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Nalbuphine is effective in decreasing the rewarding effect induced by morphine in rats

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Abstract

Nalbuphine, a κ-opioid agonist and μ-opioid partial agonist, has been widely used as an analgesic or an adjuvant with morphine in clinics. In rats, it attenuates tolerance and physical dependence caused by morphine, when co-administered. In this study, we investigated the effect of nalbuphine on morphine reward. Using the conditioned place preference (CPP) paradigm in rats, we demonstrated that co-administration of nalbuphine (1 mg/kg, i.p.) with morphine (5 mg/kg, i.p.) during conditioning could completely block the CPP induced by morphine. However, in experiments examining locomotor activity in rats, nalbuphine showed no effect on the development of behavioral sensitization induced by reported morphine administration. In microdialysis experiments, morphine induced a significant increase in the dopamine metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid in the shell region of the nucleus accumbens. Co-administration of nalbuphine blocked the increase in dopamine metabolites induced by morphine. These results may be due to the attenuating effect of nalbuphine on the dopaminergic activity of mesolimbic pathways. All of these results suggest nalbuphine could have a great potential as a pharmacotherapy for opiate abuse.

Keywords: Morphine; Nalbuphine; Conditioned place preference; Locomotor activity; Microdialysis; Nucleus accumbens; Mesolimbic pathway

1. Introduction

Nalbuphine is a κ-opioid agonist/μ-opioid partial agonist analgesic (Emmerson et al., 1996; Selley et al., 1998; Stevenson et al., 2003). With parenteral administration, it is approximately equipotent to morphine (Errick and Heel, 1983). Nalbuphine is a potent analgesic with a low side effect and dependence profile in both animals and humans (Schmidt et al., 1985). In post-operative pain management, nalbuphine infusion has been reported to be a good adjuvant for epidural morphine, which could reduce the incidence of side effects (e.g. respiratory depression, nausea, vomiting and pruritus) without decreasing the quality of pain relief (Wang et al., 1996, 1998). Although nalbuphine-induced analgesia, like that caused by the other κ-opioid agonists, is sex-dependent (Gear et al., 1999), it has been widely used in the clinical treatment of pain (Hoskin and Hanks, 1991). In studying opioid dependence, nalbuphine has been examined for its addictive potential and found to have less misuse liability in both humans and animal models (Preston and Jasinski, 1991; Pchelintsev et al., 1991). Kuzmin et al. (1997) also reported that the selective κ-opioid agonist, U50,488H could decrease the intake of morphine in the experiments of self-administration in mice. In a previous study, we demonstrated that nalbuphine co-administered with morphine prevents tolerance and naloxone-precipitated withdrawal signs in rats (Lee et al., 1997). Therefore, it is possible that nalbuphine can attenuate morphine-induced rewarding effects, which could be linked with the development of psychological dependence. In this study, we used the conditioned place preference (CPP) paradigm to investigate the co-administration effect of nalbuphine on morphine reward (Tzschentke, 1998; Huang et al., 2003). Behavioral sensitization of locomotor activity was examined in parallel with CPP experiments, as it was viewed as an important factor in the development and maintenance of addiction (Robinson and Berridge, 1993).

In conjunction with the behavioral study, microdialysis experiments were performed at the shell region of the nucleus accumbens (NAc). The concentrations of dopamine, serotonin and their

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metabolites were determined to evaluate the action of nalbuphine on morphine-induced activation of the mesolimbic pathway. Because the dopaminergic neuropathways of the mesolimbic system, especially the neurons at the ventral tegmental area (VTA) projecting to the NAc, have been recognized as a major target of addictive drugs (Bonci et al., 2003; Self, 2004), this investigation of microdialysis in free moving animals could provide more information about the possible action of nalbuphine on the neural circuits responsible for morphine-induced rewarding effects.

2. Methods

2.1. Animals

Male Sprague–Dawley rats, weighing 350–400 g, were purchased from the National Experimental Animal Centre, Taipei, Taiwan. All rats were kept in an animal room with a 12-h light:12-h dark cycle, at a temperature of $25 \pm 2\,^{\circ}C$ and humidity of 55%. Standard diet and water were provided ad libitum. The animals were acclimated for at least 1 week before the experiments were commenced. The care of animals was carried out in accordance with institutional and international standards (Principles of Laboratory Animal Care, NIH) and the protocol received prior approval from the Institutional Animal Care and Use Committee of the National Defense Medical Center, Taiwan, ROC.

2.2. Schedule of drug administration

A 13-day schedule was used for CPP tests and the tests of locomotor activity. On day −1, the animals were placed in a dark isolated room for 60 min for habituation. On day 0, locomotor activity was tested following saline administration in the afternoon. In the morning of day 1, pre-drug place preference was tested for CPP experiments in an isolated room. Following drug administration, locomotor activity of the animals was determined in the afternoon of day 1. Saline and drug conditionings were carried out on days 2–7, promptly after saline administration in the morning or drug administration [morphine (5 mg/kg, i.p.), nalbuphine (1 mg/kg, i.p.) or morphine (5 mg/kg, i.p.) + nalbuphine (1 mg/kg, i.p.)] in the afternoon. A control (saline) group of animals received saline injections and conditionings both in the morning and afternoon. The rats were placed in the saline- or drug-associated compartment for 40 min. Post-drug place preference was measured and recorded in the morning of days 8 and 13 to examine whether rats had a preference for the drug and whether the preference could last. In the afternoon of day 13, locomotor activity of the animals was measured to evaluate the behavioral sensitization.

2.3. Conditioned place preference test

The CPP apparatus, made of an acrylic plastic box (70 cm × 25 cm × 25 cm), was divided into three compartments. Two identical-sized compartments (30 cm × 25 cm × 25 cm) were constructed at both sides, separated by a narrower corridor (10 cm × 25 cm × 25 cm). The compartments were connected by guillotine doors (10 cm × 10 cm) between the central unit and the other two at both ends. One of the large compartments was covered by a mosaic type paper (checkered, 2.5 cm × 2.5 cm black and white squares) on the three walls and floor as a visual cue; another large compartment was covered by pure white paper. To aid the visual cues, a blue light bulb and a red light bulb were separately hung above the two large compartments. During the experiments, the CPP apparatus was kept in an isolated room with only blue and red light bulbs on. After each behavioral test or place conditioning, the whole box was cleaned thoroughly to prevent interference from the smell of faeces and urine.

As described in Section 2.2, the pre- and post-drug place preferences of the animals were determined, giving the rats free access to the entire box for 15 min. During conditionings, the animals were kept for 40 min in the corresponding compartment.

2.4. Locomotor activity test

The total activity of rats was measured in transparent standard polypropylene animal cages (38 cm × 22 cm × 15 cm). The test cages were placed in a photobeam activity system (San Diego Instruments, USA). A computer control unit registered interruptions to the photobeams from five individual cages. Any break of the photobeam was recorded as total activity. Activity was recorded in 5-min periods for 2 h immediately after drug or saline administration. The locomotor activity experiments were performed in an isolated noise-free room.

2.5. Microdialysis experiments

Under pentobarbital anaesthesia (50 mg/kg, i.p.), different groups of rats were implanted stereotaxically with guide cannulae (MAB 6 Guide Cannula for MAB 6.20 Microdialysis Probes, re-usable, Microbiotech, Stockholm, Sweden) at the shell of the nucleus accumbens (coordinates: AP, +1.7 mm; L, +1.0 mm with respect to bregma; V, −7.2 mm from skull). The cannulae were fixed firmly to the skull using two metal screws and dental cement. Animals were allowed 3 days for recovery. On the day of the experiment, a microdialysis probe (MAB 6.20, Microbiotech) was inserted. The probe contains 1 mm of exposed dialysis membrane (outer diameter: 0.6 mm; molecular weight cut-off: 15,000 Da) protruding from the guide cannula. The dialysis solution (artificial cerebrospinal fluid: NaCl, 145 mM; KCl, 2.8 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.2 mM; D-glucose, 0.25 mM; pH 7.4) was advanced through the probe via Santopren tubing (Microbiotech) by a microsyringe pump (CMA 102, Sweden) at a flow rate of 1 μL/min. Before the collection of dialysates, 2.5 μL of antioxidants (oxalic acid 1 mM and acetic acid 0.1 M) was added in the sampling tubes to prevent the rapid oxidation of monoamines. After 60 min of equilibration, dialysis samples were collected every 15 min and measured by high-performance liquid chromatography (HPLC). If the levels of measured monoamines remained stable (the difference between the three sequential dialysates is within 15%), the last two values were averaged to define the basal efflux. Subsequently, animals were injected with saline, morphine (5 mg/kg, i.p.), nalbuphine (1 mg/kg, i.p.) or both drugs, and the dialysates were collected every 30 min for 225 min, except for the first sample, which was collected 15 min after drug injection. The samples were immediately subjected to HPLC to analyze the monoamines (as described below). After each microdialysis experiment, the location of the probe was verified histologically in a series of 50 μm sections. The actual tip location of the microdialysis probes is illustrated in Fig. 1.

2.6. Monoamine analysis on high-performance liquid chromatography

The HPLC system was composed of a reverse-phase C-18 column (MD-150, RP-C-18, 3 μm, length: 15 cm, ESA, USA), a high-pressure pump (LC-10AD, Shimadzu, Japan), and connected with an electrochemical detector (ECD) coupled with three electrodes (Coulochem II, ESA, USA). The electrode of the guard cell was set at 350 mV, and electrodes 1 and 2 (for detection) were set at 40 and 250 mV, respectively. Under an isocratic condition, the mobile phase solvent (MD-TM, ESA, USA) was circulated at a flow rate of 0.5 mL/min in the system. To quantify the sample peaks, each chemical [3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA)] was compared with the external standards, which were freshly prepared and injected every fifth sample run.

2.7. Statistical analysis

In CPP tests, the paired *t*-test was used to analyze the data for the comparison of the place preference before and after drug administration (day 1 versus days 8 and 13). For the place preference before drug conditioning (day 1), the data were analyzed with one sample *t*-test with a hypothetical mean value of zero. Two-way repeated measures ANOVA followed by Bonferroni post-test was used to analyze the data from the locomotor activity tests and the microdialysis curves. One-way ANOVA followed by post hoc Newman–Keuls test was used to analyze the data from the area under the curve (AUC) of the microdialysis curves. The AUC was obtained from the total peak area above the horizontal line of 100%, in summation with the peak areas below the line,