The Role of Mu- and Kappa-Opioid Receptors in Cocaine-Induced Conditioned Place Preference

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ABSTRACT — Effects of buprenorphine, U-50,488H, naltrexone and lithium chloride on cocaine conditioned place preference were examined. Buprenorphine, a mixed opioid agonist-antagonist, blocked the cocaine-induced place preference. Furthermore, the kappa-receptor agonist U-50,488H and the mu-receptor antagonist naltrexone both antagonized the cocaine preference. U-50,488H or naltrexone alone induced a place aversion in a dose-dependent manner. However, the cocaine-induced conditioned place preference was not blocked by lithium chloride, although the latter induced a conditioned place aversion, indicating that the antagonism of cocaine-induced place preference by U-50,488H or naltrexone does not result from a functional antagonism. These results suggest that mu- and kappa-opioid receptors may be involved in cocaine-induced conditioned place preference.

Cocaine abuse persists as a public health problem that causes significant problems related to addiction (1) and has also increased among heroin-dependent persons (2). “Speed balls” (heroin plus cocaine) is a popular form (3). At present, there is no uniformly effective pharmacotherapy for cocaine abuse (4, 5), and cocaine-heroin dual abuse is an even more difficult treatment challenge. Heroin abuse can be treated with the opioid agonist methadone (6) and the opioid antagonist naltrexone (7, 8), but these pharmacotherapies are not useful for combined cocaine and heroin abuse (9).

Recently, Mello et al. (10) reported that buprenorphine suppresses cocaine self-administration in rhesus monkeys. Based on this finding, it was suggested that buprenorphine might be useful for the pharmacotherapies of not only cocaine abuse but also cocaine-heroin combination abuse. Moreover, recent clinical reports have suggested some efficacy of buprenorphine for the treatment of cocaine abusers (11, 12). However, Brown et al. (13) suggested that buprenorphine might enhance cocaine rewarding properties using the conditioned place preference procedure.

In the present study, we examined using the conditioned place preference procedure whether buprenorphine enhances or attenuates the rewarding properties of cocaine. Moreover, since buprenorphine has mixed opioid agonist-antagonist properties, a kappa-opioid agonist, U-50,488H and a mu-opioid antagonist, naltrexone, were used to clarify the role of opioids in cocaine-induced place preference. In addition, we examined the effect of lithium chloride-induced conditioned place aversion on the cocaine-induced place preference.
MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats (Tokyo Experimental Animal, Tokyo), weighing 160–190 g, were housed in wire cages. They were maintained at 21°C on a 12-hr light/dark cycle with food and water available ad libitum.

Place conditioning
Conditioning was conducted using the un-biased procedure according to Suzuki et al. (14, 15). The apparatus was a shuttlebox (30 × 60 × 30 cm: w × l × h) made of acrylic resin board. The box was divided into two compartments of equal size by means of a sliding partition. One compartment was white with a textured floor; the other one was black, with a smooth floor. For conditioning, rats were immediately confined to one compartment after drug injections and to another compartment after saline injections. The treatment orders of injection (drug or saline) and of compartment (white or black) were counterbalanced across subjects. Conditioning sessions (three for the drug, three for the vehicle) were conducted once daily. In each session, the animals were treated for a 50-min period. On day 7, achievement of conditioning was tested as follows: The partition separating the two compartments was raised up to 12 cm above the floor, and a neutral platform consisting of a galvanized steel mesh (5 × 2 cm: w × l) was inserted along the seam separating the compartments. Preference of rats for a particular place was assessed by observation in the drug-free state after placing the animals on the neutral platform and allowing them free access to both compartments. The time spent in each compartment during a 15-min session was then measured. The position of the rat was defined by the position of its forelimbs and head. All sessions were conducted under conditions of dim illumination and white masking noise.

Saline control
The control rats were i.p.-injected with saline (1 ml/kg) instead of drugs in each of the conditioning sessions; the rats injected with saline were confined to one compartment, and on the next day, they were confined to another compartment after saline injection. This conditioning session was repeated 3 times. Either saline control (black- or white-floored compartment place) was regarded as a substitution for the drug-associated place at random, which was decided before the start of experiments.

Effects of cocaine, buprenorphine, U-50,488H, naltrexone, morphine and lithium chloride
Cocaine (0.5–4.0 mg/kg) and saline were injected i.p. on alternate days. The rats were immediately confined to each compartment after the injection. Buprenorphine (0.02–0.5 mg/kg) and saline were injected i.p. at 30 min before the training on alternate days. Naltrexone (3.0 mg/kg) and saline were injected i.p. or s.c. at 10 min before the training on alternate days. U-50,488H (1.0–10.0 mg/kg) and saline were injected i.p. on alternate days. Morphine (1.5 mg/kg) and saline were injected s.c. on alternate days. Lithium chloride (40.0 mg/kg) and saline were injected s.c. on alternate days.

Effects of cocaine combined with buprenorphine, U-50,488H, naltrexone and lithium chloride
Buprenorphine (0.02–0.5 mg/kg) was injected i.p. at 30 min before the cocaine (4.0 mg/kg) and saline treatments. Naltrexone (3.0 mg/kg) was injected i.p. or s.c. at 10 min before the cocaine (4.0 mg/kg) and saline treatments. U-50,488H (1.0–10.0 mg/kg) and cocaine (4.0 mg/kg) or saline were injected i.p. simultaneously. Lithium chloride (40.0 mg/kg, s.c.) and cocaine (4.0 mg/kg, i.p.) or saline (1 ml/kg) were injected simultaneously.

Effect of morphine combined with lithium chloride
Lithium chloride (40.0 mg/kg) and morphine (1.5 mg/kg) or saline were injected s.c. simultaneously.
Drugs

The drugs used were cocaine hydrochloride (Sankyo Co., Ltd., Tokyo), morphine hydrochloride (Sankyo Co., Ltd., Tokyo), buprenorphine (Lepletan®; Otsuka Pharmaceutical Co., Ltd., Tokyo), U-50,488H (trans-3,4-dichloro-N-methyl-N-(2-1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide) synthesized by Dr. Nagase, naltrexone hydrochloride (RBI Co., Ltd., Garden City) and lithium chloride anhydrous (Wako Pure Chemical Industries, Ltd., Osaka). These drugs were dissolved in saline.

Data analysis

Conditioning scores represent the time spent in the drug-paired place minus the time spent in the vehicle-paired place and are expressed as means ± S.E.M. The Wilcoxon test was used to determine whether individual doses produced significant conditioning.

RESULTS

Saline control

Preference for the injection-associated place, which was regarded as a substitution for the drug, was calculated. As shown in Fig. 1, the saline-control rats exhibited no preference. The mean conditioning score was +6.8 ± 99.7 sec (n = 8).

Effects of cocaine, buprenorphine, naltrexone, U-50,488H, morphine and lithium chloride

As shown in Fig. 1, cocaine induced a place preference in a dose-dependent manner. Significant conditionings were observed at doses of 2.0 and 4.0 mg/kg, at which all animals had positive conditioning scores (P < 0.05). Morphine (1.5 mg/kg, s.c.) induced a significant place preference (265.0 ± 64.3 sec). Buprenorphine (0.5 mg/kg, i.p.) induced a slight place preference (Fig. 2). On the other hand, U-50,488H (10.0 mg/kg, i.p.) induced a strong place aversion (P < 0.05, Fig. 2). Naltrexone (3.0 mg/kg, s.c.) also induced a place aversion (P < 0.05), but naltrexone (3.0 mg/kg, i.p.) did not induce a place aversion (Fig. 2). Lithium chloride (40.0 mg/kg, s.c.) induced a strong place aversion (P < 0.05, Fig. 4). Significance was shown by the ANOVA using 15 groups from Figs. 1, 2 and 4 (F14,103 = 3.262, P < 0.01).

Fig. 1. Dose-response curves of the cocaine-induced place preference. The ordinate represents the preference for the drug-paired place. Each point represents the mean with S.E.M. of 8 animals. The star denotes significant preference conditioning (Wilcoxon test, *P < 0.05).
Effects of cocaine combined with buprenorphine, U-50,488H, naltrexone and lithium chloride

The cocaine (4.0 mg/kg)-induced conditioned place preference was antagonized by pretreatment with buprenorphine in a dose-dependent manner. Dosing of buprenorphine (0.5 mg/kg) significantly (P < 0.05) antagonized the cocaine-induced place preference (Fig. 3). Dosing of U-50,488H (10.0 mg/kg) signifi-

Fig. 2. Place conditionings produced by buprenorphine, U-50,488H and naltrexone. The ordinate represents the preference for the drug-paired place. Each column represents the mean with S.E.M. of 8 animals. The star denotes significant aversion conditioning (Wilcoxon test, *P < 0.05).

Fig. 3. Effects of cocaine combined with buprenorphine, U-50,488H or naltrexone. The ordinate represents the preference for the drug-paired place. Each column represents the mean with S.E.M. of 8–12 animals. The stars denote significant difference from cocaine alone (Wilcoxon test, *P < 0.05, **P < 0.01).
cantly antagonized the cocaine-induced place preference (P < 0.05, Fig. 3). Cocaine (4.0 mg/kg)-induced place preference was significantly antagonized by s.c. naltrexone pretreatment (P < 0.05) and showed a tendency to be inhibited by i.p. pretreatment (Fig. 3). In the case of combination with s.c.-naltrexone, the rats showed a slight place aversion. As shown in Fig. 4, lithium chloride (40.0 mg/kg, s.c.) produced a place aversion (P < 0.05). However, the cocaine-induced place preference was not antagonized by the combination with lithium chloride. The intensity of the place aversion by lithium chloride (40.0 mg/kg, s.c.) was similar to that by U-50,488H (10.0 mg/kg). Significance was shown by the ANOVA using 19 groups from Figs. 2, 3 and 4 (F18,153 = 2.464, P < 0.01).

**Effects of morphine combined with lithium chloride**

Morphine (1.5 mg/kg, s.c.)-induced place preference was not antagonized by the combination with 40 mg/kg of lithium chloride (192.0 ± 128.2 sec).

**DISCUSSION**

It has been reported that cocaine can produce a conditioned place preference (16–18). In the present study, 2.0 and 4.0 mg/kg of cocaine significantly produced a conditioned place preference. A high dose (0.5 mg/kg) of buprenorphine also exhibited a tendency to produce a conditioned place preference. By buprenorphine treatment 30 min before cocaine injection, the cocaine-induced conditioned place preference was blocked dose-dependently. Our results support the findings in the self-administration study done by Mello et al. (10). Furthermore, Kosten et al. (11, 12) demonstrated that buprenorphine is useful for the treatment of cocaine abusers. In another paradigm, pretreatment (30 min) with greater than 0.3 mg/kg of buprenorphine provided a significant protection against the lethal effects of cocaine (19, 20). Witkin et al. (20) also reported that pretreatment (30 min) with buprenorphine protected against convulsions and lethality induced by cocaine. In addition, pretreatment (30 min) with buprenorphine produced dose-dependent protection against the lethal effects of cocaine at 60 min and 24 hr after the injection. Recently, Brown et al. (13) reported that the appetitive effect of cocaine was potentiated by coadministering buprenorphine. However, since buprenorphine shows relatively slow dissociation from opioid receptors (20, 21), opioid receptors might be effectively occupied by buprenorphine when cocaine was injected later. Therefore, pretreatment with buprenorphine may be important in blocking the cocaine-induced place preference.

On the other hand, studies of receptor binding have demonstrated that buprenorphine has affinity for both mu- and kappa-receptors (22). In addition, it has been reported that buprenorphine acts as a mu-receptor antagonist and kappa-receptor agonist (23, 24). Therefore, we used naltrexone, a mu-antagonist, and U-50,488H, a selective kappa-agonist, for comparison with the effect of buprenorphine in combination with cocaine. It has been re-
ported that both naltrexone (25) and U-50,488H (26) produced conditioned place aversion. In the present studies, U-50,488H induced a strong conditioned place aversion dose-dependently, and naltrexone (3.0 mg/kg, s.c.) also induced a conditioned place aversion with the same intensity as U-50,488H (3.0 mg/kg, i.p.). In the combination studies, the cocaine-induced conditioned place preference was blocked by both naltrexone and U-50,488H. However, it is possible that the drugs which induced a conditioned place aversion may attenuate drugs-induced place preference, i.e., functional antagonism. Although lithium chloride alone induced a conditioned place aversion (27), the cocaine-induced place preference was not attenuated by the combination with lithium chloride in the present study. Moreover, morphine (1.5 mg/kg)-induced place preference was not affected by lithium chloride. Inversely, lithium chloride-induced place aversion also was not affected by cocaine in the same procedure (data not shown). From these results, it is suggested that the antagonism of cocaine-induced place preference by both U-50,488H and naltrexone does not result from a functional antagonism.

Opioid antagonists, such as naloxone and naltrexone, do not suppress cocaine self-administration in primates (27, 28) or rodents (29). However, other investigators reported that naltrexone partially suppressed the cocaine self-administration in primates (30). Additionally, naloxone attenuates the effect of cocaine on rewarding brain stimulation (31). Moreover, Hammer et al. (32) suggested that mu-opioid receptors may be involved in the reinforcing effect of cocaine. Accordingly, it is considered that the cocaine-induced place preference was inhibited by mu-opioid receptor blockade in our study.

The role of kappa-opioid receptor in cocaine-induced place preference has not been documented. It is not clear whether the inhibitory effect of buprenorphine on the cocaine-induced place preference results from the kappa-antagonistic action of buprenorphine. However, we found that cocaine-induced place preference was blocked by the selective kappa-opioid receptor agonist U-50,488H. It is well-known that cocaine-induced place preference is mediated by the stimulation of mesolimbic dopamine neurons (33, 34). U-50,488H decreases dopamine levels in the nucleus accumbens (35), and it inhibits the stimulation of dopamine neurons including the mesolimbic neurons (36). In the present study, the blockade of U-50,488H on cocaine-induced place preference may result from decreasing dopamine levels or inhibiting the stimulation of dopamine neurons.

In conclusion, our findings suggest that cocaine-induced place preference may involve both mu- and kappa-opioid receptors. Moreover, the buprenorphine blockade of cocaine-induced place preference may result from mu-antagonistic and/or kappa-agonistic properties of buprenorphine.

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