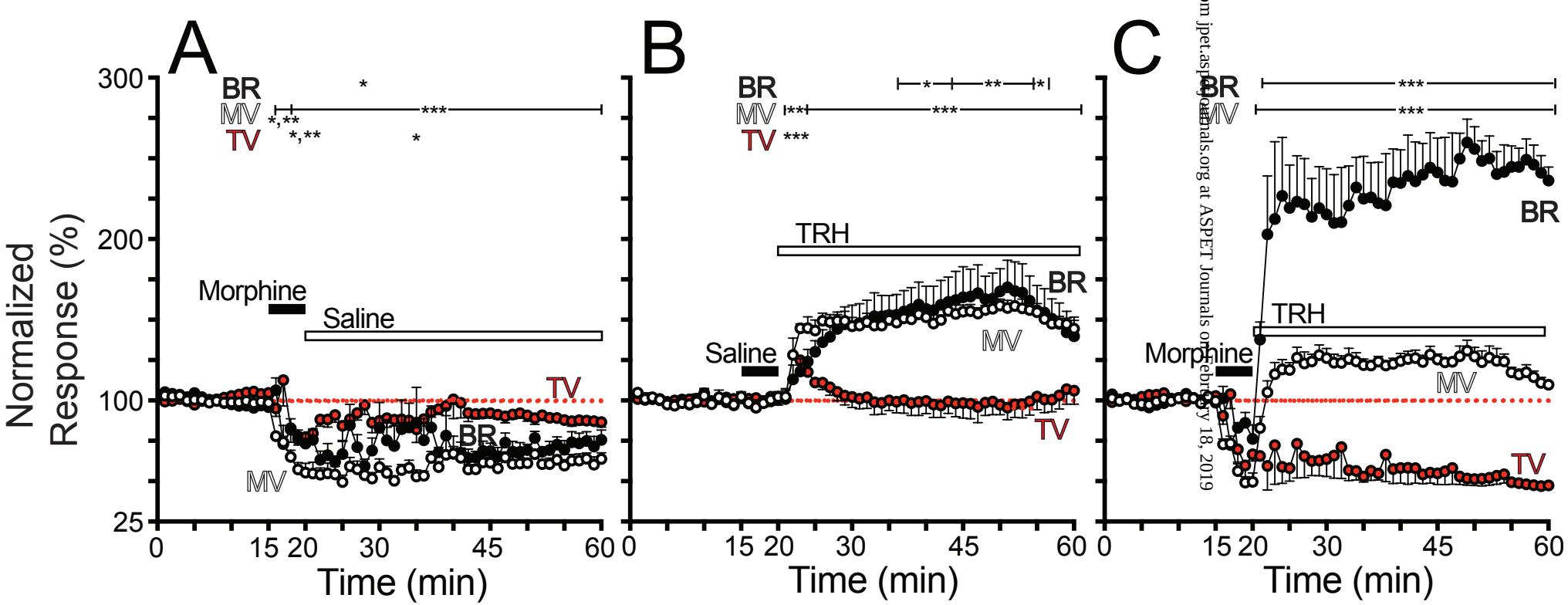

Figure 2

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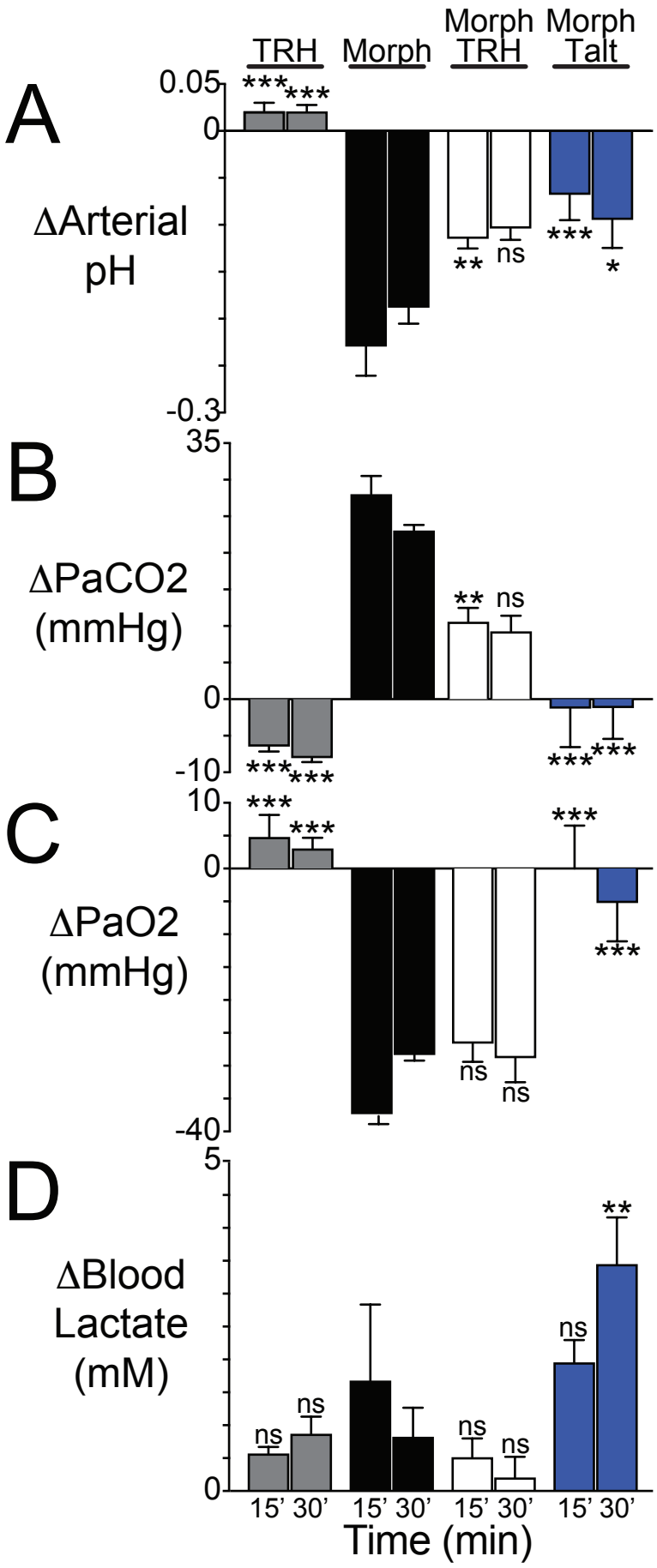

Figure 3

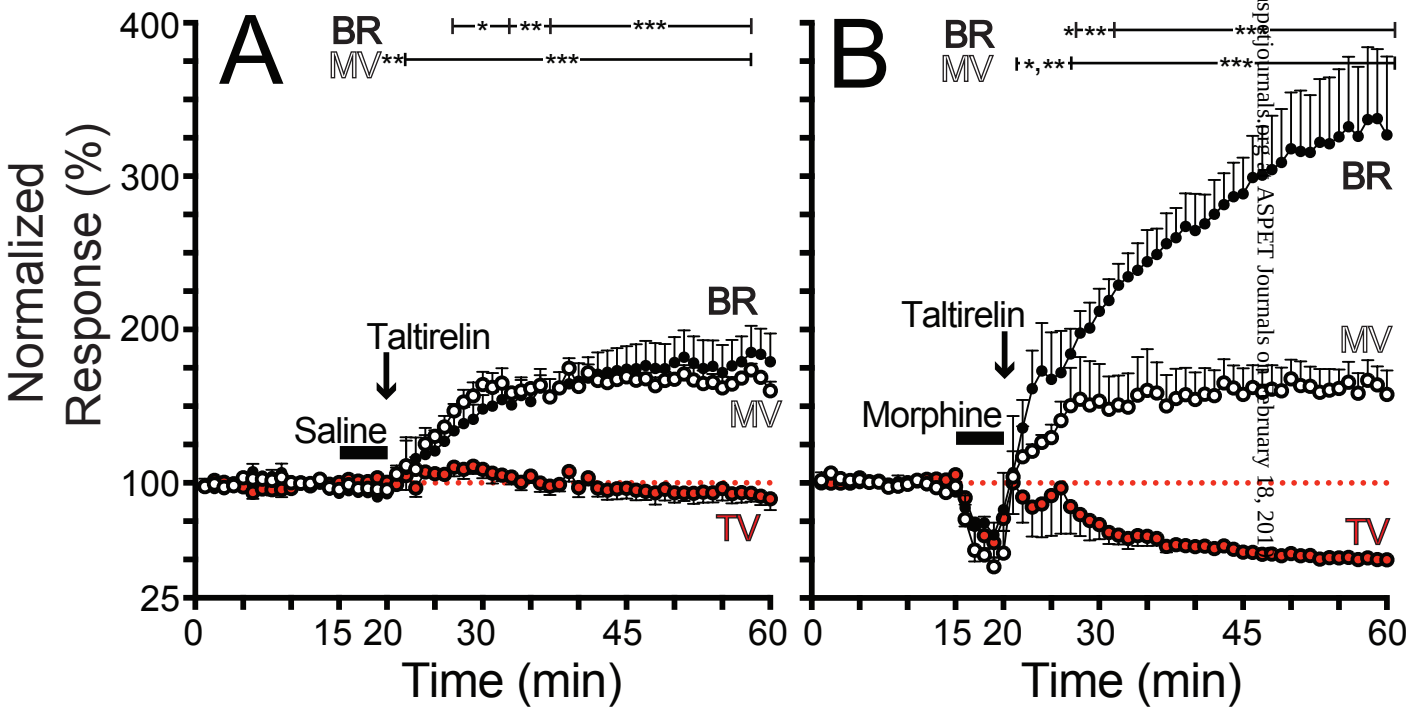

Figure 4

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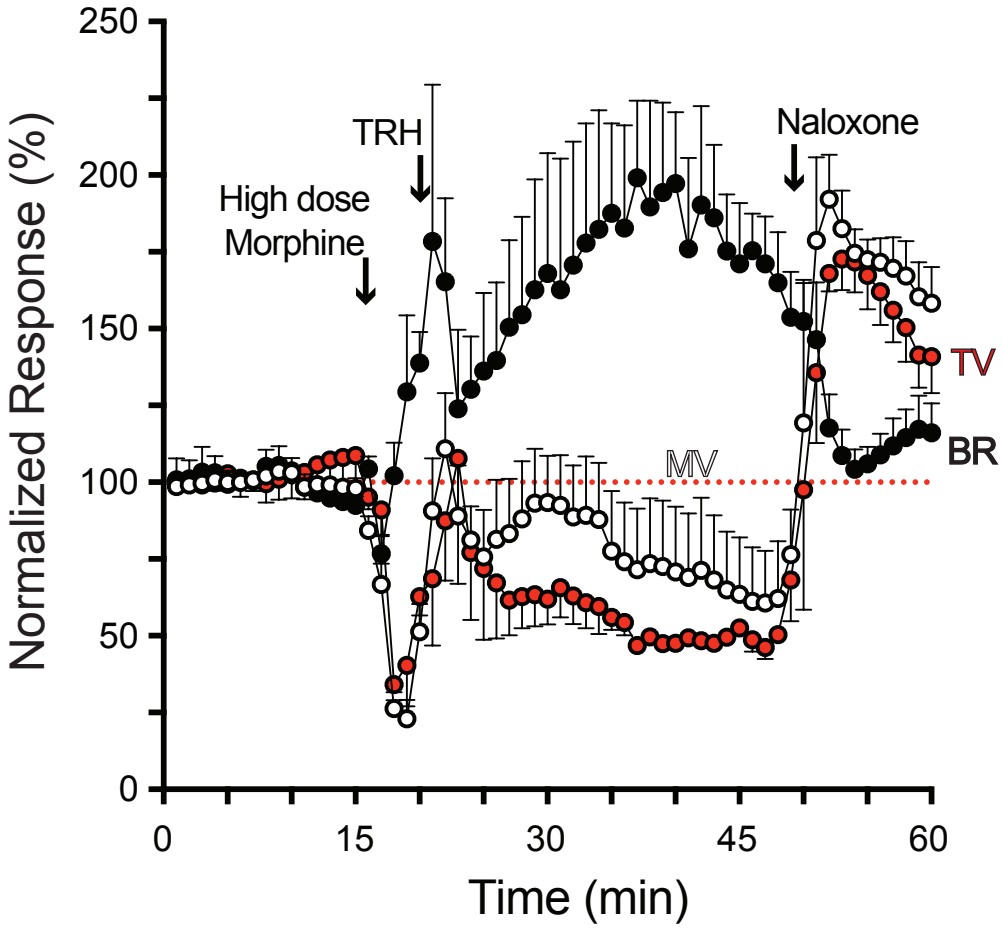

Figure 5

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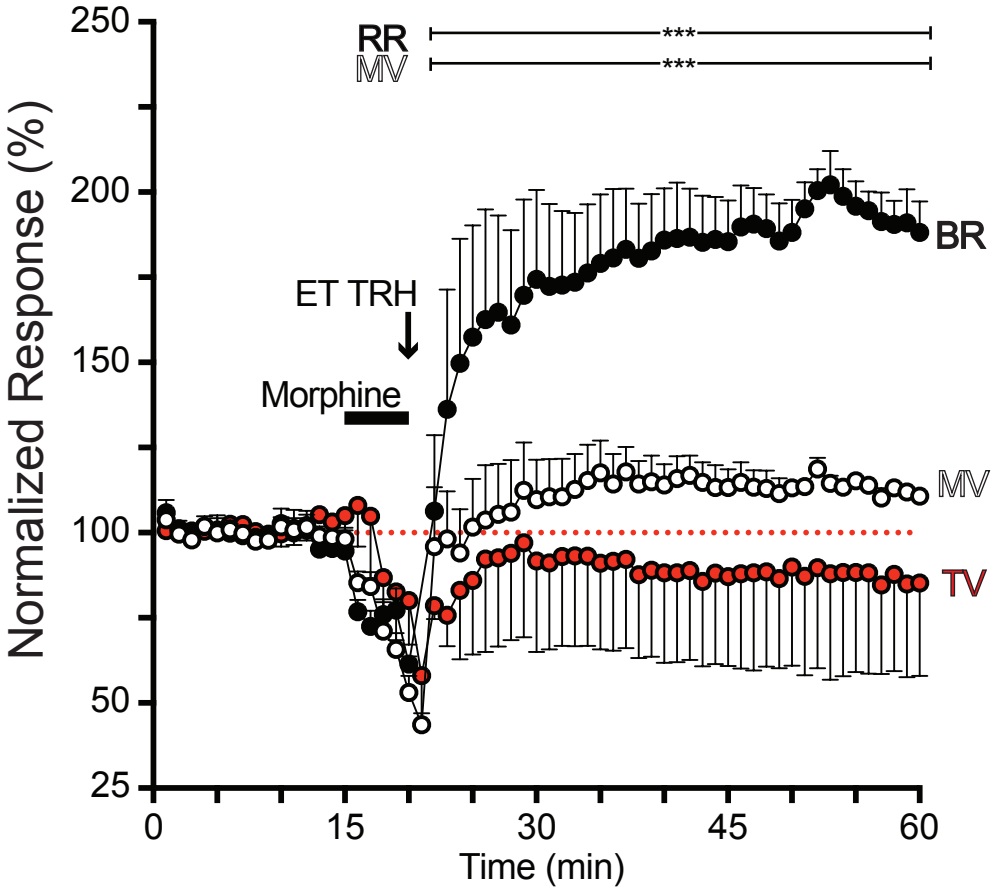
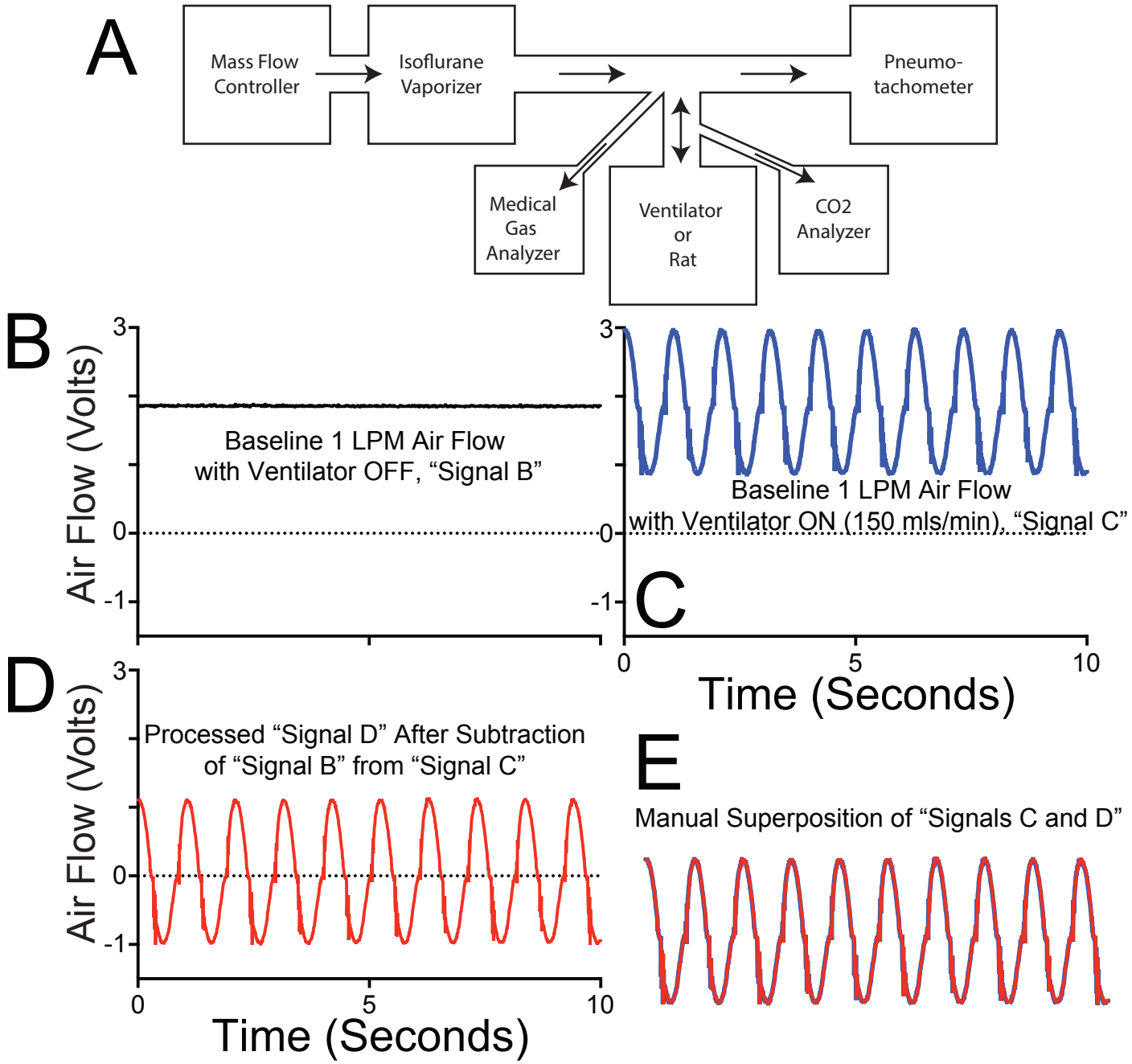


Figure 6

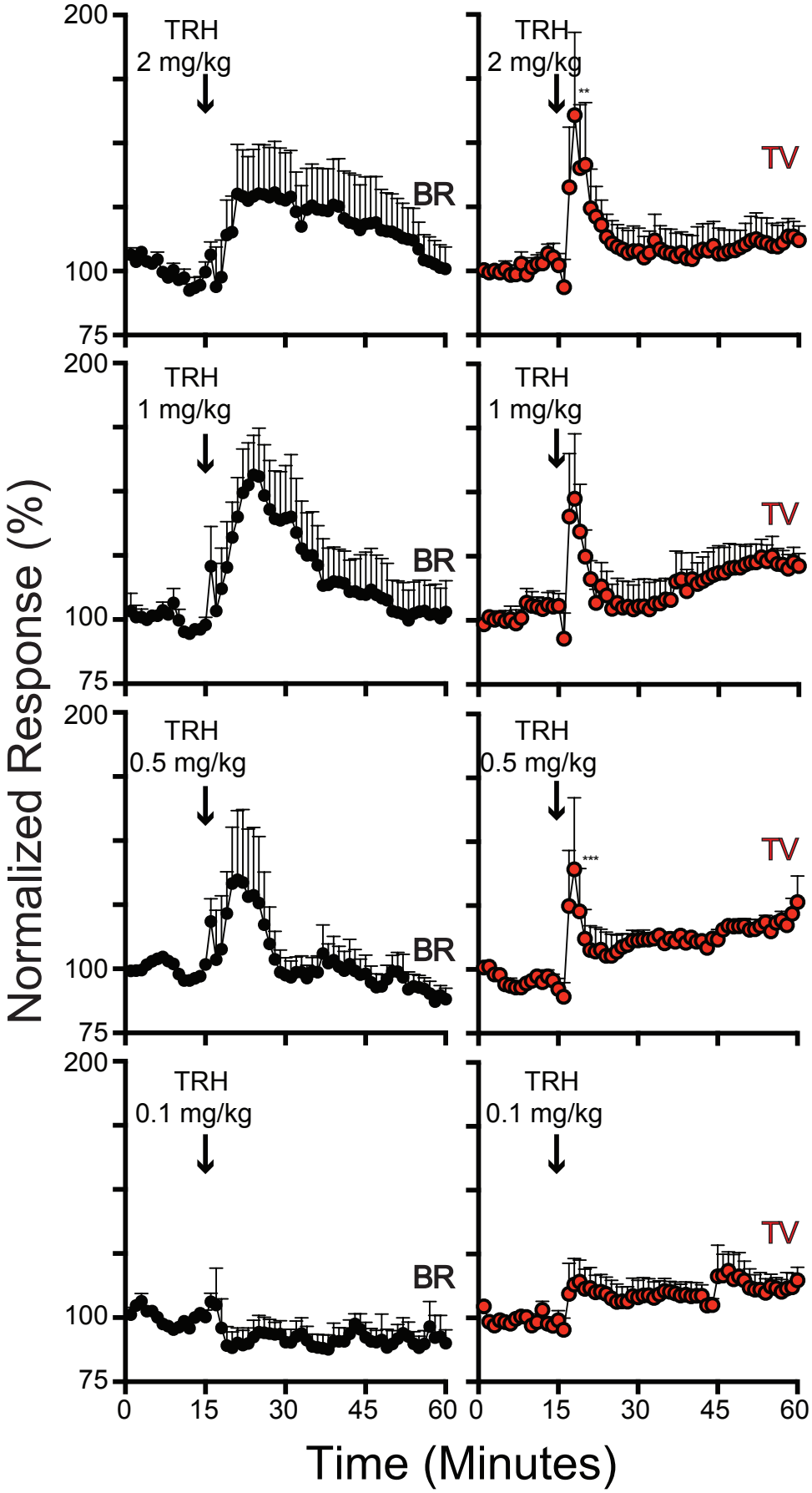
"Intravenous and intratracheal thyrotropin releasing hormone and its analog taltirelin reverse opioid-induced respiratory depression in isoflurane anesthetized rats". James D. Boghosian, Anita Luethy, and Joseph F. Cotten. *Journal of Pharmacology and Experimental Therapeutics.*



Supplemental Figure 1. Gas flow configuration and pneumotachometer signal processing required for estimating rat ventilation. A, gas flow direction (arrows) and component configuration used in this study. B, pneumotachometer electrical signal during 1 liter per minute (LPM) air flow with ventilator turned OFF. C, pneumotachometer signal with ventilator turned ON (150 mls/min at 60 breathes/min). D, processed alternating pneumotachometer signal after digital subtraction of the the direct current component using "AC & DC Estimator" and "Subtract" functions in Labview. E, manual superposition of the processed signal in D with the alternating component in C demonstrating preservation of the ventilator signal component. Data were acquired as described in the Methods.

Supplemental Figure 1

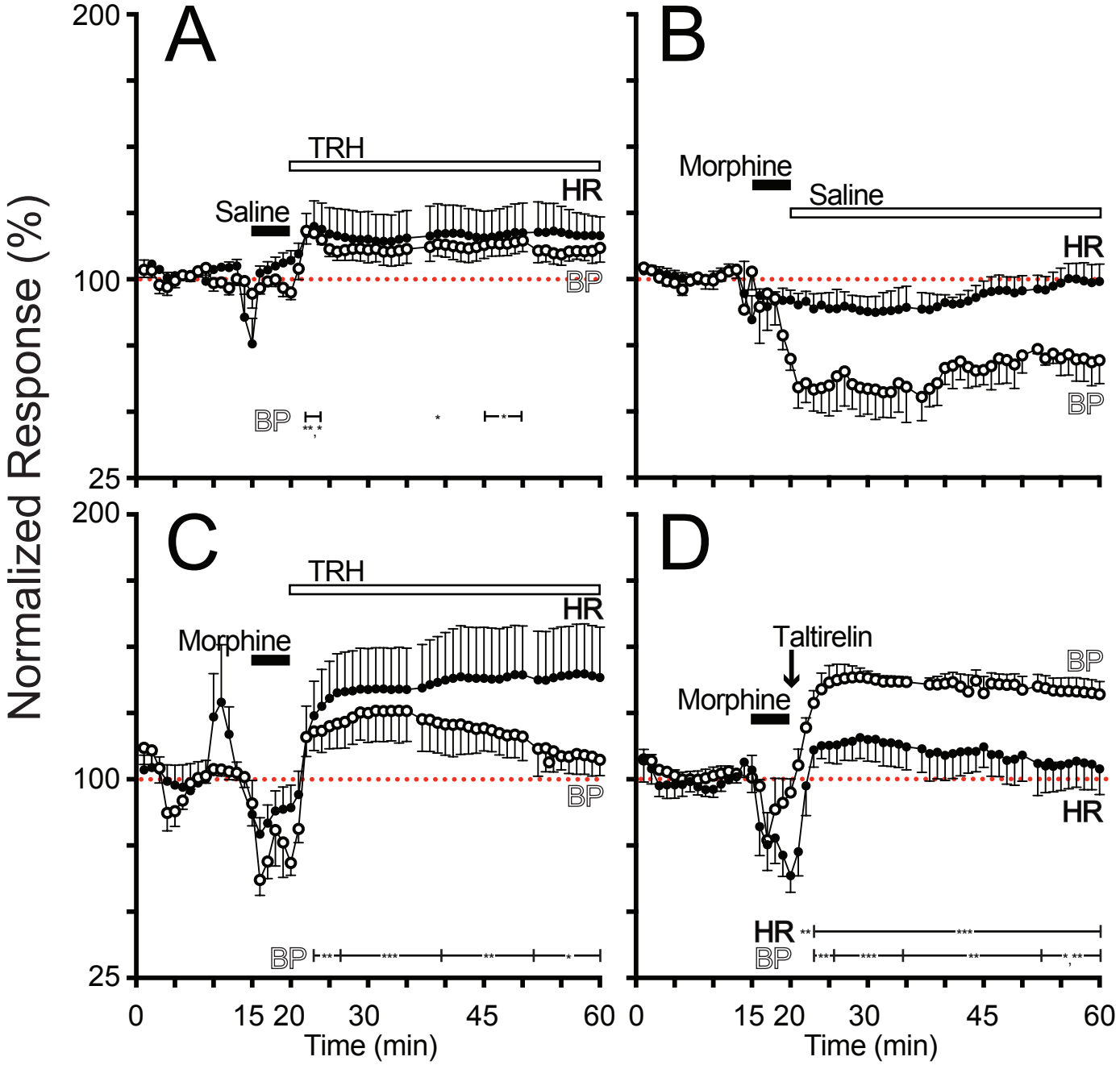
"Intravenous and intratracheal thyrotropin releasing hormone and its analog taltirelin reverse opioid-induced respiratory depression in isoflurane anesthetized rats". James D. Boghosian, Anita Luethy, and Joseph F. Cotten. *Journal of Pharmacology and Experimental Therapeutics.*



Supplemental Figure 2. Effect of intravenous bolus TRH on breathing rate (BR; black) and tidal volume (TV; red). Data were collected from the same animals/study as those of Figure 1 and were analyzed in the same manner. Down arrow indicates IV TRH administration. Data were normalized to the averaged 15 mins of baseline breathing prior to TRH administration. Baseline breathing rate and tidal volume were, respectively, 66±2 min^{-1} and 0.4±0.01 ml/100 g (n = 16 ± SEM for each). Each data point is the average of 4 animals, using 1 min of averaged data from each animal (n=4 + SEM); error bars may not be visible when smaller than the data point. Asterisks (** and ***) indicate statistical significance ($P<0.01$ and $P<0.001$, respectively; ANOVA with a Dunnett's posttest) relative to the data point just prior to TRH administration.

Supplemental Figure 2

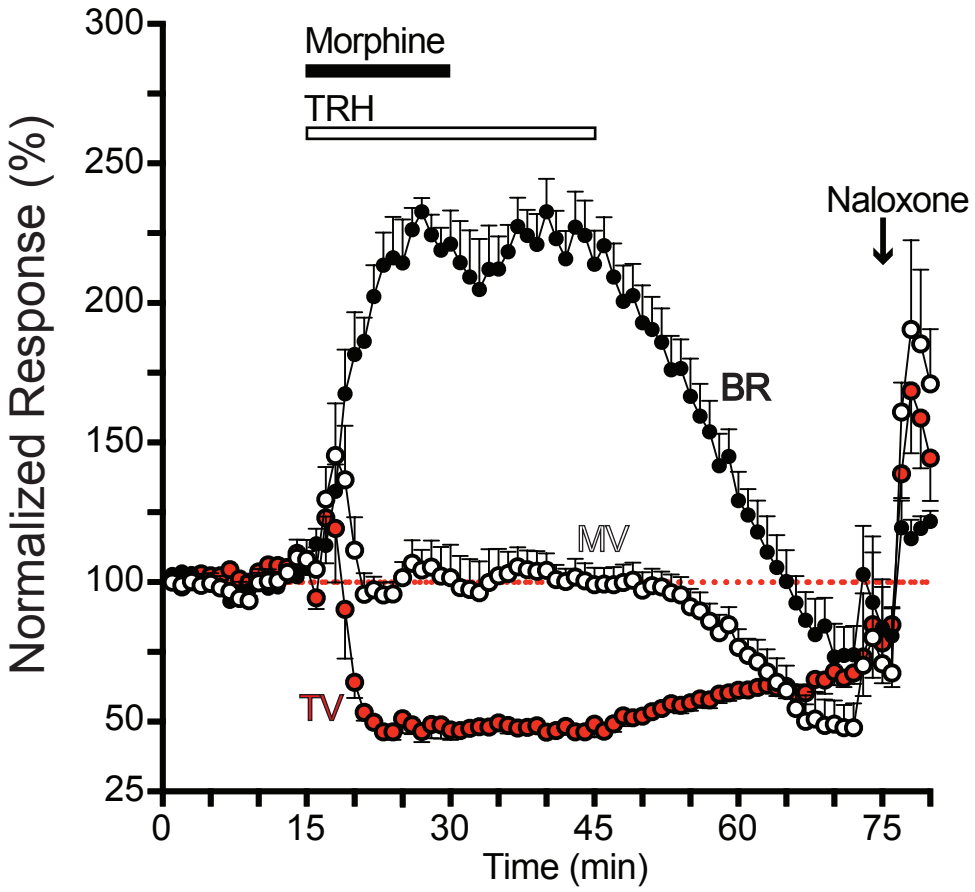
"Intravenous and intratracheal thyrotropin releasing hormone and its analog taltirelin reverse opioid-induced respiratory depression in isoflurane anesthetized rats". James D. Boghosian, Anita Luethy, and Joseph F. Cotten. *Journal of Pharmacology and Experimental Therapeutics.*



Supplemental Figure 3. Effect of intravenous morphine, TRH, and taltirelin on heart rate (HR; black) and mean blood pressure (BP; white). Data in A through C and in D were collected from the same animals/study as those of Figures 2 and 3, respectively, and normalized similarily. Drugs were dosed as in Figures 2 and 3. Each data point is the average of 3 to 4 animals, using 1 min of averaged data from each animal (n=3 to 4 ± SEM). Asterisks (*, **, and ***) indicate statistical significance ($P<0.05$, $P<0.01$ and $P<0.001$, respectively; ANOVA with a Dunnett's posttest) relative to the data point just prior to TRH administration (in A and C), morphine (in B), and Taltirelin (in D). Baseline heart rate and mean blood pressure in all groups were 360±33/min and 73±10 mmHg (n = 11 ± SEM).

Supplemental Figure 3

"Intravenous and intratracheal thyrotropin releasing hormone and its analog taltirelin reverse opioid-induced respiratory depression in isoflurane anesthetized rats". James D. Boghosian, Anita Luethy, and Joseph F. Cotten. *Journal of Pharmacology and Experimental Therapeutics.*



Supplemental Figure 4. Intravenous TRH prevents morphine-induced respiratory depression. Normalized minute ventilation (white circles; MV), breathing rate (black circles; BR), and tidal volume (red circles; TV) in orotracheally intubated rats spontaneously breathing 1.5% isoflurane in air. Morphine (15 mg/kg IV over 15 min; black bar); TRH (1 mg/kg IV bolus PRIOR to morphine followed by 5 mg/kg/h IV infusion; white bar); and naloxone (0.4 mg/kg IV bolus; down arrow). Each data point is the average of 4 animals using 1 min of averaged data from each animal (n=4 ± SEM). Baseline minute ventilation, breathing rate, and tidal volume were 31±1 ml/min/100 g, 70±2 min^{-1} , and 0.4±0.02 ml/100 g, respectively (n = 4 ± SEM).

Supplemental Figure 4