Interaction between Discriminative Stimulus Effects of Cocaine and Morphine

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ABSTRACT—We recently demonstrated that combining cocaine and morphine could enhance their reinforcing effects which may be mediated by the dopaminergic system. In the present study, the effects of cocaine and morphine on the discriminative stimulus effects of morphine and cocaine, respectively, were examined. Furthermore, dopaminergic mediation in the discriminative stimulus effects of morphine was also examined. Pretreatment with 1.0 or 3.0 mg/kg morphine shifted the dose-response curve for cocaine to the left, and 3.0 mg/kg morphine significantly potentiated the discriminative stimulus effects of cocaine. On the other hand, neither 1.25 nor 2.5 mg/kg cocaine affected the discriminative stimulus effects of morphine. These results suggest that potentiation of the discriminative stimulus effects of cocaine by morphine may reflect the enhancement of reinforcing effects in the combination of cocaine and morphine. Furthermore, neither SCH23390 (D1-receptor antagonist) nor haloperidol (D2-receptor antagonist) affects the discriminative stimulus effects of morphine, while combining these drugs slightly attenuated the effects of morphine. Thus, another neurotransmitter rather than dopamine may play an important role in the discriminative stimulus effects of morphine. Therefore, the discriminative stimulus effects of morphine are apparently not potentiated by cocaine, unlike those of reinforcing effects.

Keywords: Cocaine, Morphine, Drug discrimination, Drug interaction, Dopamine

It is well known that cocaine as well as μ-opioid receptor agonists themselves induce reinforcing effects and subjective effects. Furthermore, the simultaneous intake of several drugs by drug abusers has been reported in recent years. One common combination is that of cocaine and heroin (μ-opioid receptor agonist), which is referred to by street abusers as “speed ball”. Therefore, some investigators have sought to characterize the interactions of these drugs (1–3). We recently demonstrated that combining cocaine and morphine (μ-opioid receptor agonist) could enhance their reinforcing effects in rats (2). However, the pharmacological basis of the interaction of these drugs is still unknown.

Drug discrimination procedures have provided important information about neuropharmacological mechanisms underlying subjective effects and are potentially valuable for characterizing interactions between cocaine and other psychoactive drugs (1). Furthermore, it is believed that discriminative stimulus effects of abused drugs may be linked to their reinforcing effects. Spealman and Bergman (1, 3) demonstrated that morphine can potentiate the discriminative stimulus effects of cocaine in the monkey. Nevertheless, effects of cocaine on the discriminative stimulus effects of morphine are yet unclear. In the present study, to examine more fully the enhancement of the reinforcing effects by the combination of cocaine and morphine in rats, the effects of cocaine and morphine on the discriminative stimulus effects of morphine and cocaine, respectively, were examined in rats that had been trained to discriminate between either 3.0 mg/kg morphine or 10 mg/kg cocaine and saline.

Many studies have shown that the dopaminergic system plays a primary role in the discriminative stimulus effects of cocaine (4–7), while reinforcing effects of cocaine and morphine are also mediated by the dopaminergic system (8–12). Nevertheless, involvement of dopaminergic mediation in the discriminative stimulus effects of morphine is not sufficiently clear. Therefore, the effects of dopamine antagonists such as SCH23390 (D1-receptor antagonist) and haloperidol (D2-receptor antagonist) on the discriminative stimulus effects of morphine were also examined.
MATERIALS AND METHODS

Animals
Twenty male Fischer 344 rats (Charles River Japan, Inc., Atsugi) were maintained at 200–230 g (80% free-feeding weight). Water was available ad libitum for all of the rats in their home cages. The rats were housed in individual cages at a room temperature of 22±1°C with a 12-hr light-dark cycle (light on 8:00 a.m. to 8:00 p.m.).

Apparatus
Experiments were conducted in operant chambers (Model GT 8810; O’Hara & Co., Ltd., Tokyo) equipped with 2 levers, with a foodcup mounted midway 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. A 20-mg food pellet (O’Hara & Co., Ltd.) was delivered by lever-pressing behavior.

Discrimination training
Discrimination training was performed according to the method of Suzuki et al. (13). Briefly, after the response rates had stabilized under a fixed ratio (FR) 10 and the rat received 40 reinforcements during 4 consecutive sessions, the rats were divided into two experimental groups. One group of rats (n=12) was trained to discriminate between morphine (3.0 mg/kg) and saline. Morphine or saline was administered s.c. 30 min before each session in a daily sequence of SDDSSDDSSD (D=drug, S=saline). On the other hand, another group of rats (n=8) was trained to discriminate between cocaine (10 mg/kg) and saline. Cocaine or saline was administered i.p. 15 min before each session. Training sessions were 15 min in duration, and this phase of training was continued until all of the rats performed up to the required criterion [accuracies of at least 83% (First Food Pellet ≥12 responses) for 5 consecutive sessions]. Moreover, discrimination training was continued even after the criterion was attained.

Testing procedure
After the animals attained the criterion, dose-response, generalization, combination and antagonism tests were initiated. Test sessions were performed after the discrimination criterion described above had been satisfied for at least 3 consecutive sessions. In the dose-response test or generalization to the discriminative stimulus effects of cocaine and in the combination test with morphine on the discriminative stimulus effects of cocaine, test sessions consisted of four FR components to determine a four-point cumulative dose-response curve. A cumulative dosing procedure was performed according to the method of Suzuki et al. (13). Briefly, during each component, rats were placed in the operant chamber until either they made 10 responses on either lever or 5 min (component time) had elapsed without reinforcer. After the first component was finished (5 min elapsed), the drugs were administered again. This procedure was repeated three times.

The generalization of morphine to the discriminative stimulus effects of cocaine and the combination with cocaine on the discriminative stimulus effects of morphine were performed according to the method of Cunningham and Callahan (4). In the combination test, doses of drugs that produced less than 20% drug-lever responses in the generalization tests were used.

In the antagonism tests, SCH23390, haloperidol or saline was administered i.p. before morphine (3.0 mg/kg) was administered. The pretreatment times and doses of drugs used were 15 min for 1.25–10 mg/kg cocaine, 30 min for 0.3–10 mg/kg morphine and 1 hr for 0.1 mg/kg naloxone, 0.003–0.03 mg/kg SCH23390 and 0.03–0.3 mg/kg haloperidol. If the rats did not make 10 responses during each component, the response was judged to have been disrupted.

Drugs
The drugs used in the present study were cocaine hydrochloride (Takeda Pharmaceutical Industries, Inc., Osaka), morphine hydrochloride (Sankyo Co., Tokyo), naloxone hydrochloride and SCH23390 hydrochloride (Research Biochemicals, Inc., Wayland, MA, USA) and haloperidol (Serena injection®, Dainippon Pharmaceutics, Osaka). All drugs were dissolved in saline and injected in a volume of 1.0 ml/kg.

Data analyses
During the training sessions, accuracy was defined in terms of the number of correct responses as a percentage of the total responses before the first food pellet. During the test sessions, performance was expressed in terms of the number of drug-lever responses as a percentage of the total number of responses on completion of FR 10. The drug was considered to have generalized to the discriminative stimulus properties of cocaine if more than 80% of the responses were on the drug lever. The response rate was calculated as the total number of responses before the completion of 10 responses on either of the levers, divided by the time (minutes) taken to complete the first ratio. Statistical analyses for the comparison of pretreatment with saline and drugs was performed by a two-factor (groups × cumulative dose) repeated measures analysis of variance (ANOVA) or the paired Student’s t-test.
RESULTS

During the dose-response tests, both cocaine (1.25–10 mg/kg) and morphine (0.3–3.0 mg/kg) produced an increase in drug-appropriate responses in all of the rats (Figs. 1 and 2).

In the generalization test, 0.3–10 mg/kg morphine did not engender cocaine-appropriate responses and decreased response rates as the dose of morphine increased (Fig. 1). On the other hand, cocaine (1.25–10 mg/kg) did not generalize to the discriminative stimulus effects of morphine and decreased response rates in a dose-dependent manner (Fig. 2). The responses of 3 of the 8 rats were disrupted at doses of 5.0 and 10 mg/kg cocaine.

In the combination tests, pretreatment with either 1.0 or 3.0 mg/kg morphine dose-dependently shifted the dose-response curve for cocaine to the left; the effect of 3.0 mg/kg morphine was significant compared to pretreatment with saline ($F_{[1,40]}=10.52, P<0.01$) (Fig. 3A). Morphine (1.0 and 3.0 mg/kg) with saline did not engender cocaine-appropriate responses (<20%). Furthermore, the potentiating effect of 3.0 mg/kg morphine on the discriminative stimulus effects of cocaine ($F_{[1,40]}=7.31, P<0.01$) was significantly blocked by 0.1 mg/kg naloxone ($F_{[1,40]}=7.31, P<0.01$) (Fig. 3B).

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**Fig. 1.** Dose-response and generalization of cocaine (circles) and morphine (squares) on the discriminative stimulus effects of cocaine (top panel) and on the response rates (bottom panel) in rats trained to discriminate between 10 mg/kg cocaine and saline. Each point is the mean percentage of cocaine-appropriate responses and response rates with S.E.M. of 4–8 animals. The percentage of cocaine-appropriate responding and the response rates were not calculated in cases where a rat made fewer than 10 responses.

**Fig. 2.** Dose-response and generalization of morphine (squares) and cocaine (circles) on the discriminative stimulus effects of morphine (top panel) and on the response rates (bottom panel) in rats trained to discriminate between 3.0 mg/kg morphine and saline. Each point is the mean percentage of morphine-appropriate responses and response rates with S.E.M. of 5–8 animals. The percentage of morphine-appropriate responding and the response rates were not calculated in cases where a rat made fewer than 10 responses.
Fig. 3. Effects of morphine or morphine plus naloxone on the discriminative stimulus effects of cocaine. A: Influences of 1.0 and 3.0 mg/kg of morphine or saline on the discriminative stimulus effects of cocaine (top panel) and on response rates (bottom panel) in rats trained to discriminate between 10 mg/kg cocaine and saline. Rats were injected with saline (circles), or 1.0 (squares) or 3.0 (triangles) mg/kg morphine before treatment with cocaine. B: Influence of 0.1 mg/kg naloxone or saline on the potentiating effects of 3.0 mg/kg morphine on the discriminative stimulus effects of cocaine (top panel) and on response rates (bottom panel) in rats trained to discriminate between 10 mg/kg cocaine and saline. Rats were injected with saline plus saline (circles), saline plus morphine (squares) or naloxone plus morphine (triangles) before treatment with cocaine. Each point represents the mean percentage of drug-appropriate responses and response rates with S.E.M. of 6 animals.

Table 1. Influences of 1.25 and 2.5 mg/kg cocaine on the discriminative stimulus effects of morphine in rats trained to discriminate between 3.0 mg/kg morphine and saline

| Drug               | Cocaine (mg/kg, i.p.) | % Morphine-lever responding (S.E.M.) | Responses/min (S.E.M.) | n/N  |
|--------------------|-----------------------|-------------------------------------|------------------------|------|
| Morphine 0.3 mg/kg, s.c. | 0                    | 16.7 (11.2)                         | 45.2 (7.3)             | 6/6  |
|                    | 1.25                  | 28.0 (19.6)                         | 36.3 (10.0)            | 5/6  |
|                    | 2.5                   | 28.3 (15.2)                         | 26.7 (4.5)             | 6/6  |
| Morphine 1.0 mg/kg, s.c. | 0                    | 65.0 (14.8)                         | 41.5 (6.9)             | 6/6  |
|                    | 1.25                  | 64.0 (17.5)                         | 48.1 (5.8)             | 5/6  |
|                    | 2.5                   | 56.0 (18.6)                         | 25.1 (10.5)            | 5/6  |
| Morphine 3.0 mg/kg, s.c. | 0                    | 100.0 (0.0)                         | 53.7 (7.4)             | 6/6  |
|                    | 1.25                  | 90.0 (10.0)                         | 70.6 (12.3)            | 5/6  |
|                    | 2.5                   | 90.0 (10.0)                         | 52.9 (20.7)            | 5/6  |

n/N, number of subjects that completed the FR10 (n) out of the total number of subjects tested (N). The percentage of morphine-appropriate responses and the response rates were not calculated in cases where a rat made fewer than 10 responses.
Fig. 4. Effects of several doses of SCH23390 (A), haloperidol (B) or saline on the discriminative stimulus effects (top panel) and response rates (bottom panel) of morphine in rats trained to discriminate between 3.0 mg/kg morphine and saline. Each point represents the mean percentage of morphine-appropriate responses and response rates with S.E.M. of 5 animals.

However, 0.1 mg/kg naloxone did not affect the dose-response curve for cocaine or the response rates (data not shown). On the other hand, 1.25 and 2.5 mg/kg cocaine did not affect the dose-response curve for morphine (Table 1), and some rats showed a disrupted response. Cocaine (1.25 and 2.5 mg/kg) with saline did not engender morphine-appropriate responses (<20%).

In the antagonism tests, both SCH23390 and haloperidol scarcely attenuated the discriminative stimulus effects of morphine (Fig. 4). At their highest doses, SCH23390 (0.03 mg/kg) and haloperidol (0.3 mg/kg) significantly reduced the response rates as compared with saline pretreatment (P < 0.05). In addition, neither SCH23390 (0.03 mg/kg) nor haloperidol (0.3 mg/kg) engenders morphine-appropriate responding (<10%) and decreases the response rate about 60 and 45%, respectively, as compared with saline. The responses in 1 out of 6 rats were disrupted at a dose of 0.03 mg/kg SCH23390. Pretreatment with the combination of haloperidol (0.1 mg/kg) and SCH23390 (0.003–0.03 mg/kg) slightly attenuated the discriminative stimulus effects of morphine, but these changes were not significant, as compared with saline pretreatment, and combining haloperidol and 0.03 mg/kg SCH23390 significantly reduced the response rates as compared with saline plus saline pretreatment (P < 0.001) (Fig. 5).
DISCUSSION

The simultaneous intake of cocaine and the µ-opioid receptor agonist heroin ("speed ball") by drug abusers has been reported in recent years. The combination of these drugs reportedly results in a greater "rush" than that experienced with heroin alone (14, 15). Furthermore, place-preference is reportedly enhanced by the combination of cocaine and the µ-opioid receptor agonist morphine in rats (2). In the present study, discriminative stimulus effects of morphine were not potentiated by cocaine in rats. This result is somewhat surprising, since discriminative stimulus effects of abused drugs may be linked to their reinforcing effects. On the other hand, morphine potentiated the discriminative stimulus effects of cocaine in rats, which is consistent with the previous results in monkeys (1, 3). These results suggest that the effect of morphine on the discriminative stimulus effects of cocaine, rather than the effect of cocaine on the discriminative stimulus effects of morphine, may reflect the enhancement of the reinforcing effects in the combination of cocaine and morphine in rats.

Various lines of evidence suggest that the discriminative stimulus effects of cocaine are mediated by the dopaminergic system. Cocaine and morphine increase synaptic dopamine concentration by inhibiting dopamine uptake and increasing dopamine release from the terminal, respectively (16). In addition, the dopamine-releasing effects of µ-agonists might be expected to augment the dopamine uptake-inhibiting effects of cocaine (17, 18). Masukawa et al. (2) suggested that combining a dopamine releaser (opioids) with a dopamine-uptake inhibitor (cocaine) could potentiate their reinforcing effects. Thus, morphine might potentiate the discriminative stimulus effects of cocaine.

The dopaminergic mediation of the discriminative stimulus effects of morphine is not sufficiently clear. It is well known that the dopaminergic system may play an important role in the reinforcing effects of morphine. Nevertheless, neither the D₁-receptor antagonist SCH23390 nor the D₂-receptor antagonist haloperidol attenuated the discriminative stimulus effects of morphine. The latter results are consistent with the report that haloperidol does not produce reliable attenuation of the discriminative stimulus effects of morphine (19) in rats. Furthermore, combining SCH23390 and haloperidol only partially attenuated the discriminative stimulus effects of morphine, suggesting that the dopaminergic system may play a limited role in the discriminative stimulus effects of morphine. On the other hand, Ukai et al. (7) demonstrated that combining SCH23390 and haloperidol completely antagonize the discriminative stimulus effects of cocaine. Thus, another neurotransmitter rather than dopamine may play an important role in the discriminative stimulus effects of morphine, unlike those of cocaine. Furthermore, activation of the mesolimbic dopaminergic system may be critically linked to the expression of morphine's reinforcing effects. Injection of morphine into the ventral tegmental area, which consists of cell bodies of the mesolimbic dopaminergic system, produces partial generalization to the discriminative stimulus effects of morphine (20), while previous studies have demonstrated that microinjection of morphine into the periaqueductal grey (21) or parabrachial nucleus (22) partially or completely generalized to the discriminative stimulus effects of morphine. In addition, we already demonstrated that the reinforcing effects of morphine are mediated through µ-opioid receptors (12), while the discriminative stimulus effects of morphine are mediated through µ₁-opioid receptors (Suzuki et al., unpublished data). These results suggest that the mechanisms of discriminative stimulus effects of morphine may differ from those of reinforcing effects. Therefore, the discriminative stimulus effects of morphine are apparently not potentiated by cocaine, unlike the reinforcing effects of morphine.

In the present study, 1.25 and 2.5 mg/kg cocaine slightly increases the morphine-appropriate responding at a dose of 3.0 mg/kg morphine. With regard to these results, since the discriminative stimulus effects of morphine may be, at least partially, mediated by the dopaminergic system (see above), discriminative stimulus effects of morphine would be slightly increased by dopamine uptake inhibiting effects of cocaine. On the other hand, cocaine slightly increased or decreased the response rate of morphine, while morphine slightly decreased the response rate of cocaine. Several reports demonstrated that the dopaminergic system might be involved in the rate-altering effects of psychostimulants and opioids. For example, dopamine receptor antagonists increase or decrease the response rates of cocaine and opioid (5, 6, 23, present results). Thus, involvement of the dopaminergic system in the rate-altering effects of cocaine and morphine is yet not fully clarified. Further investigations are needed to verify the involvement of the dopaminergic system in the response rates of cocaine and morphine.

In conclusion, we suggest that potentiation of the discriminative stimulus effects of cocaine by morphine may reflect the enhanced reinforcing effects of the combination of cocaine and morphine. Furthermore, another mechanism rather than involvement of the dopaminergic system may play an important role in the discriminative stimulus effects of morphine, unlike those of cocaine. Therefore, the discriminative stimulus effects of morphine are apparently not potentiated by cocaine, unlike the reinforcing effects of morphine.
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