Mode of Potentiating Action of Cocaine in Morphine Analgesia

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Abstract—The mechanism of antinociceptive interactions among morphine, cocaine and alcohol was studied in mice, guinea pigs and rabbits. In the tail-pressure test in mice, cocaine and alcohol alone showed almost no antinociceptive effects at doses up to 8 mg/kg, s.c., and 4 g/kg, i.g., respectively. Alcohol at 2 g/kg, i.g., also did not influence the effect of morphine, while cocaine at 4 mg/kg, s.c., significantly potentiated the antinociceptive effects of not only morphine but also pentazocine. In an analysis of serum and brain concentration levels of morphine in mice, when morphine and cocaine were simultaneously administered at 2 mg/kg, s.c., and 4 mg/kg, s.c., respectively, both serum and brain levels of morphine showed neither increase nor decrease in comparison with the levels in mice administered morphine alone. In myenteric plexus-longitudinal muscle preparations of isolated guinea pig ileum, 1 μM cocaine enhanced the agonistic effects of both pentazocine and ethylketocyclazocine. Furthermore, cocaine as well as ethylketocyclazocine showed naloxone-reversible agonistic effects in isolated rabbit vas deferens. These results indicate that cocaine may potentiate the antinociceptive effects of morphine and pentazocine by acting on the κ-opioid receptors as an agonist.

It is well-known that although opioid analgesics have been used as the most effective drugs for treating severe pain, this series of drugs commonly produces an unwanted side-effect, namely physical dependence (1). Consequently, studies for the development of potent analgesics with low or no physical dependence-producing potential have been intensively pursued in animals (2–4) and man (5, 6). Furthermore, methods to produce potent analgesic effects with low doses of opioid analgesics in combination with other drugs have also been investigated. Indeed, oral administration of morphine in combination with cocaine and alcohol, the so-called “Brompton mixture”, has been clinically examined in patients suffering from pain, and it has been reported that the mixture was highly effective in controlling the chronic pain of malignant disease as well as cancer pain without opioid withdrawal signs and symptoms (7, 8). Nevertheless, experimental studies on the interaction, especially antinociceptive interaction, between opioid analgesics and cocaine or alcohol has not been extensively performed. Kakunaga et al. (9) reported no antinociceptive interaction between morphine and cocaine. Nott (10) observed markedly potentiated morphine analgesia by cocaine in mice and suggested that the potentiation was a result of the ability of cocaine to potentiate endogenously released noradrenaline in the central nervous system. It has been also observed that, similar to cocaine, other central nervous system stimulants such as amphetamines also enhance morphine analgesia (10–13) and that the enhancement of morphine analgesia by amphetamines was due to increased whole brain levels of morphine that were modified by changes of the activity of the sympathetic nervous system (13). However, whether the potentiation by cocaine is due to the same mechanism is unclear. On the other hand, various results have been obtained regarding the antinociceptive effects of alcohol that were either mediated (14, 15) or not mediated (16, 17)
by opioid receptors in common with morphine, and antinociceptive interactions between opioid analgesics and alcohol are as of yet unclear.

Therefore, the tail-pressure test, analysis of serum and brain concentration levels of morphine, and experiments on myenteric plexus-longitudinal muscle preparations of the guinea pig ileum and on the rabbit vas deferens preparations were conducted to examine the mechanism of antinociceptive interaction among opioid analgesics, cocaine and alcohol.

Materials and Methods

Drug-naive male Jcl-ICR mice (Clea Japan Inc., Tokyo), male Hartley guinea pigs (Funabashi Farm, Chiba), and male KBL-JW rabbits (Kitayama Labs., Nagano) were used in the various experiments. The mice and guinea pigs were housed for more than a week before the start of the experiment in group cages in quarters regulated for temperature, humidity and light cycle. The rabbits were housed in individual cages under the same conditions as the other animals. The animals were fed on a solid diet (CE-2 for mice, CG-3 for guinea pigs and CR-1 for rabbits; Clea Japan Inc., Tokyo) with access to tap water ad lib. In the case of the in vivo tests, the drug solutions respectively consisted of morphine hydrochloride (Takeda Chemical Ind. Co., Ltd., Osaka), cocaine hydrochloride (Takeda Chemical Ind. Co., Ltd., Osaka) and pentazocine hydrochloride (Winthrop Laboratories, Tokyo) dissolved in 0.9% w/v saline, and ethyl alcohol (Wako Pure Chemical Industries, Ltd., Osaka) and pentazocine hydrochloride (Winthrop Laboratories, Tokyo) dissolved in 0.9% w/v saline, and ethyl alcohol (Wako Pure Chemical Industries, Ltd., Osaka) diluted with distilled water. In the case of the in vitro tests, morphine, pentazocine, cocaine, alcohol, ethylketocyclazocine methanesulfonate (EKC, Winthrop Laboratories, Tokyo), D-Ala²-D-Leu⁵-enkephalin (DADLE, Peptide Institute, Inc., Osaka) and naloxone hydrochloride (Sankyo Co., Ltd., Tokyo) were dissolved and/or diluted with distilled water.

Statistical analysis was performed using a two-tailed Student’s t-test. When the value of P was less than 0.05, the difference was considered significant.

Experiment 1. Observation of antinociceptive effects determined by the tail-pressure test in mice

For observation of the nociceptive response, a sharp-edged plastic plate was placed on the base of the mouse’s tail, and pressure was gradually applied at a constant rate of increase by a motor-driven air-compressor. After the start of the stimulation, the mouse’s responses of squeaking, turning the head or biting the plastic plate were regarded as nociceptive responses. When the mouse responded to the stimulation, the pressure value was recorded on a polygraph recorder. When either the mouse showed a response or the pressure reached 700 mmHg, the stimulation was completely removed. Immediately after this, pressure was again applied to the tail through the plate. This procedure was repeated three times in succession at each test, with the mean value being regarded as the threshold value. Mice were used in the experiment only when they had shown a reflex response to the nociceptive stimulation at pressures below 70 mmHg in the preliminary test conducted 4 hr prior to the experiment. Morphine, cocaine and pentazocine were separately administered subcutaneously to the nape of the neck and the skin of the back, and alcohol was intragastrically administered by gavage at a fixed injection volume of 10 ml/kg. When only one drug was used, saline was also administered to a different site from that of the drug. The test was conducted 30 min after the drug administration.

a) Dose-response relationships in the antinociceptive effects of morphine, pentazocine, cocaine and alcohol: Fifteen groups of 6 to 8 mice each weighing between 16.5 and 26.4 g were used in this experiment. The dose ranges that were administered were: morphine, 0.5 to 4 mg/kg; pentazocine, 2 to 16 mg/kg; cocaine, 2 to 8 mg/kg; and alcohol, 1 to 4 g/kg. Saline, 10 ml/kg, was also administered to 26 mice as the control, and any antinociceptive effects were observed.

b) Antinociceptive interaction between morphine and alcohol: Three groups of 6 mice each weighing between 19.7 and 23.4 g were used in this experiment. Simultaneous administration of morphine at 1 mg/kg, s.c., and alcohol at 2 g/kg, i.g., was conducted. The effects of morphine and alcohol alone at
these doses were also observed for comparison.

c) Antinociceptive interaction between morphine and cocaine: Fourteen groups of 6 or 8 mice, each weighing between 18.6 and 25.9 g, were used in this experiment. Morphine was administered at doses of 0.5, 1 and 2 mg/kg, s.c., simultaneously with cocaine at 2 and 4 mg/kg, s.c., and antinociceptive effects were observed. The effects of morphine and cocaine alone at these doses were also determined for comparison. Then the potency ratio and the confidence limit at 95% were calculated from the linear regression expression obtained by using the least squares method.

d) Antinociceptive interaction between pentazocine and cocaine: Seven groups of 8 mice, each weighing between 20.5 and 23.9 g, were used in this experiment. Pentazocine was administered at doses of 2, 4 and 8 mg/kg, s.c., simultaneously with cocaine at 4 mg/kg, s.c., and antinociceptive effects were observed. The effects of pentazocine and cocaine alone at these doses were also determined for comparison. Then the potency ratio and the confidence limit at 95% were calculated from the linear regression expression obtained by using the least squares method.

Experiment 2. Analysis of serum and brain concentration levels of morphine in mice

The same two groups of 6 mice each that in Experiment 1 were administered either with the combination of 10 ml/kg saline, s.c., and 2 mg/kg morphine, s.c., or the combination of 4 mg/kg cocaine, s.c., and 2 mg/kg morphine, s.c., were also used in this experiment.

Blood samples of 1 ml each were collected from the vena cava inferior, and whole brains were isolated under the condition of ether anesthesia at 30 min after administration of the drugs; i.e., immediately after the tail-pressure test. Blood samples were centrifuged for 20 min at 1500×g, and then the serum samples were collected. Each brain was homogenized in distilled water. Morphine was extracted from the serum and from 20% w/v brain homogenate as follows: A mixture of 1 ml of 0.1 M borate buffer (pH 9.0), 99.0 ng naloxone and 4 ml of chloroform-n-butanol (9:1) were added to each 100 μl serum sample or 250 μl brain sample and the mixture was shaken and then centrifuged. A 4 ml aliquot of the chloroform-n-butanol layer was removed and evaporated to dryness with nitrogen gas at 40°C. The residue was dissolved in 1 ml of 0.1 N HCl and washed with the organic solvent. To the aqueous layer, 0.1 ml of 1.0 N NaOH, 1 ml of ammonium chloride buffer (pH 9.0) and 4 ml of chloroform-n-butanol (9:1) were added, and the mixture was shaken and centrifuged. The organic layer was then removed and evaporated as described above.

Morphine concentrations in the serum and the brain were determined by using a high-performance liquid chromatograph (Shimadzu Seisakusho, Ltd., Kawasaki) with an electronchemical detector. A stainless steel column, 3.9 mm in internal diameter and 30 cm in length and packed with Nucleosil 10C18, was used for the chromatographic separation of morphine at 35°C. The working electrode was maintained at an applied potential of +0.79 V against an Ag/AgCl reference electrode. The chromatography was performed at ambient temperature using an isocratic mobile phase composed of 0.07 M KH₂PO₄ containing 0.5 mM EDTA-2Na, 8% acetonitrile and 5% methanol. The flow rate was 1.0 ml/min. Serum and brain morphine levels were determined by calculating the peak height ratio of morphine to the internal standard, naloxone.

Experiment 3. Experiments on isolated organ preparations of guinea pigs and rabbits

Four groups of 4 guinea pigs, each weighing between 620 and 960 g, and 4 groups of 4 rabbits, each weighing between 4.0 and 4.9 kg, were used in the experiments. The preparations of myenteric plexus-longitudinal muscles of guinea pig ilea and rabbit vasa deferentia were prepared according to the methods of Rang (18) and Oka et al. (19), respectively. A preparation was attached to an isotonic strain gauge transducer under a resting tension of approximately 200 mg and then suspended in a 10 ml organ bath containing Krebs’ solution at a bath temperature of 38°C. The Krebs’ solution had the following composition: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 25.0 mM NaHCO₃ and 11.7 mM glucose. The solution in the bath was con-
continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. Electric field stimulation was applied to the preparation as an amplified square wave at 0.1 Hz for 1 msec and with a maximum voltage of 80 V, through a pair of platinum electrodes placed on the top and bottom of the preparation. The maximum muscle contraction evoked by the electrical stimulation was isotonically registered on a polygraph recorder. Drugs were applied at several bath concentrations after stable twitch responses to the stimuli had been obtained, and the concentration of the drug needed to precipitate 50% inhibition of the twitch response (IC50) was calculated from the linear regression expression obtained by using the least squares method. Finally, the IC50 values for the drugs were compared before and 15 min after pretreatment with cocaine or naloxone.

**a) Myenteric plexus-longitudinal muscle preparation of the guinea pig ileum:** Influences of morphine, pentazocine, EKC and DADLE on the contraction induced by electrical stimulation were observed, after which the IC50s before and after pretreatment with 1 μM cocaine were compared.

**b) Rabbit vas deferens preparation:** Influences of morphine, EKC, DADLE and cocaine on the contraction induced by electrical stimulation were observed, after which the IC50s before and after pretreatment with 1 μM naloxone were compared.

**Results**

**Experiment 1. Observation of antinociceptive effects by the tail-pressure test in mice**

**a) Dose-response relationships in the antinociceptive effects of morphine, pentazocine, cocaine and alcohol:** The 26 control mice, which were subcutaneously administered saline alone, responded to the nociceptive stimulation at 59.9±4.3 (mean±S.E.) mmHg of pressure. Morphine and pentazocine dose-dependently increased the pressure threshold at doses ranging between 0.5 to 4 mg/kg and 2 to 16 mg/kg, respectively. Cocaine and alcohol, on the other hand, showed but little suppression of the response to the stimulation (Fig. 1).

**b) Antinociceptive interaction between morphine and alcohol:** The pressure thresholds for morphine and alcohol alone were 278.3±54.3 mmHg and 71.9±10.2 mmHg, respectively. The value for the combination of morphine and alcohol at these doses was almost the same as for morphine alone (Fig. 2).

**c) Antinociceptive interaction between morphine and cocaine:** The pressure threshold for cocaine 2 mg/kg alone was 87.6±7.7 mmHg. The effects of morphine at 0.5 and 1.0 mg/kg were unchanged by simultaneous administration of cocaine at 2 mg/kg, but the combination of morphine at 2 mg/kg and cocaine at 2 mg/kg produced significantly greater antinociceptive effect than with morphine alone (Fig. 3). When the dose of
cocaine was increased to 4 mg/kg, the pressure threshold for cocaine alone was 92.0±19.9 mmHg, and the antinociceptive effects of all combinations of morphine and cocaine were always significantly greater than those of morphine alone (Fig. 4). The effect of morphine at 2 mg/kg was potentiated by cocaine.

d) Antinociceptive interaction between pentazocine and cocaine: When pentazocine and cocaine were simultaneously administered to mice at a dose combination of 2 mg/kg and 4 mg/kg, respectively, the antinociceptive effect was similar to that of pentazocine alone. However, the higher doses of pentazocine, 4 and 8 mg/kg, in combination with cocaine at 4 mg/kg produced significantly greater effects than those of pentazocine alone (Fig. 5). The potency ratio against pentazocine alone was 2.0.

Experiment 2. Analysis of serum and brain concentration levels of morphine in mice

The morphine concentration levels in the serum and the brain were 269.7±29.9 and 105.5±6.0 ng/ml, respectively (mean±S.E.), in the control mice which had been administered morphine at 2 mg/kg in combination with saline. No change was noted in either the serum or the brain levels even when morphine was simultaneously administered with cocaine at 4 mg/kg (Table 1).

Experiment 3. Experiments on isolated organ preparations of guinea pigs and rabbits

a) Myenteric plexus-longitudinal muscle preparation of the guinea pig ileum: Cocaine showed no agonistic effect on the electrically evoked contractions in this preparation at a bath concentration of 1 μM, but increased the contractile force of the muscle at 100 nM. The contraction enhanced by cocaine was not antagonized by naloxone. Morphine, pentazocine, EKC and DADLE all concentration-dependently inhibited the muscle contraction, and the IC50s of these drugs were 49.3±5.0, 731.3±77.5, 0.22±0.04 and 1742±580.9 nM, respectively (mean±S.E.). Cocaine did not influence the effect of morphine. The effect of DADLE tended to be inhibited by cocaine, but no statistically significant difference was observed in comparison with the control value. On the other hand, the IC50s for both pentazocine and EKC were significantly decreased by pretreatment with cocaine in comparison with the control values (Table 2).
### Table 1. Influence of cocaine on morphine concentration levels in serum and brain in mice

| Drug                        | Morphine concentration (ng/ml or g) |
|-----------------------------|-------------------------------------|
|                             | Serum$^b$ | Brain$^b$ |
| Saline 10 ml/kg, s.c.       | 269.7±29.9 | 105.5±6.0 |
| Morphine 2 mg/kg, s.c.      |           |           |
| Cocaine 4 mg/kg, s.c.       | 265.2±22.8 | 101.5±3.5 |
| Morphine 2 mg/kg, s.c.      |           |           |

Each value represents the mean and standard error for 6 mice.  
$^a$: Drugs were simultaneously administered.  
$^b$: Samples were collected 30 min after drug administration.

### Table 2. Influence of cocaine on IC50s for opioid receptor agonists in the myenteric plexus-longitudinal muscle preparation of guinea pigs

| Drug     | IC50 (nM)                      |
|----------|--------------------------------|
|          | No treatment | Pretreatment with cocaine 1 $\mu$M$^a$ |
| Morphine | 49.3±5.0      | 46.8±5.5                      |
| Pentazocine | 731.3±77.5  | 426.2±19.9$^*$               |
| EKC     | 0.22±0.04     | 0.04±0.01$^*$                |
| DADLE   | 1742.8±580.9  | 2845.5±1038.6               |

Each value represents the mean and standard error for 4 preparations.  
$^a$: Preparation was pretreated with cocaine for 15 min.  
EKC: Ethylketocyclazocine.  
DADLE: D-Ala$^2$-D-Leu$^5$-enkephalin.  
$^*: $Significantly different from the value of the no treatment group.

### Table 3. Influence of cocaine on the contraction induced by electrical stimulation in the isolated vas deferens of rabbits

| Drug     | IC50 (nM)                      |
|----------|--------------------------------|
|          | No treatment | Pretreatment with naloxone 1 $\mu$M$^a$ |
| Cocaine  | 4561.6±1180.3 | 25222.9±7686.0$^*$            |
| Morphine | No effect$^b$ | —                              |
| EKC     | 8.0±3.7        | 387.0±67.6$^*$                |
| DADLE   | No effect$^b$ | —                              |

Each value represents the mean and standard error for 4 preparations.  
$^a$: Preparation was pretreated with naloxone for 15 min.  
$^b$: at the concentration up to 100 $\mu$M.  
EKC: Ethylketocyclazocine.  
DADLE: D-Ala$^2$-D-Leu$^5$-enkephalin.  
$^*: $Significantly different from the value of the no treatment group.
Discussion

When administered alone, both morphine and pentazocine dose-dependently suppressed the responses of mice to nociceptive stimuli in the tail-pressure test, while cocaine and alcohol did not show marked suppression of the response. Simultaneous administration of morphine with a dose of alcohol which never induced marked behavioral changes showed neither more nor less effect than did morphine alone. Alcohol’s antinociceptive effects have been observed in the phenylquinone writhing test in mice (16), in the tail flick test in mice (14) and rats (17), and in men (15). The discrepancy of the results in the experiment from those of other investigators seems to be based on the differences of experimental conditions such as doses and administration routes of the drug and in the methods used. The results in the present experiment, at least under the experimental conditions used in this study, indicate that alcohol neither has any analgesic action nor influences morphine analgesia.

Cocaine at the dose of 2 mg/kg tended to potentiate morphine’s antinociceptive effects, and by 4 mg/kg, morphine’s effects were dramatically potentiated to 3.4 times as great as those of morphine alone. Cocaine at 4 mg/kg also enhanced the antinociceptive effects of pentazocine to 2 times greater than those of pentazocine alone. Although there was a report that cocaine did not influence the antinociceptive effects of morphine (9), Nott (10) observed that 4 mg/kg cocaine, s.c., markedly potentiated morphine analgesia in the tail flick test in mice. This potentiation of morphine’s effects has been also observed in the production of the Straub tail reaction in mice (20). The present investigation also demonstrated that cocaine potentiated the antinociceptive effects not only of morphine but also of pentazocine. The results in the antinociceptive interaction in mice further indicate that cocaine, but unlike alcohol, plays an important role in the effectiveness of mixtures of opioid analgesics, cocaine and alcohol.

In the analysis of serum and brain concentration levels of morphine, the level in mice which showed significant potentiation of morphine’s antinociceptive effect did not change in comparison with that in mice administered morphine alone. Concerning the mechanisms of the potentiation of morphine analgesia, Nott (10) has suggested that enhancement of morphine analgesia by cocaine was a result of the ability of cocaine to potentiate endogenously released noradrenaline in the central nervous system. It has been also observed that similar to cocaine, other central nervous system stimulants such as amphetamines enhance morphine analgesia (10–13) or morphine-induced Straub tail and toxicity (21). Sprague and Takemori (13) reported that enhanced whole brain levels of morphine evident after methamphetamine pretreatment may have been the result of peripheral changes induced by methamphetamine such as increased blood flow to the site of morphine and absorption or reduction in the extent of morphine metabolism. Since cocaine did not in the present experiment produce any alteration of morphine contents in the serum and the brain, the mechanism of potentiation of morphine analgesia by cocaine may be different from that in the case of amphetamines. However, relationships between the potentiating action of cocaine in morphine analgesia and opioid receptors were not examined in these reports.

In the experiment on isolated organs, cocaine potentiated both agonistic effects of pentazocine and EKC, but did not influence those of either morphine or DADLE in the myenteric plexus-longitudinal muscle preparation of the guinea pig ileum. Cocaine as well as EKC also showed clear-cut naloxone-reversible agonistic effects in the vas deferens preparation of the rabbit. However, neither morphine nor DADLE showed any agonistic effects in this preparation. Few studies have attempted to elucidate the opioid receptor-mediated mechanism in explaining the effects of cocaine alone or interactions between opioid analgesics and cocaine. It is well-known that the myenteric plexus-longitudinal muscle of the guinea pig ileum is considered to possess \( \mu \)-, \( \kappa \)- and \( \delta \)-receptors (22, 23), whereas the rabbit vas deferens contains only \( \kappa \)-receptors (19, 24, 25); and furthermore, morphine, pentazocine, EKC and DADLE are
known to act as agonists on the $\kappa$- and $\nu$-,$\kappa$-,$\kappa$- and $\delta$-receptors, respectively (26–29). Based on this knowledge, the results in the present experiment indicate that cocaine acts on the $\kappa$-receptors as an agonist.

Thus, the results in the present study suggest that cocaine may potentiate the antinociceptive effects of opioid analgesics by acting on the $\kappa$-opioid receptors as an agonist. On the other hand, alcohol does not seem to play any important role in the antinociceptive interaction among morphine, cocaine and alcohol. If a mixture of an opioid analgesic with cocaine is tested in patients suffering from pain and not found to be as effective as the same mixture with alcohol, then it may be that alcohol plays a role in a mechanism that is not directly related to modification of the pain sensation.

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