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Short communication

Discriminative stimulus properties of morphine mediated by μ_1-opioid receptors

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Abstract

The μ-opioid receptor subtypes involved in the discriminative stimulus properties of morphine were investigated in rats that had been trained to discriminate between 3.0 mg/kg morphine and saline. The discriminative stimulus properties of morphine were significantly attenuated by β-funaltrexamine (an irreversible μ-opioid receptor antagonist: 10 and 20 mg/kg) and naloxonazine (an irreversible μ_1-opioid receptor antagonist: 20 mg/kg). These results suggest that the discriminative stimulus properties of morphine may be mediated by μ_1-opioid receptors.

Keywords: Morphine; Drug discrimination; μ_1-Opioid receptor

1. Introduction

The discriminative stimulus properties of drugs in animals are considered to be a model of subjective effects in humans and are believed to be related to neuropharmacological mechanisms. Furthermore, the rewarding effects, as well as the discriminative stimulus properties, of morphine are thought to be critically involved in drug-seeking behavior; both are mediated by μ-opioid receptors. Morphine increases the release of dopamine from the nucleus accumbens as measured by microdialysis techniques (Di Chiara and Imperato, 1988), and this may play an important role in the rewarding effects of morphine.

Several binding studies have demonstrated that μ-opioid receptors consist of μ_1- and μ_2-opioid receptor subtypes (Pasternak and Wood, 1986). Many investigators have sought to characterize the involvement of μ_1- and μ_2-opioid receptors in the pharmacological effects of morphine (Kamei et al., 1993; Pasternak and Wood, 1986). Suzuki et al. (1993) recently reported that morphine-induced place preference, which may be linked to its rewarding effects, is mediated by μ_2-opioid receptors. In addition, the dopamine-releasing effects of morphine are also regulated by μ_2-opioid receptors (Pasternak and Wood, 1986). However, although it is well known that the discriminative stimulus properties of morphine are mediated by μ-opioid receptors, it is not yet clear which receptor subtype, μ_1 or μ_2, is involved. In the present study, we attempted to identify the μ-opioid receptor subtype(s) involved in the discriminative stimulus properties of morphine (3.0 mg/kg) using β-funaltrexamine (an irreversible μ-opioid receptor antagonist) and naloxonazine (an irreversible μ_1-opioid receptor antagonist).

2. Materials and methods

2.1. Animals

Eighteen male Fischer 344 rats (Charles River Japan, Atsugi, Japan) maintained at 210–230 g (80% free-feeding weight) were used. Each rat was approximately 80 days old at the beginning of the experiment. Water was available ad libitum for all rats in their home cages. The rats were housed in individual cages at a

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room temperature of 22 ± 1°C with a 12-h light-dark cycle (light on 8:00 a.m. to 8:00 p.m.).

2.2. Apparatus

Experiments were conducted in operant-chambers (model: GT 8810; O'Hara & Co., Tokyo, Japan) equipped with two levers, with a reinforcement cup mounted between the levers. White lamps were installed above each of the levers. Chambers were enclosed within sound- and light-attenuating boxes and supplied with white noise to mask extraneous sound. Reinforcement consisted of 20-mg food pellets (O'Hara & Co., Tokyo, Japan).

2.3. Discrimination training

Discrimination training was performed according to the methods of Suzuki et al. (1994). Briefly, before they were trained to discriminate between morphine and saline, all rats were trained to press a lever. Training began under a fixed-ratio (FR 1) reinforcement schedule in which the rat was presented with a food pellet each time it pressed a lever. When reinforcement was provided, the light above the lever was illuminated. After the response rates had stabilized, the FR requirement was increased steadily to a reinforcement schedule of FR 10. After the response rates had stabilized under FR 10, and the rat received reinforcement 40 times in four consecutive sessions, both levers were presented. The rats were trained to discriminate between morphine (3.0 mg/kg s.c.) and saline. Drug or saline was administered 30 min before each session. The rats were required to respond on the stimulus-appropriate lever to obtain reinforcement; there were no programmed consequences for responding on the incorrect lever. Training sessions were 15 min in duration, and this phase of training continued until all of the rats performed up to the criterion [accuracy of at least 83% (First Food Pellet ⩽ 12 responses) for five consecutive sessions]. Discriminative training was continued even after the criterion was attained.

2.4. Testing procedure

After the animals attained the criterion, dose-response and combination tests were initiated. In the dose-response tests, morphine (0.3–3.0 mg/kg) was administered s.c. 30 min before the test sessions. In the combination tests, a cumulative dosing procedure (half log unit) was used. Test sessions consisted of three or four FR components; morphine was administered s.c. 30 min before the first component. For each component, rats were placed in the operant box until they had made ten responses on either lever or until 5 min (duration of the component) had elapsed. Morphine was administered again immediately after the first component was completed. This procedure was repeated two or three times. In the combination tests, saline, 10 or 20 mg/kg β-funaltrexamine, or 20 mg/kg naloxonazine was administered s.c. 24 h before the first component. The doses and pretreatment time of β-funaltrexamine and naloxonazine were based on the reports by Suzuki et al. (1993) and Geoffrey et al. (1986), respectively.

2.5. Drugs

The drugs used in the present study were morphine hydrochloride (Sankyo Co., Tokyo, Japan), β-funaltrexamine hydrochloride (Research Biochemicals, MA, USA) and naloxonazine hydrochloride. Naloxonazine was synthesized by us. All drugs were dissolved in saline.

2.6. Data analysis

During the training sessions, accuracy was defined as the number of correct responses, expressed as a percentage of the total number of responses before the first food pellet. During the test sessions, performance was expressed as the number of drug-lever responses as a percentage of the total number of responses upon completion of FR 10. Response rate (responses/min) was also evaluated during the test sessions. The response rate was calculated as the total number of

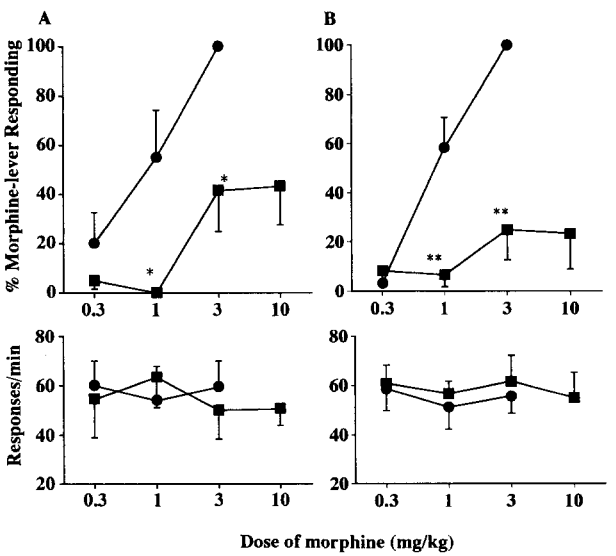

Fig. 1. Effects of 10 (A) and 20 (B) mg/kg β-funaltrexamine (squares) or saline (circles) on the discriminative stimulus properties of morphine (top panel) and on the response rates (bottom panel) in rats that had been trained to discriminate between 3.0 mg/kg morphine and saline. Each point represents the mean percentage of morphine-appropriate responses or the response rate with S.E.M. for six animals. * $P < 0.05$ and ** $P < 0.01$ vs. saline control.

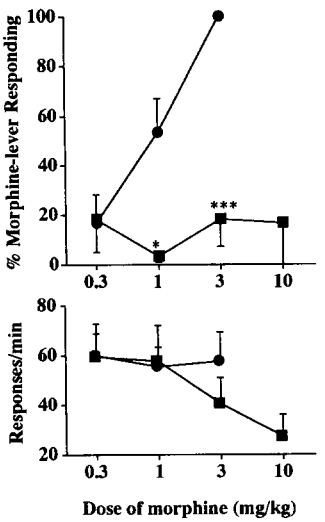

Fig. 2. Effects of 20 mg/kg naloxonazine (squares) or saline (circles) on the discriminative stimulus properties of morphine (top panel) and on the response rates (bottom panel) in rats that had been trained to discriminate between 3.0 mg/kg morphine and saline. Each point represents the mean percentage of morphine-appropriate responses or the response rate with S.E.M. for six animals. * $P < 0.05$ and *** $P < 0.001$ vs. saline control.

responses before the completion of ten responses on either lever, divided by the time (minutes) taken to complete the first ratio. The paired Student's t-test was used to compare the percentage of responses on the morphine-lever and the response rate during the test sessions to those following pretreatment with saline.

3. Results

The rats acquired the ability to discriminate between morphine and saline in an average of approximately 21 sessions. Once rats attained the criterion, drug-saline discrimination stabilized and was maintained with a high degree of accuracy. During the dose-response tests, morphine (0.3–3.0 mg/kg) produced a dose-related increase in morphine-appropriate responses, and a high dose of morphine (3.0 mg/kg) produced morphine-appropriate responses more than 80% of the time in all rats (data not shown).

The discriminative stimulus properties of morphine (1.0 and 3.0 mg/kg) were significantly reduced by pretreatment with either β-funaltrexamine or naloxonazine (Figs. 1 and 2). After pretreatment with 10 or 20 mg/kg β-funaltrexamine or 20 mg/kg naloxonazine, morphine (10 mg/kg) produced 43.3%, 23.3% or 21.7% morphine-appropriate responses, respectively. The response rates during the combination tests with β-funaltrexamine or naloxonazine did not differ from those with saline (Figs. 1 and 2).

4. Discussion

The present result that the irreversible μ-opioid receptor antagonist β-funaltrexamine significantly attenuated the discriminative stimulus properties of morphine supports previous reports that such properties are mediated by μ-opioid receptors (Picker et al., 1990; Walker et al., 1994). Furthermore, our result agrees with the previous finding of Locke and Holtzman (1986) that intracerebroventricular administration of β-funaltrexamine shifts the dose-response curve for morphine to the right as compared with pretreatment with saline. To the best of our knowledge, this is the first demonstration that the μ_1-opioid receptor antagonist naloxonazine completely attenuates the discriminative stimulus properties of morphine. This result strongly suggests that μ_1-opioid receptors play an important role in the discriminative stimulus properties of morphine.

The discriminative stimulus properties of drugs of abuse may be linked to their rewarding effects. Recently, Suzuki et al. (1993) demonstrated that naloxonazine does not modify morphine-induced place preference, and that morphine induces place preference in μ_1-opioid receptor-deficient CXBK mice, using the conditioned place preference paradigm. Since self-administered drugs are effective in producing place preference (Hoffman, 1989), and since place preference induced by drugs of abuse may be linked to their rewarding effects, morphine-induced rewards may be mediated by μ_2-opioid receptors. The present result may be inconsistent with previous results regarding morphine-induced place preference (Suzuki et al., 1993). Regarding this discrepancy, the dopaminergic (especially the mesolimbic) system is believed to play an important role in the rewarding effects of morphine (Funada et al., 1993), and the dopamine-releasing effect of morphine from dopaminergic nerve terminals is regulated by μ_2-opioid receptors (Pasternak and Wood, 1986). Neither the dopamine D_1 receptor antagonist SCH23390 (*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzozepine-OH) nor the dopamine D_2 receptor antagonist haloperidol produces reliable attenuation of the discriminative stimulus properties of morphine (Higgins et al., 1994; Suzuki et al., 1995), suggesting that the dopaminergic system plays a limited role in the discriminative stimulus properties of morphine. Therefore, the discriminative stimulus properties of morphine in rats may not necessarily reflect the rewarding effects of morphine.

In summary, the results of the present study demonstrated that the discriminative stimulus properties of morphine were significantly attenuated by β-funaltrexamine and naloxonazine. These results suggest that such properties may be mediated by μ-opioid (especially μ_1-opioid) receptors.

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