Changes in murine respiratory dynamics induced by aerosolized carfentanil inhalation: Efficacy of naloxone and naltrexone

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AR T I C L E  I N F O

Keywords: Carfentanil Naloxone Naltrexone Inhalation exposure Prophylaxis Respiratory dynamics Physiology based pharmacokinetics

ABSTRACT

Carfentanil (CRF) is an extremely potent opioid capable of inducing fatal respiratory depression. Naloxone (NX) and naltrexone (NTX) are opioid antagonists for which the efficacy against CRF remains largely unexplored. In this study, the effects of aerosolized CRF on respiratory function were investigated using adult male CD-1 mice. Mice were exposed to 0.4 mg/m³ of CRF for 15 min using custom whole-body plethysmograph units. Minute volume (MV), respiratory frequency (f), duty cycle (DC), and tidal volume (TV) were monitored and compared to control animals exposed to aerosolized H₂O. CRF exposure induced respiratory depression, characterized by a marked decrease in MV, which was sustained throughout 24 h post-exposure. Prophylactic and therapeutic treatment with intramuscular (i.m.) NX marginally improved MV, with slight dose-dependent effects. Analogous treatment with i.m. NTX returned MV to baseline levels, with all doses and intervention times performing similarly. Despite improvements in MV, treatment administration did not reverse changes in DC, a measure of respiratory timing. Overall, NX and NTX administration alleviated volumetric aspects of opioid-induced respiratory toxicity, while changes in respiratory timing remained unresolved throughout post-exposure observation. These sustained changes and differences in recovery between two aspects of respiratory dynamics may provide insights for further exploration into the underlying mechanism of action of opioids and opioid antagonists.

1. Introduction

Opioids are a class of lipid soluble analgesics with widely accepted applications in a variety of clinical settings. Side effects of opioid use are well-characterized and may include nausea, vomiting, physical dependence, and respiratory and central nervous system depression. Despite strict regulations in place for opioid usage, deaths resulting from opioid-induced respiratory depression have been regularly reported, and the number of deaths related to opioid misuse has risen significantly over the past two decades (Warner et al., 2014; Kolodny et al., 2015). In addition, a striking relationship has been observed between opioids prescribed and rates of opioid misuse and associated adverse outcomes, indicating the severity of the opioid misuse crisis and emphasizing the need to better understand opioid toxicity and potential therapeutics (Dart et al., 2015; Kolodny et al., 2015).

Opioids exert their effects via the activation of opioid receptors belonging to three major families: µ, δ, and κ (Mansour et al., 1988; Feng et al., 2012). These receptors are located throughout the central nervous system (Mansour et al., 1988) and widely expressed in several peripheral tissues, including the heart, lung, kidney and small and large intestine (Wittert et al., 1996). This widespread tissue distribution results in physiological effects at both the central and peripheral level. Opioid receptors play central roles in physiological processes such as regulation of ionic homeostasis, neuroprotection, nociception, regulation of emotional response, modulation of the immune response, regulation of feeding, and respiratory and cardiovascular control, and are implicated in disease states such as obesity and epileptic seizures (Pattinson, 2008; Feng et al., 2012). Due to these diverse roles, a host of potentially lethal adverse effects resulting from excessive opioid usage exist, including respiratory depression, hypotension, vasodepression,
and bradycardia (Feng et al., 2012).

Carfentanil (CRF) is a synthetic opioid with a potency 10,000 times that of morphine and 100 times that of fentanyl (George et al., 2010) and is not intended for therapeutic use in humans. It is classified as a Schedule II drug under the Controlled Substances Act, meaning that it has a high potential for abuse and that its use may lead to severe psychological or physical dependence. Commercially, CRF is most commonly marketed as a tranquilizing agent (Wildnil, Wildlife Laboratories) for large animals, including livestock and wildlife. Due to its high binding affinity, CRF has also been employed in medical laboratories to map μ-opioid receptors in the brain by positron emission tomography (PET) (Frost et al., 1989; Titeler et al., 1989; Eriksson and Antoni, 2015). Aside from commercial uses and the potential for drug abuse, the possible use of CRF as a lethal agent was brought to the world’s attention as a result of the 2002 Moscow theater incident, where an aerosol, later identified as a mixture of CRF and remifentanil, was introduced into the ventilation system, resulting in 125 deaths, in a poor attempt to manage a hostage crisis (Krechetnikov, 2012; Riches et al., 2012). The pharmacokinetics of CRF have been briefly explored in humans (Minkowski et al., 2012) and non-human species (Mutfow et al., 2004; Cole et al., 2006). For non-human animal models, CRF was rapidly absorbed with a half-life on the order of hours (Mutfow et al., 2004; Cole et al., 2006); for healthy humans, the half-life was less than an hour (Minkowski et al., 2012).

Naloxone (NX) and naltrexone (NTX) are FDA-approved opioid antagonists capable of reversing the adverse effects of opioids. Both drugs are thought to be competitive antagonists devoid of agonistic effects and function by inhibiting opioid-opioid receptor interactions responsible for downstream physiological effects (Martin, 1976; Booth, 1982). Prior studies in human and non-human species have shown that NX and NTX are capable of reversing respiratory depression induced by morphine and fentanyl exposure (Freye et al., 1983; Lewanowitsch and Irvine, 2002; Olofson et al., 2010). However, studies on the therapeutic potential of these antagonists on extremely potent opioids, such as CRF, are limited and largely preliminary (Moresco et al., 2001; Mutfow et al., 2004; Cole et al., 2006; Wong et al., 2017). In particular, the relatively short duration of action observed for NX may reduce its effectiveness against opioids with high binding affinities, and episodes of renarcotization may occur after the initial recovery (Dahan et al., 2010). While NTX has a longer duration of action and higher antagonistic potency compared to NX (Freye et al., 1983), renarcotization events have also been reported after treatment with NTX (Miller et al., 1996). Additional studies on the efficacies of various treatment regimens utilizing different opioid antagonists would provide valuable information for the development and optimization of potential therapeutics against opioid-induced toxicity.

In the present study, mice were exposed to aerosolized CRF using custom whole-body plethysmograph (WBP) units modified to monitor respiratory dynamics in real-time during aerosol exposure. A host of physiological parameters, including minute volume (MV), tidal volume (TV), respiratory frequency (f), and duty cycle (DC), were used to assess CRF-induced toxicity, specifically respiratory depression. Treatment regimens involving NX and NTX at varying doses and intervention times were evaluated against their ability to reverse CRF-induced changes in respiration.

2. Materials and methods

2.1. Animals

Adult male albino CD-1 mice (25–30 g) were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Mice were housed in groups of 10 prior to exposure and individually afterwards. Accommodations consisted of polycarbonate micro-isolator cages kept in climate-controlled rooms with temperatures maintained between 20 °C and 26 °C and a relative humidity of 30–70%. Animals were fed ad libitum and subjected to a 12-h light/dark cycle with no twilight. All research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, published by the National Academy Press, 2011, and the Animal Welfare Act of 1966, as amended. The study protocol was approved by the Institutional Animal Care and Use Committee, United States Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD.

2.2. Chemicals

Carfentanil (CRF), methyl 4-(N-(1-oxopropyl)-N-phenylamino)-1-(2-phenylethyl)-4-piperidinacarboxylate, > 95% pure (C₇₄H₇₂N₂O₅; MW 394.51), was obtained in crystalline form as a citrate salt from the chemical synthesis laboratory at the US Army Combat Capabilities Development Command Chemical Biological Center, APG, MD. From this, stock solutions of 2.5 mg/mL in sterile water were prepared for inhalation exposures. Xylazine and ketamine were purchased from Webster Veterinary Supplies (Devens, MA). NX, 17-allyl-4,5a-epoxy-3,14-dihydroxyxymorphinan-6-one hydrochloride dihydrate, and NTX, (S)-17-(cyclopromethyl)-4,5-epoxy-3,14-dihydroxyxymorphinan-6-one hydrochloride, were obtained from Sigma Chemical Co. (St. Louis, MO).

2.3. Inhalation exposures

Exposures were conducted using a custom inhalation system enclosed within a custom-designed certified glovebox (Baker Co., Sanford, ME), as described previously by Wong et al. (2017). Briefly, animals were exposed within WBP chambers (FinePointe Series Whole Body Plethysmography Rat Chamber, Data Sciences International, St. Paul, MN) modified to record respiratory dynamics during inhalation exposure and exposed to either H₂O or CRF at a concentration-time product (Ct) of 6 mg × min/m³ (0.4 mg/m³ for 15 min) (Fig. 1). Before the start of exposure, all mice were allowed to acclimate for 10 min within the exposure chamber, and baseline respiratory dynamics were recorded for 30 min. Following baseline, animals were exposed to aerosolized H₂O (control) or CRF for 15 min. Aerosols were generated using Collison nebulizers (BGI Incorporated, Waltham, MA) pressurized with a medical air compressor (Jun-Air, Benton Harbor, MI) to 25–30 psi to generate CRF aerosol, which was then fed into a custom-designed air manifold connected to four WBP chambers. Bias flow generators (Bias Flow Fresh Air Pump; Data Sciences International; St. Paul, MN) connected to the WBPs generated a slight vacuum to pull gas and aerosolized CRF from the manifold into the chamber, ensuring agent flow to each exposure unit at physiologically compatible and sufficient rates. Exit flows from the manifolds and bias flow units passed through a custom-built activated charcoal decontamination unit.

Measured respiratory dynamic parameters of interest included MV, TV, f, and DC. The data acquisition software (FinePointe, Data Sciences International, St. Paul, MN) recorded every 15 s and utilized a rejection index to exclude statistical outliers and noise. After the conclusion of exposure, mice remained in the WBP for 24 h with continuous respiratory dynamics collection. During post-exposure, animals were provided with a high protein rodent liquid diet (Bio-Serv, Frenchtown, NJ).

2.4. Treatment protocol

Animals were divided into groups based on exposure (H₂O or CRF), countermeasure (none, NX, or NTX), countermeasure dose (0.05, 1, or 5 mg/kg), and intervention time (N/A; prophylactic, 15 min prior to exposure; or therapeutic, 15 min after conclusion of exposure), as shown in Table 1; for each group, n = 11–18. Doses were chosen based...
on a single dose of the FDA-approved opioid overdose treatment, Narcan Nasal Spray (4 mg naloxone), scaled to the mouse equivalent dose (approximately 1 mg/kg) (FDA, 2005). Lower and higher doses were selected to explore dose-dependent effects. Naltrexone doses were selected to mirror naloxone for comparison. For prophylaxis, mice in the treatment groups were first allowed to acclimate to the WBPs before being removed, given a single intramuscular (i.m.) administration of NX or NTX, and then returned to the WBP chamber for a shortened baseline respiratory dynamics collection period of 15 min. For therapy, mice in the treatment groups were removed from the WBPs at 15 min post-exposure, given a single i.m. administration of NX or NTX, and immediately returned to the WBP chamber for data collection.

2.5. Data analysis

Mathematical comparisons of time traces were performed using GraphPad Prism (GraphPad Prism v7, Graph Pad Software Inc. San Diego, CA) to generate heat maps illustrating the severity of respiratory effects or degree of recovery throughout the experiment. Heat map severity was calculated using the upper and lower limits of the 95%
Table 2
Heat map group assignments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mathematical evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Effects/Normal</td>
<td>Average is within 95% confidence interval of control</td>
</tr>
<tr>
<td>Moderate Effects</td>
<td>Partial Recovery</td>
</tr>
<tr>
<td>Severe Effects</td>
<td>No Recovery</td>
</tr>
</tbody>
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confidence interval (CI) for both the exposed untreated and the control untreated groups. The averages for each parameter at each time point were assigned comparison values using the following ranges: below the lower 95% CI, within the 95% CI, or above the upper 95% CI. Each time point received two values, one as compared to the exposed untreated ranges and the second as compared to the control untreated ranges. Values were then summed and combined to create categories for severity analysis; evaluations were assigned to one of three categories according to Table 2: normal, moderate or severe.

CRF pharmacokinetics during and post-exposure were simulated using GastroPlus v9.6 (Simulations Plus Inc., Lancaster, CA). Individual subjects were created using the Populations Estimates for Age-Related (PEAR) Physiology program internal to GastroPlus. Cardiac outputs were calculated from subject weight by a 34 power law, tissue perfusion was scaled linearly to cardiac output, and tissue volume was scaled linearly to subject weight. CRF doses were calculated using an integral estimate of each subject’s MV over time and the volumetric concentration of CRF (Table C1). Pulmonary deposition fractions were estimated from regional deposition data presented in Raabe et al. (1988) assuming a particle radius of 2 μm and are given in Table C2.

3. Results

3.1. Effects of exposure and treatment on volumetric aspects of respiration

Time traces of MV over the course of exposure and 24 h post-exposure are shown in Fig. 2. Exposure to CRF induced a decrease in MV as compared to control animals exposed to aerosolized sterile H2O. This depression is maintained throughout the post-exposure observation period. Prophylactic and therapeutic treatment with NX marginally improved MV post-exposure, with slight dose-dependent effects. Comparable treatment with NTX returned MV to baseline levels, with all doses and intervention times performing similarly.

A mathematical comparison of individual time traces was generated to provide an indication of the severity of effect and degree of recovery and is presented as a heat map in Fig. 3. Categories of normal physiology or complete recovery, moderate effects or partial recovery, and severe effects or no recovery were calculated from 95% CI and are presented as green, yellow, and red boxes, respectively. Animals exposed to H2O and administered treatment exhibited moderate MV depression shortly after the conclusion of exposure (3–8 h); however, these effects were not sustained and MV was fully recovered thereafter. NX and NTX administration fully alleviated effects of CRF on MV within the first two hours after exposure. This was followed by a transient relapse lasting several hours, with MV fully recovered at 15 and 22 h post-exposure for NTX and NX, respectively.

3.2. Effects of exposure and treatment on respiratory timing

DC during baseline, exposure, and post-exposure is shown in Fig. 4 for control, exposed untreated, and exposed treated animals. Exposure to aerosolized CRF in the absence of treatment induced a marked increase in DC during exposure. Upon conclusion of exposure, DC immediately began to decrease and later equilibrated at a slightly elevated level as compared to baseline within seven hours post-exposure. Effects of therapeutic treatment with either NX or NTX mirrored those of the untreated, exposed group. Prophylactic administration, on the other hand, resulted in a less pronounced increase in DC during exposure, although DC ultimately equilibrated to levels akin to those observed for all exposed animals regardless of treatment regimen.

An analogous heat map was generated for DC using 95% CI comparisons to illustrate recovery and is shown in Fig. 5. Comparisons were assigned to one of three categories: normal physiology or full recovery, moderate effects or partial recovery, and severe effects or no recovery. Animals exposed to aerosolized H2O and given NX or NTX after exposure exhibited minimal changes in DC. Controls given prophylactic treatment exhibited slightly more deviations in respiratory timing; however, these changes were completely reversed within 24 h post-exposure. Neither NX nor NTX treatment was effective in reversing variations in DC induced by CRF exposure, with DC remaining severely affected for up to 24 h after the conclusion of exposure. Although a brief period of complete recovery (5–12 hrs post-exposure) was observed in animals given NTX prior to exposure, DC later relapsed and remained elevated for up to 24 h post-exposure.

3.3. Simulated CRF pharmacokinetics

Tissue distribution of CRF as determined by physiology based pharmacokinetics (PBPK) simulation is given in Fig. 6 as concentrations per volume of tissue. Initially following CRF exposure (0.4 mg/m3 CRF for 15 min), CRF was present at relatively high concentrations in the brain, liver, and kidney. Over the course of 24 h post-exposure, concentrations in most tissues decreased; however, concentration in the adipose tissue increased substantially, while concentrations in the muscle and brain increased to a comparatively lesser extent. For comparison, the amount of CRF sequestered in each tissue is presented as a fraction of the initial dose in Table C3.

4. Discussion

Opioid misuse can cause severe and sometimes fatal respiratory depression and represents an increasing public health concern. Opioids exert their effects by interacting with opioid receptors located throughout the central nervous system and peripheral tissues (Wittert et al., 1996; Feng et al., 2012). A high density of opioid receptors are located in the respiratory center of the brain, and the direct action of opioids on these receptors is thought to be the cause of opioid-induced respiratory depression (Mutolo et al., 2007; Feng et al., 2012). Hypoventilation following opioid exposure may result from several possible mechanisms, including a diminished chemoreceptor response to CO2 and O2, prolonged expiratory time, and reduced depth of breathing (Jungquist et al., 2011). Combined, these effects result in decreased O2 availability and may self-potentiate to progress to respiratory arrest and death. Yet studies have found that the vast majority of these adverse events could have been prevented with better respiratory monitoring (Lee et al., 2015). An improved understanding of changes in various aspects of respiration as a result of exposure to aerosolized CRF would therefore provide valuable information for evaluating and treating opioid toxicity.

Reduced ventilation following CRF exposure was observed immediately and manifested as a reduction in MV, which persisted for up to 24 h after the conclusion of exposure (Fig. 2). MV represents an ideal
respiratory parameter to assess, as it is the product of TV and $f$, both of which are noted as indicators of opioid-induced respiratory depression (Heard et al., 1996; Bouillon et al., 2003; Lalley et al., 2014; Montandon and Horner, 2014). MV depression was most pronounced around 8–10 h after exposure, with MV reduced by approximately 200% compared to controls. This time period corresponded to increased activity in control animals due to the diurnal cycle of mice. One interpretation of the lack of activity in exposed animals during this time period may be that CRF exposure is altering the brain, resulting in behavioral changes that persist up to 24 h post-exposure. These results are consistent with clinical observations collected in a prior study utilizing the same exposure apparatus and experimental conditions, where differential behaviors persisted throughout exposure and post-exposure observation (Wong et al., 2017). Collectively, these findings indicate possible cognitive impairment following CRF exposure. Further studies are warranted to elucidate the severity and permanence of these conditions.

Treatment regimens involving two common opioid antagonists, NX

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**Fig. 2.** MV of mice following exposure to CRF at a Ct of $6 \text{ mg} \times \text{min/m}^3$ (0.4 mg/m$^3$ for 15 min). Mice were prophylactically (15 min prior to exposure) or therapeutically (15 min after the conclusion of exposure) treated with NTX (2a) or NX (2b). Treatment time points are shown as dotted lines. Untreated control and exposed groups are shown with corresponding 95% CIs for reference (shaded grey for control and purple for exposed). CRF exposure induced a pronounced decrease in MV as compared to controls, with most notable differences observed from 7 to 13 h post-exposure. Treatment with NTX returned MV to baseline levels, although the heightened MV observed around 10 h post-exposure for controls was not present in exposed groups, with or without treatment. Minimal improvements in MV were observed for NX-treated animals. $n = 11–18$ for each group.
and NTX, were evaluated as potential medical countermeasures for CRF exposure. Control animals administered either drug exhibited respiratory dynamics comparable to those of untreated controls, verifying that neither compound elicits undesirable respiratory side effects (Figs. A1, A2). Prophylactic and therapeutic treatment with NX reduced CRF-induced respiratory depression to some extent, although MV remained moderately to severely diminished (approximately 50–70% of control) throughout the majority of post-exposure observation with slight dose-dependent effects (Fig. 3). Similar treatments with NTX appear to be more successful in reversing MV effects as compared to NX, with complete MV recovery observed 15 h after the conclusion of exposure.

Observationally, exposed animals exhibited normal behavior and were responsive prior to full MV recovery. The difference in recovery timescales in terms of CRF-induced behavioral and physiological changes highlights the possibility of permanent or semi-permanent alterations in the brain as a result of CRF exposure. These alterations may be related to tissue-specific selectivities and binding affinities for CRF, which remain largely unexplored to date. Interactions between CRF and opioid receptors located in various tissues could provide insight on the longer lasting effects observed in this study.

Additionally, it is worth noting that opioid receptors are widely expressed in lung tissue, and CRF may have a direct effect on the function of that tissue as well as a central effect on respiratory function (Wittert et al., 1996; Zembraski et al., 2000). While systemic treatment administration alleviated CRF-induced MV depression to some extent, it is possible, due to the route of countermeasure administration and the route of CRF exposure, that the central effects of CRF are mitigated with treatment, but the direct effects on the peripheral tissues are not fully reversed. The temporally extended effects of CRF may also be due to its high lipid solubility, which not only results in rapid analgesic effects but also renders it capable of being stored in adipose tissue (Lanthier et al., 1999; Yong et al., 2014; Leen and Juurlink, 2019). This explanation is consistent with PBPK simulations using experimental subject physiologies, where CRF concentrations were highest in adipose tissue 24 h post-exposure (Fig. 6). Possible release of CRF from these depots may occur after clearance of antagonists, resulting in re-narcotization. Alternatively, the relatively rapid elimination of both antagonists may prevent full reversal of CRF effects due to its high binding affinities (Miller et al., 1996; Dahan et al., 2010). Currently, the clinical practice for treating severe opioid toxicity involves continuous infusion of an opioid antagonist, which may be a possible treatment regimen for future investigations into carfentanil exposure.

Despite improvements in respiratory ventilation, treatment administration did not reverse changes in DC, a measure of respiratory timing. While periods of moderate recovery were dispersed throughout post-exposure observation, the effects on DC predominantly remained severe (Fig. 5). Again, the underlying message conveyed from these results is twofold. On one hand, the difference in recovery timescales and lack of recovery may indicate possible persisting alterations in the brain as a result of opioid-opioid receptor interactions. These alterations may mirror structural and functional changes observed in various brain regions following long-term opioid usage (Upadhyay et al., 2010). Moreover, the differential recovery observed for MV and DC may be related to the specific brain regions in which these alterations or interactions occur. For instance, CRF may interact primarily with neurons from the dorsal or ventral respiratory group, both of which are responsible for setting the rhythm of respiration (Lumb and Horner, 2013). Irreversible changes or prolonged interactions related to either respiratory group may therefore manifest as longer lasting effects on respiratory parameters involving timing and pattern (DC and f, Figs. 4 and A3), thus resulting in the different recovery timescales observed for behavior and physiology. Similarly, CRF may interact to a lesser extent with the pontine respiratory group, which is thought to control lung volumes, thereby leading to less severe or shorter effects on volumetric respiratory parameters (MV and TV, Figs. 2 and A3). Conversely, persistent signs and symptoms may be due to countermeasure metabolism, as only a single dose of either opioid antagonist was administered in this study. Combination treatment regimens utilizing opioid antagonists with different receptor specificities and multiple dose regimens accounting for drug elimination may be worth further evaluation. For instance, delta opioid antagonists have been evaluated for their therapeutic potential in reversing respiratory depression induced by highly potent opioids (Freye et al., 1991, 1992).

5. Conclusions

Mice exposed to aerosolized CRF exhibited reduced ventilation and altered breathing patterns characteristic of opioid-induced respiratory depression. A single i.m. administration of NX or NTX reduced the amount of time animals experienced respiratory depression. Although
not all CRF-induced respiratory changes were alleviated by the treatment regimens assessed, improvements observed in this study suggest that systemic treatment administration may be an additional option for inhalation exposures, especially in situations where treatment at the site of exposure may not be feasible.

In general, NTX performed more favorably than NX, diminishing the effects on MV and reducing the duration of respiratory depression. However, while effects on respiratory ventilation were moderated with treatment, changes in respiratory timing were not reversed up to 24 h post-exposure. Furthermore, prophylactic treatment proved as effective as therapeutic treatment, indicating the possibility of pretreatment for first responders in the event of aerosolized CRF release. The presence of sustained changes highlights a need to further characterize opioid-opioid receptor selectivity, activity, and affinity. Insight into these interactions may provide valuable information for the development and optimization of therapeutics. Given the increasing availability and usage of more potent opioids, therapeutics with longer durations of action or higher antagonist potencies may also be worthwhile areas of

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Fig. 4. DC of mice following exposure to CRF at a C2 of 6 mg × min/m³ (0.4 mg/m³ for 15 min). Mice were prophylactically (15 min prior to exposure) or therapeutically (15 min after the conclusion of exposure) treated with NTX (4a) or NX (4b). Treatment time points are shown as dotted lines. Untreated control and exposed groups are shown with corresponding 95% CIs for reference (shaded grey for control and purple for exposed). CRF exposure induced an immediate, marked increase in DC during exposure. An increase of similar magnitude was observed for all therapeutically treated groups. Prophylactic treatment with NX or NTX diminished this effect, with only a slight DC increase observed. Post-exposure, DC equilibrated within 7 h post-exposure, but remained slightly elevated compared to baseline for all exposed groups. n = 11–16 for each group.
Given the high metabolism of mice and the ability to perform high dose studies with low mortality, the mouse model presented in this study is ideal for conducting longer term studies to assess potential prolonged opioid-opioid receptor interactions and evaluate additional therapeutic regimens.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Dr. Michael Perkins, Ashley Rodriguez, and Jennifer Devorak for their various contributions to this project. The views expressed herein are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. This work was supported by the Defense Threat Reduction Agency (DTRA-CB, CB3950). W.Y.T., S.A.P., and M.C.R. were supported in part by an appointment to the Postgraduate Research Participation Program at the US Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and USAMRDC.
Appendix A. Supplementary data


Toxicology Letters 316 (2019) 127–135

References


