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Nalmefene reverses carfentanil-induced loss of righting reflex and respiratory depression in rats

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ABSTRACT

Reversing the respiratory depression induced by carfentanil involves intravenous administration of naloxone or naltrexone, but this treatment has disadvantages. Hence, finding a more appropriate treatment to counter the depressive actions of carfentanil is needed. In the present study, with the naloxone as a control, we investigated the efficacy of nalmefene for countering the depressive actions of carfentanil. Rats were treated successively with carfentanil (10 μg/kg, i.v.) and nalmefene (9.4–150.0 μg/kg, i.m.), and the duration of loss of righting reflex (LORR) recorded. Respiratory parameters were measured in free-moving rats using a whole-body plethysmograph after rats were administered carfentanil (20 μg/kg, i.v.) and nalmefene (9.4–150.0 μg/kg, i.m.) sequentially. The parameters of arterial blood gases were also examined. Nalmefene (9.4–150.0 μg/kg, i.m.) treatment dose-dependently decreased the duration of carfentanil-induced LORR. The respiratory rate after 60 min of nalmefene (150.0 μg/kg, i.m.) treatment increased from 34.3 ± 5.3 bursts/min to 117.8 ± 18.9 bursts/min, and enhanced pause decreased from 1.1 ± 0.1 to 0.4 ± 0.1, and was close to those of normal rats. Furthermore, nalmefene (37.5–150.0 μg/kg) treatment could enable the PaO_2, SaO_2 and $PaCO_2$ to approach normal levels 10 min (15 min after carfentanil injection) or 30 min (25 min after carfentanil injection) after injection. While, a single injection of naloxone (150.0 μg/kg, i.m.) only achieved partial remission of respiratory depression. These data suggest that nalmefene more effectively counters the depressive actions induced by carfentanil and is a more appropriate treatment to antagonize carfentanil toxicity compared with naloxone.

1. Introduction

Carfentanil is an analog of the synthetic opioid analgesic fentanyl. It is one of the most potent opioids, with a quantitative potency 10,000-times that of morphine and 100-times that of fentanyl (Lust et al., 2011). Initially, carfentanil was intended for use only as a tranquilizing agent to rapidly incapacitate large animals for examinations and procedures by veterinarians (Ramsay et al., 1995; Shaw et al., 1995). Nowadays, carfentanil is also used for *in vivo* positron emission tomography studies of mu-opioid receptors in the laboratory and clinic (Hagelberg et al., 2012; Ly et al., 2013).

In recent years, there have been reports of humans developing symptoms of drug toxicity induced by carfentanil (George et al., 2010). As with other opioid analgesics, carfentanil can produce loss of righting reflex (LORR) and even induce respiratory depression (recognized as one of the most serious adverse events) (Moresco et al., 2001). Therefore, finding an appropriate treatment to counter the depressive actions of carfentanil is needed.

Naloxone is an opioid receptor antagonist. Several studies have shown that naloxone can reverse the respiratory depression induced by morphine or fentanyl in an effective and rapid manner (Goodman et al., 2007; Longnecker et al., 1973). The short elimination and biophase equilibration half-lives (as well as rapid receptor kinetics) of naloxone complicate the reversal of high-affinity opioids such as carfentanil (Dahan et al., 2010). However, the duration of action of carfentanil far exceeds that of naloxone. The course of naloxone treatment should be kept under continued surveillance, and repeat doses should be administered as needed (Sarton et al., 2008).

Naltrexone, as a long-acting antagonist, has also been reported to reverse carfentanil-induced respiratory depression (Miller et al., 1996). However, "recycling" of carfentanil occurs because it has affinity for adipose tissues; it is stored there and also has a longer half-life than that of naltrexone. Once naltrexone has been metabolized completely and cleared from the body, carfentanil can be released from adipose tissue and recirculate, thereby causing a "renarcotization event" (Storms et al., 2006). New types of opioid antagonist with longer durations of action and higher

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efficiency against carfentanil would be useful for avoiding such recycling as well as for alleviating side effects.

Nalmefene is an opiate derivative similar in structure and activity to the opiate antagonist naltrexone (Keating, 2013). The advantages of nalmefene relative to naltrexone include: a longer half-life; greater oral bioavailability; more competitive binding with subtypes of opioid receptors; no observed dose-dependent liver toxicity (Ingman et al., 2005). Nalmefene is used to completely or partially reverse the effects of narcotics and alcohol by blocking opiate receptor sites, thereby reversing or preventing the toxic effects of narcotics and alcohol (Soyka and Rosner, 2010).

In the present study, we hypothesized that nalmefene would effectively counter the depressive actions of carfentanil. Thus, with the naloxone as a control, we investigated the efficacy of different doses of nalmefene for antagonizing carfentanil-induced LORR and respiratory depression in rats.

2. Materials and methods

All experiments were conducted after approval of the study protocol by the Animal Care and Use Committee of the Beijing Institute of Pharmacology and Toxicology (Beijing, China). The study protocol conformed to guidelines on the ethical use of animals set by the National Institutes of Health (Bethesda, MD, USA). All efforts were made to minimize the number of animals used and their suffering.

2.1. Animals

Experiments were completed on adult male Sprague-Dawley rats (180–220 g) provided by the Beijing Animal Center (Beijing, China). Animals were housed five per cage in a room maintained at 25 ± 1 °C with an alternating 12-h light–dark cycle. Rats had free access to water and food.

2.2. Laboratory reagents

Nalmefene hydrochloride was purchased from Haisco Pharmaceutical Co., Ltd. (Sichuan, China), and naloxone from Sigma (St. Louis, MO, USA), and dissolved in sterile physiological saline (0.9% NaCl) to the final concentrations used in this study. Carfentanil oxalate was obtained from Humanwell Healthcare Group (Hubei, China) and also dissolved in physiological saline before experimentation. Nalmefene or naloxone was injected intramuscularly (0.5 ml/kg body weight) and carfentanil was injected intravenously (2 ml/kg).

2.3. LORR

To study the effect of nalmefene on carfentanil-induced LORR, rats were injected with carfentanil (10 μg/kg, i.v.) according the result of our preliminary experiment. Five minutes later, rats were injected with varying doses of nalmefene (9.4–150.0 μg/kg, i.m.) or naloxone (150.0 μg/kg, i.m.). The duration of LORR was recorded individually for each rat. It was calculated by subtracting the time of onset of LORR from the time at recovery from carfentanil-induced sleep. The righting reflex was deemed to be "lost" if an animal did not right itself within 15 s of being placed on its back.

2.4. Determination of respiratory parameters

The dose-dependent effect of nalmefene against carfentanil-induced respiratory depression was studied in freely moving rats. A Whole-body Plethysmograph (EMKA Technologies, Paris, France) was used to measure respiratory parameters, *i.e.*, respiratory rate and enhanced pause (an index of airway obstruction). Rats were stabilized in the plethysmograph chamber for 30–40 min until a stable baseline was produced. They were then injected with carfentanil (20 μg/kg, i.v.) based on the preliminary result. After 10 min, rats were treated with different doses of nalmefene (9.4–150.0 μg/kg, i.m.) or naloxone (150.0 μg/kg, i.m.). Data for respiratory parameters were collected for an additional 60 min and analyzed using IOX software (EMKA Technologies).

2.5. Measurement of arterial blood gases

To characterize the effects of nalmefene against carfentanil-induced respiratory depression of arterial blood gases, rats were injected with carfentanil (20 μg/kg, i.v.), and then, 5-min later, given different doses of nalmefene (9.4–150.0 μg/kg, i.m.) or naloxone (150.0 μg/kg, i.m.). At 0, 5, 15, and 30 min after injection with carfentanil, arterial blood samples (1.0 ml) were drawn from the abdominal aorta with a self-filling polypropylene syringe containing 60 IU of dry, electrolyte-balanced heparin (PICO70; Radiometer Medical; Copenhagen, Denmark). At the point time of 0 min, rats were anesthetized by ether before acquiring arterial blood samples. Air pockets were expelled immediately after sampling. Thereafter, samples were mixed gently until testing on an ABL90 Flex Blood Gas Analyzer (Radiometer Medical). The partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) as well as oxygen saturation (SO_2) in arterial blood were recorded and analyzed at the corresponding time point. Five animals per time point were used for measurement of arterial blood gases.

2.6. Statistical analysis

For LORR and measurement of arterial blood gases, data are the mean ± S.E.M. They were analyzed by two-way ANOVA followed

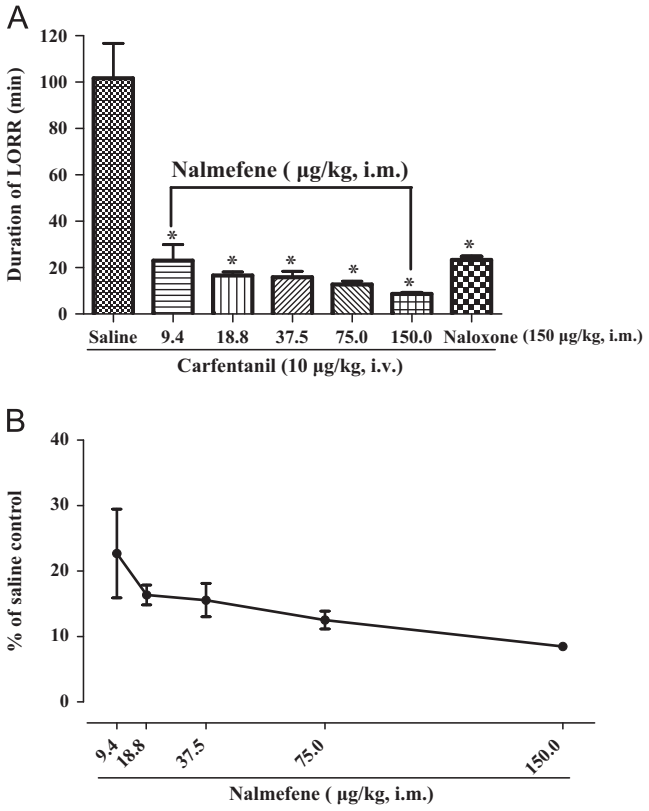

Fig. 1. (A) Effect of nalmefene or naloxone and (B) dose-related effect of nalmefene on the duration of carfentanil-induced LORR in rats. Nalmefene or naloxone was injected via the intramuscular route 5 min after carfentanil injection. Each column (A) and each point (B) represent the mean and S.E.M. of 5 rats. $^{*}P < 0.01$ *vs* respective saline-treated rats. ($n = 5$ for each group).

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