European Journal of Pharmacology 758 (2015) 11–15

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Nalbuphine could decrease the rewarding effect induced by tramadol in mice while enhancing its antinociceptive activity

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ARTICLE INFO

Article history:
Received 8 November 2014
Received in revised form
24 March 2015
Accepted 25 March 2015
Available online 3 April 2015

Keywords:
Nalbuphine
Tramadol
Reward
Psychological dependence

ABSTRACT

Nalbuphine, a kappa-opioid agonist and mu-opioid partial agonist, has been used as an analgesic or an adjuvant with morphine to attenuate the development of morphine dependence and rewarding effect. In this study, we investigated the effect of nalbuphine on tramadol rewarding effect and antinociception. Using the conditioned place preference (CPP) paradigm in mice, we demonstrated that co-administration of nalbuphine (7 mg/kg, s.c.) with tramadol (70 mg/kg, s.c.) during conditioning completely blocked the CPP induced by tramadol. Co-administration of nalbuphine blocked the increase in dopamine level in the nucleus accumbens induced by tramadol. These actions were accompanied by an increase rather than attenuation of the antinociceptive effect of tramadol. These results suggest that nalbuphine could have a great potential as a pharmacotherapy for tramadol abuse.

1. Introduction

Opioids are strong analgesics used to control moderate to severe pain (Jang et al., 2006); however, their repeated administration can lead to dependence and tolerance (Jang et al., 2006). Opioids act through three types of receptors; μ, δ, and κ (Jang et al., 2006). Most of the opioids used clinically are *mu*-opioid receptor (MOR) agonists (Nakamura et al., 2008).

The mesolimbic reward system is activated by opioids and generates signals in the ventral tegmental area (VTA) that results in the release of dopamine (DA) in the nucleus accumbens (NAc) causing feeling of pleasure (Kosten and George, 2002). It has been reported that the NAc shell is highly correlated with reward (Di Chiara et al., 2004) and dopamine release within the nucleus accumbens shell in rats is used as a criterion for dependence (Sprague et al., 2002). Morphine was reported to increase extracellular level of dopamine in the NAc which was blocked by microinjection of κ-opioid agonist into the NAc (Narita et al., 2005).

Tramadol is an effective analgesic in step 2 of the World Health Organization's guidelines for the treatment of patients with cancer pain (Lee et al., 1993). Tramadol acts through opioid and nonopioid (norepinephrine and serotonin reuptake inhibition) mechanisms (Liu et al., 1999; Raffa et al., 1992). Tramadol is a synthetic analog of codeine with affinity for the MOR approximately tenfold less than that of codeine and 6000-fold less than that of morphine (Raffa et al., 1992). The analgesic potency of tramadol was found to be equal to codeine and 10 times less than morphine (Marquardt et al., 2005), and one-fifth as potent as nalbuphine (Lee et al., 1993).

Some studies have reported that tramadol possesses considerably lower abuse potential than morphine (Budd, 1994); however, tramadol abuse has been shown in humans (Cicero et al., 1999; Epstein et al., 2006; Zacny, 2005) associated with physical and psychological dependence (Sprague et al., 2002).

Subcutaneous injection of tramadol produced a significant place preference in mice accompanied by an increase in dopamine level in the nucleus accumbens which was suppressed by pretreatment with the MOR antagonist β-funaltrexamine (Nakamura et al., 2008).

Nalbuphine is a κ agonist/μ partial agonist (Emmerson et al., 1996; Selley et al., 1998; Stevenson et al., 2003) which is more active on κ than on μ receptors (Picker et al., 1993).

Nalbuphine is a potent analgesic with a low side effect and dependence profile (Errick and Heel, 1983; Schmidt et al., 1985) which is used clinically in postoperative pain management (Dix, 2001; Renner et al., 2010; Schultz-Machata et al., 2014). Nalbuphine produces morphine-like effects at low doses and dysphoric effects at higher doses (Peachey, 1987).

Coadministration of nalbuphine with morphine dose-dependently blocked the development of morphine tolerance, dependence

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http://dx.doi.org/10.1016/j.ejphar.2015.03.062
0014-2999/

and respiratory depression in rats without attenuation of the antinociceptive effect of morphine (Dabrowska-Wojciak and Piotrowski, 2008; Jang et al., 2006; Lee et al., 1997).

The present study was designed to investigate the effect of co-administration of nalbuphine with tramadol on a tramadol-induced rewarding effect which corresponds to psychological dependence.

2. Material and methods

2.1. Chemicals

Tramadol hydrochloride was purchased from MinaPharm Co. and Nalbuphine hydrochloride was purchased from Amoun pharmaceuticals Co. All drugs were diluted in saline.

2.2. Animals

Adult male balb/C mice weighing 20–30 g were purchased from Theodor Bilharz Research Institute, Cairo, Egypt. The mice were kept under standard environmental and nutritional conditions throughout the investigation. All experimental procedures were approved by the Ethical Committee for Animal Handling at Zagazig University (ECAHZU).

Mice were randomly distributed into 4 groups ($n=8$) and were injected subcutaneously with tramadol (70 mg/kg), nalbuphine (7 mg/kg), tramadol + nalbuphine (10:1) or saline.

The dose of tramadol was selected based on a previous study (Nakamura et al., 2008), while the ratio of nalbuphine coadministered with tramadol was selected based on a previous report using nalbuphine with morphine (Jang et al., 2006) and the same dose of nalbuphine was used alone to study its actions. In addition, nalbuphine was previously reported to exert antinociceptive action at similar dose level in rats (Khasar et al., 2003) and mice (Patrick et al., 1999).

In our pilot experiment, we have tested the antinociceptive activity of different doses of tramadol (17.5, 35, 70 and 100 mg/kg) and nalbuphine (7 mg/kg) alone or combined together.

2.3. Evaluation of the rewarding effect using the conditioned place preference (CPP) apparatus

The conditioned place preference (CPP) test is a well-recognized means to quantify the rewarding effect of drugs (Tao et al., 2006). In the place-conditioning paradigm, mice and rats prefer an environment associated with administration of μ agonists (Suzuki et al., 1991).

The conditioned place preference apparatus is composed of three distinct compartments, two equally sized large outer compartments; A: white colored with mesh floor (drug-paired), B: black colored with rod floor (saline-paired), separated by white, smooth floor small central area (C, communicating tunnel) (Carlezon, 2003).

The animals were made psychologically dependent and tested for rewarding effects (Nakamura et al., 2008) as follows.

Preconditioning: On day 1, each mouse was placed separately into compartment C in the apparatus for 10 min with free access to all compartments, and the time spent in each compartment was recorded to assess unconditioned preference. A normal unconditioned mouse prefers to remain in compartment B.

Conditioning: on days 2, 4, and 6, animals were subcutaneously injected with tramadol, nalbuphine, or tramadol + nalbuphine; then the animals were immediately placed in compartment A for 1 h (the compartment opposite to that in which they had spent the most time in the preconditioning test). On days 3, 5 and 7, the animals received subcutaneous injection of saline and were immediately placed in compartment B for 1 h. Access to compartment C was blocked during conditioning sessions.

Postconditioning: on day 8, the animals were allowed free access to all compartments for 10 min and no drug injection was given. The time spent in the drug-paired compartment (A) was recorded for each animal and the change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment (A) and the time spent in this compartment in the preconditioning session.

2.4. Quantification of dopamine by ELISA

After recording the preference profile for each animal, animals were sacrificed by decapitation, the whole brain was isolated and the shell region of the nucleus accumbence was dissected according to mouse brain atlas (Paxinos and Franklin, 2001). Dopamine level in the shell region of the nucleus accumbence was quantified using ELISA kit purchased from Uscn Life Science Inc. according to the manufacturer's instructions.

2.5. Evaluation of the antinociceptive activity using the hotplate test

The latency time (in seconds) taken by the mouse to lick its paws or to jump within a plexiglas cylinder placed on a hotplate surface (adjusted to 55 °C) was taken as the end point and the increase in it after drug administration was taken as a measure of the antinociceptive activity as previously described by Abdel-Zaher et al. (2011) with slight modification. Prior to administration of drugs, mice were tested on the hotplate for 4 days in order to obtain a stable control response level (pre-drug latency), during these 4 days, animals that failed to respond within 30 s were removed from the hotplate and retested again after 30 min and animals that failed to respond within 30 s were excluded from the subsequent test. The antinociceptive effect of tramadol, nalbuphine, tramadol plus nalbuphine was determined 60 min after drug injection (post-drug latency). A maximum cut-off time of 30 s was chosen to prevent tissue injury and mice that did not respond within 30 s were removed from the hotplate and assigned a score of 30 s.

2.6. Statistical analysis

Data are expressed as mean ± standard error of mean. Statistical analysis was performed using one way analysis of variance (ANOVA) to compare multiple groups followed by Tukey's post-hoc test using Graph pad Prism software version 5. For all analysis, the level of statistical significance was set at $P<0.05$.

3. Results

3.1. Rewarding effect

The difference in time spent in the drug-paired compartment before and after conditioning was used as a measure of place preference. All mice showed no significant place preference for the drug-paired compartment before conditioning (day 1). For the saline (C) and nalbuphine (N) groups, no change of place preference was observed. Administration of tramadol resulted in a significant increase in time spent in the drug-paired compartment compared with the time spent in the same compartment before conditioning (335 vs 135 s). When nalbuphine was co-administered with tramadol, the drug-paired place preference was significantly decreased (199 vs 335 s) in comparison to tramadol group (Fig. 1).