release from rat brain synaptosomes (Shimazu et al., 2003b), rather than potentiating the release. If binding of (−)-BPAP at noradrenaline transporters contributes to an inhibitory effect of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior, selective noradrenaline uptake inhibitors would mimic the effect. So far, no studies in rats have reported pre-session treatment effect of selective noradrenaline uptake inhibitors on reinstatement of drug-seeking behavior, whereas a few studies in squirrel monkeys and humans have reported such effects. In squirrel monkeys, pre-session treatment with selective noradrenaline uptake inhibitors nisoxetine and talsupram both produced leftward shift of dose-effect curve of reinstatement of cocaine-seeking behavior induced by cocaine-priming injections; however, neither of the two selective noradrenaline uptake inhibitors affected the dose-effect curve of reinstatement of cocaine-seeking behavior induced by priming injections of GBR 12909, a selective dopamine uptake inhibitor (Platt et al., 2007). Furthermore, priming injections of nisoxetine and talsupram alone reinstated cocaine-seeking behavior (Platt et al., 2007). On the contrary, one clinical study has found positive results of the selective noradrenaline uptake inhibitor reboxetine to maintain cocaine abstinence (Szerman et al., 2005). Although the findings of clinical and preclinical studies seem to be inconsistent, the clinical evidence may support possible involvement of monoamine uptake inhibition in blocking effect of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior, especially via noradrenaline transporters.

Alternatively, (−)-BPAP has been reported to be a highly potent enhancer (0.1 μg/kg s.c.; Yoneda et al., 2001) of electrically-stimulated monoamine release (Miklya and Knoll, 2003), whereas standard monoamine uptake inhibitors do not share this effect (Miklya and Knoll, 2003). Therefore, these findings suggest (−)-BPAP as an atypical monoamine uptake inhibitor. Meanwhile, previous studies reported "atypical" dopamine uptake inhibitors, including benztropine analogues with pharmacological profiles unlike that of cocaine (Newman et al., 1995; Katz et al., 1999; Beuming et al., 2008; Loland et al., 2008). Among benztropine analogues, several *N*-substituted benztropine analogues exhibited reduced cocaine-like effects (Katz et al., 2004), antagonized cocaine-stimulated activity (Desai et al., 2005), and failed to substitute for cocaine in rats trained to self-administration cocaine (Hiranita et al., 2009). One of the possible targets underlying the "atypical" property of *N*-substituted benztropines analogues appear to be the σ1 receptor (σ1-R), because (1) *N*-substituted benztropine analogues have a high affinity for this protein with the nanomolar order of Ki values (Katz et al., 2004), (2) rimcazole, a σ1-R antagonist with a high affinity for the dopamine transporters (Cao et al., 2003), shows reduced cocaine-like behavioral effects (Katz et al., 2003) and (3) these two analogues show different molecular interactions at the dopamine transporters from cocaine (Loland et al., 2008). Interestingly, (−)-BPAP has been reported to be a ligand at σ1-R (Hamabe et al., 2000). Furthermore, *in vitro* study demonstrated that enhancement of cellular survival activity on cortical neurons by incubation with (−)BPAP was blocked by pre-incubation with *N*-[2-(3, 4-dichlorophenyl) ethyl]-4-methylpiperazine (BD 1063), a σ1-R antagonist (Hamabe et al., 2000). A behavioral study also demonstrated that (1) methamphetamine self-administration upregulated σ1-R mRNA and protein levels in several limbic regions (Stefanski et al., 2004). Meanwhile, we have reported that donepezil, an inhibitor of choline esterase, dramatically attenuated the reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues and methamphetamine-priming injections (Hiranita et al., 2006). Interestingly, donepezil has been reported to have very high affinity for σ1-Rs (IC_{50} value; 14.6 nM) (Kato et al., 1999). Although the involvement of σ1-Rs in the reinstatement of methamphetamine-seeking behavior has not been well understood, the σ1-Rs may be considered as the possible target underlying blocking effect of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior.

In the present study, pre-session treatment with SCH-23390 dose-dependently reversed the blocking effect of SKF-81297 on the reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues and methamphetamine priming injections. In contrast, at even the tenfold higher dose, pre-session treatment with SCH-23390 appreciably failed to reverse the effect of the single pre-session treatment with (−)-BPAP on the reinstatement of methamphetamine-seeking behavior. The role of dopamine D_1-like receptors on reinstatement of drug-seeking behavior seems complicated. Systemic administration of the agonist (SKF-81297) and antagonist (SCH-23390) both have been reported to attenuate cocaine-seeking behavior induced by cocaine-priming injections (15 mg/kg i.p.) or cocaine-associated cues in rats (Alleweireldt et al., 2002, 2003). On the contrary, systemic administration of SCH-23390 (up to 10 μg/kg) has been reported to fail to attenuate reinstatement of cocaine-seeking behavior induced by priming injections of cocaine (5.0, 10 and 20 mg/kg i.p.) or a selective dopamine uptake inhibitor, WIN 35,428 (Schenk and Gittings, 2003). The mechanisms underlying the inhibitory effect of both the dopamine D_1-like receptor agonist and antagonist on the reinstatement of cocaine-seeking behavior and inconsistent results of SCH-23390 on the primed-cocaine-induced reinstatement are unknown. However, considering the inhibitory effect of SCH-23390 on reinstatement of cocaine-seeking behavior in the studies by Alleweireldt et al. (2002, 2003), inability of SCH-23390 to reverse the attenuating effect of pre-session treatment with (−)-BPAP on the reinstatement of methamphetamine-seeking behavior in the present study might result from the possible inhibitory action of SCH-23390 on reinstatement of drug-seeking behavior.

On the other hand, pre-session treatment with amisulpride up to 10 mg/kg appreciably failed to reverse the effect of the single pre-session treatment with (−)-BPAP. In Chinese hamster ovary cells expressed with human dopamine D_2 and D_3 receptors, amisulpride has been reported to have very high selectivity (Ki values; 21 and 2.9 nM) (Schoemaker et al., 1997). The effective dose of amisulpride for 50% occupancy for dopamine $D_{2/3}$ recep-

tors in rat brain has been reported to be 4.68 mg/kg (s.c.) (Natesan et al., 2008). Furthermore, pretreatment with 10 mg/kg of amisulpride has been reported to reverse amphetamine (1.0 or 2.0 mg)-stimulated locomotor activity to the vehicle level (Perrault et al., 1997; Natesan et al., 2008). In addition, administration of a higher dose of amisulpride alone (20 mg/kg i.p.) has been reported to suppress 10% sucrose feeding in rats (Schneider et al., 1986). Therefore, it is unlikely that the dose of amisulpride tested (10 mg/kg) is insufficient to work as a dopamine D_2-like receptor antagonist without impairment of non-specific operant behavior in the present study. Alternatively, systemic administration of dopamine D_2-like (eticlopride (Schenk and Gittings, 2003), raclopride (Cervo et al., 2003) and haloperidol (Gal and Gyertyan, 2006)) and selective dopamine D_3 receptor antagonists (SB-277011-A (Vorel et al., 2002; Gilbert et al., 2005; Gal and Gyertyan, 2006; Cervo et al., 2007) and NGB 2904 (Gilbert et al., 2005; Xi et al., 2006; Xi and Gardner, 2007)) were found consistently to attenuate reinstatement of cocaine-seeking behavior induced by cocaine-priming injections or cocaine-associated cues in rats. Therefore, lack of amisulpride effect to reverse the attenuating effect of pre-session treatment with (−)-BPAP on the reinstatement of methamphetamine-seeking behavior in the present study might be also masked due to the possible inhibitory action of amisulpride on reinstatement of drug-seeking behavior.

CONCLUSION

In summary, activation of dopamine D_1-like receptors resulted in attenuation of the reinstatement of methamphetamine-seeking behavior in rats. Although the attenuating effect of pre-session treatment with (−)-BPAP may be unrelated to dopamine D_1-like receptors, our results suggest a specific blocking effect of pre-session treatment with (−)-BPAP without affecting the reinforcing effect of methamphetamine. Extending this conclusion to the treatment of drug dependence, (−)-BPAP and dopamine D_1-like receptor agonists may be useful as anti-relapse agents in methamphetamine dependence.

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Olanzapine Suppresses the Rewarding and Discriminative Stimulus Effects Induced by Morphine

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KEY WORDS olanzapine; morphine; abuse potential; dopamine; discriminative stimulus effect

ABSTRACT Atypical antipsychotic medications are effective for treating both the positive and negative symptoms of schizophrenia. Olanzapine is an atypical antipsychotic that blocks dopaminergic, serotonergic, adrenergic, histaminergic, and muscarinic receptors. In this study, we used rodents to investigate whether olanzapine could suppress the hyperlocomotion, rewarding effect, and discriminative stimulus effect induced by the prototypic μ-opioid morphine, which are all considered to reflect the abuse potential or psychoactive effects of μ-opioids. Olanzapine at doses that failed to induce motor coordination produced a dose-dependent reduction in hyperlocomotion induced by morphine in mice. Olanzapine at a dose that did not produce motor dysfunction also inhibited the significant place preference induced by morphine in mice. Furthermore, the discriminative stimulus effect induced by morphine in rats was dose-dependently and significantly attenuated by olanzapine at the dose that did not induce the motor dysfunction. These results suggest that treatment with both μ-opioids and olanzapine at a dose lower than that at which it induces motor dysfunction could be very useful for preventing the abuse potential and/or psychoactive effects of μ-opioids. **Synapse 66:174–179, 2012.**

INTRODUCTION

Morphine has been used as a "gold standard" for the treatment of patients who suffer moderate to severe cancer pain (WHO, 1996). When morphine is used to treat pain, the adverse effects of opioids, such as emesis, constipation, drowsiness, hallucination, and delirium must be reduced to improve the quality of life of the patient. Against this background, antipsychotic drugs, many of which are dopamine receptor antagonists, have been used to reduce opioid-induced emesis and other dopamine-related side effects, including hallucination and delirium. However, the adverse effects of antipsychotic drugs, such as extrapyramidal symptoms, can be a disadvantage of their use (Swegle and Logemann, 2006).

Ever since chlorpromazine was shown to have an antipsychotic effect more than 50 years ago, the number of antipsychotic medications has continued to grow. Typical or first-generation antipsychotic drugs such as chlorpromazine and haloperidol are effective for the treatment of delusions and hallucinations, while such compounds have a high risk of adverse extrapyramidal side effects (Barnes and McPhillips, 1998). Atypical or second-generation antipsychotic drugs, especially chlorpromazine derivatives such as clozapine and olanzapine, have been developed and used for the treatment of dopamine-, serotonin-, acetylcholine-, and/or histamine-related symptoms and show a relative lack of extrapyramidal side effects (Kumar and Sachdev, 2009). Opioid-induced changes in emotionality and multiple physiological functions

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are likely to result from the regulation of multiple neurotransmitters.

Olanzapine is an atypical thienobenzodiazepine antipsychotic that blocks dopaminergic, serotonergic, adrenergic, histaminergic, and muscarinic receptors for multiple neurotransmitters. We previously demonstrated that olanzapine clearly showed affinities for these receptors in the rodent brain and was effective for the treatment of morphine-induced emesis, for reducing neuropathic pain, and for improving pain-related sleep disturbance (Torigoe et al., in press). We proposed that olanzapine could be used as a single adjunct agent against a background of increasing concern about "polypharmacy" and can be given in a state-dependent dose, which should improve the quality of life for patients while greatly reducing the side effects of opioids.

In this study, we used rodents to further investigate whether olanzapine could suppress the hyperlocomotion, rewarding effect, and discriminative stimulus effect induced by morphine, which are all considered to reflect the abuse potential or psychoactive effects of μ-opioids.

MATERIALS AND METHODS

Animals

In this study, male Institute of Cancer Research (ICR) mice (20–25 g) (Tokyo Laboratory Animals Science Co., Tokyo, Japan) and seven male Fischer 344 rats (Charles River Japan, Atsugi, Japan) maintained at 200–230 g (80% free-feeding weight) were used. Food and water were available *ad libitum* for mice, and water was available *ad libitum* for all of the rats in their individual home cages. Animals were housed in a room maintained at 22 ± 1°C with a 12-h light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments.

Rota-rod assay

To determine the doses of olanzapine to be tested, which would not affect animal behavior, we first used a rota-rod assay. The motor coordination produced by olanzapine in mice was evaluated in terms of the time until they fell from a rota-rod at 8 rpm (KN-95; Natsume Seisakusyo, Co., Tokyo, Japan). The apparatus consists of a rotating acrylic rod (3 cm in diameter) divided into four 8-cm sections by circular Plexiglas dividers (18-cm high). First, mice were trained to be able to walk on the rota-rod for 180 s. This training was performed for 3 days. On the day after the final training session, the time until the mouse fell from the rota-rod was measured for up to 180 s at 30 min after the administration of olanzapine (0.03–1 mg/kg, s.c.). In addition, any behavioral changes were also observed by comparison with the vehicle control.

Locomotor activity

The locomotor activity of mice was measured by an ambulometer as described previously (Narita et al., 1993). Briefly, a mouse was placed in a round tilting-type activity cage 20 cm in diameter and 19-cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the mouse, was detected by three microswitches. The total activity counts in each 10-min segment were automatically recorded for 180 min following the administration of saline or morphine (10 mg/kg, s.c.). Olanzapine (0.03, 0.1, and 0.3 mg/kg, s.c.) was administered 30 min prior to treatment with morphine.

Place conditioning

Place-conditioning studies were conducted using a shuttle box (15 cm × 30 cm × 15 cm: $w \times l \times h$) composed of an acrylic resin board divided into two equal-sized compartments (Suzuki et al., 1991). One compartment was white with a textured floor, and the other was black with a smooth floor to create equally inviting compartments. The place-conditioning schedule consisted of three phases (preconditioning test, conditioning, and postconditioning test). The preconditioning test was performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and mice that had not been treated with either drugs or saline were then placed on the platform. The time spent in each compartment during a 900-s session was then recorded automatically using an infrared beam sensor (KN-80, Natsume Seisakusyo Co., Tokyo, Japan). Conditioning sessions (three for morphine: three for saline) were started the day after the preconditioning test and conducted once daily for 6 days. Mice were pretreated with olanzapine (0.1 or 0.3 mg/kg, s.c.) or vehicle 30 min before the injection of morphine or saline. Immediately after the injection of morphine (5 mg/kg, s.c.), the animals were placed in the compartment opposite that in which they had spent the most time in the preconditioning test for 1 h. On alternate days, the animals were treated with saline after pretreatment with olanzapine or vehicle and placed in the other compartment for 1 h. On the day after the final conditioning session, a postconditioning test that was identical to the preconditioning test was performed.

Drug discrimination procedure

Experiments were conducted in operant-conditioning chambers (model ENV-008; Med Associates, St.

Albans, VT) equipped with two levers and a food cup mounted midway between the levers. White lamps were installed above each of the levers. White noise was used to mask extraneous sound. Reinforcement consisted of 45-mg food pellet (Bio-Serv, Frenchtown, NJ).

Discrimination training was performed as described previously (Mori et al., 2002). Briefly, before they were trained to discriminate between drugs and saline, all rats were trained to press a lever. Training began under a reinforcement schedule of fixed ratio 1 (FR 1), 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. The FR requirement for food reinforcement was gradually increased to a value of 10. After the response rates had stabilized under FR 10, rats were trained to discriminate between 3.0 mg/kg of morphine and saline. In the discrimination training, training morphine (D) and saline (S) were administered in a session-to-session sequence of DDSS (double alternation schedule), and the assignment of left and right levers to drug and saline states was counterbalanced. Rats were required to respond on the stimulus-appropriate lever to obtain reinforcement; there were no programmed consequences for responding on the incorrect lever. Substitution tests were only performed after the discrimination criterion described below had been satisfied for at least five consecutive daily discrimination-training sessions (accuracy of at least 83% and fewer than 12 responses to obtain the first reinforcement).

After the animals attained the criterion, a dose-response test was initiated; test sessions were performed only when FRF $\leq$ 12 for at least three consecutive discrimination training sessions. In the dose-response test, rats were placed in the operant box until they had made 10 responses on either lever or 5 min had elapsed. In the combination test, doses of olanzapine that produced <20% drug-lever responses and did not affect the response rates in the generalization tests were used in generalization tests. The pretreatment time and doses of drugs used were 30 min and 0.03–0.3 mg/kg of olanzapine. 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 morphine hydrochloride (Daiichi-Sankyo, Tokyo, Japan) and olanzapine (Toronto Research Chemicals, Toronto, Ontario, Canada). Morphine was dissolved in saline, and olanzapine was dissolved in DMSO: Tween80: saline (5:5:90). All drugs were administrated in a volume of 1.0 ml/kg (rats) or 10 ml/kg (mice).

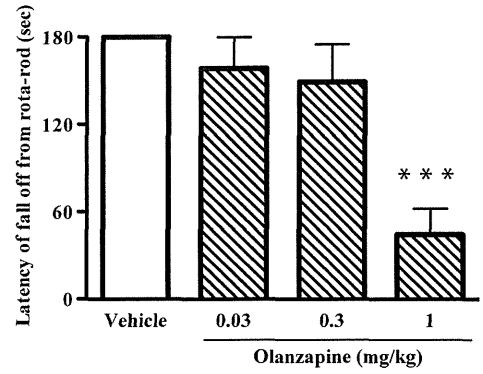

Fig. 1. Motor coordination produced by olanzapine (0.03, 0.3, and 1 mg/kg, s.c.) in mice. The motor coordination produced by olanzapine was evaluated in terms of the amount of time until the mouse fell from a rota-rod at 8 rpm. Each column represents the mean ± S.E.M. of 6 mice. ***$P < 0.001$ vs. vehicle.

Statistical analysis

Data are expressed as the mean with S.E.M. The statistical significant of differences between the groups was assessed by a one-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparisons test or two-tailed paired t-test. All statistical analyses were performed using Prism software (version 5.0a, GraphPad Software, La Jolla, CA). A P value of < 0.05 was considered to reflect significance.

RESULTS

Motor coordination produced by olanzapine in the mouse rota-rod assay

In the rota-rod test, olanzapine (1 mg/kg, s.c.) produced significant motor impairment in mice compared with vehicle-treated mice ($F(3,23) = 10.19$, $P < 0.0001$), whereas olanzapine at doses of 0.03–0.3 mg/kg did not induce motor dysfunction (Fig. 1).

Suppression of morphine-induced hyperlocomotion by pretreatment with olanzapine at doses that failed to induce motor dysfunction

A single injection of morphine at 10 mg/kg (s.c.) produced a significant increase in locomotion. Pretreatment with olanzapine at 0.03, 0.1, and 0.3 mg/kg (s.c.) induced a dose-dependent suppression of this morphine-induced hyperlocomotion, and these effects of olanzapine were significant at all of the doses tested ($P < 0.05$ for 0.03, $P < 0.01$ for 0.1, $P < 0.001$ for 0.3 vs. vehicle pretreatment) (Fig. 2A). In contrast, locomotion activity was not changed by olanzapine itself at dose of 0.3 mg/kg with saline.

Inhibition of morphine-induced place preference by pretreatment with olanzapine at a dose that did not induce motor impairment

Consistent with our previous results with morphine using the same procedure, morphine (5 mg/kg, s.c.)

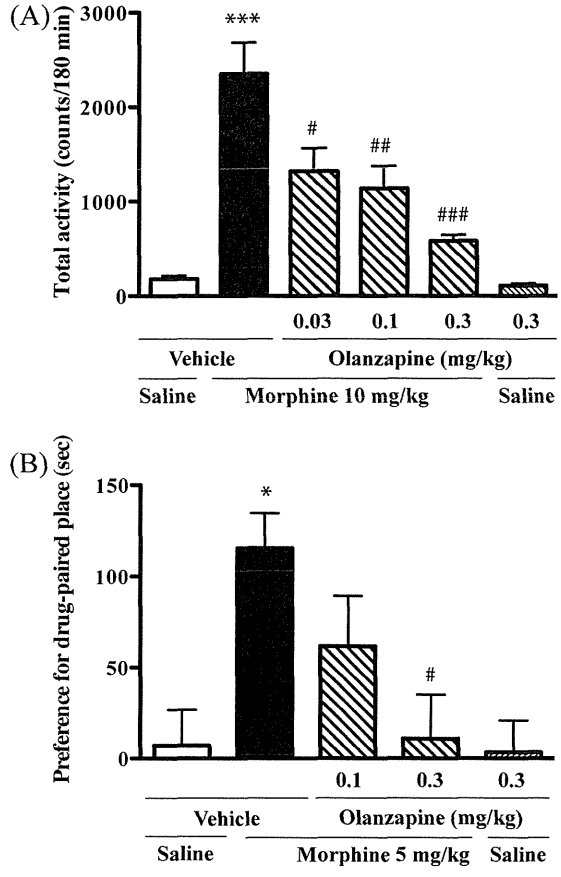

Fig. 2. Effects of olanzapine on the morphine-induced hyperlocomotion (**A**) and place preference (**B**) in mice. A: Each column represents the mean total activity for 180 min ± S.E.M. of 8–10 mice. B: Ordinate: mean differences between time spent in the postconditioning and preconditioning tests. Each column represents the mean ± S.E.M. of 6–8 mice. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. vehicle-saline. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. vehicle-morphine.

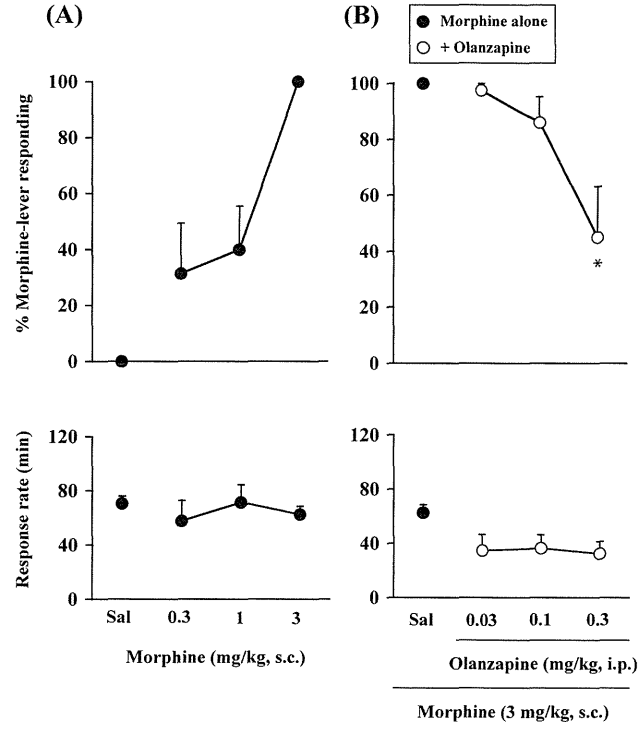

Fig. 3. **A**: Dose-response curve for % morphine-lever responses (upper panel) and response rates (bottom panel) in rats that had been trained to discriminate between 3 mg/kg morphine and saline. **B**: Effects of olanzapine on the discriminative stimulus effects (upper panel) and response rates (bottom panel) for morphine in rats that had been trained to discriminate between 3 mg/kg morphine and saline. Each point represents the mean percentage of morphine-appropriate responding with S.E.M. of 5–7 animals. $^{*}P < 0.05$ compared with the saline pretreatment.

produced a significant place preference in mice. A significant place preference or place aversion was not observed by olanzapine itself at the dose of 0.3 mg/kg with saline. Under these conditions, pretreatment with olanzapine at 0.3 mg/kg (s.c.) significantly suppressed the rewarding effects of morphine (Fig. 2B).

Suppression of the drug discriminative effect of morphine by olanzapine at doses that failed to induce motor dysfunction

Rats required 23 sessions to acquire morphine–saline discrimination. Once rats attained the criterion, morphine–saline discrimination stabilized and was maintained with a high degree of accuracy. During the dose-response tests, morphine at 0.3–3.0 mg/kg (s.c.) produced a dose-dependent increase in morphine-appropriate responses in all of the rats (Fig. 3A). In the combination test, olanzapine dose-dependently and significantly attenuated the discriminative stimulus effects of the training dose (3.0 mg/kg) of morphine without affecting the response rates ($P = 0.0294$ vs. saline pretreatment) (Fig. 3B).

DISCUSSION

In humans, morphine induces several kinds of subjective and psychotic effects, while in animals morphine clearly has reinforcing/rewarding effects. The place-conditioning procedure is used to evaluate rewarding and aversive effects (Suzuki et al., 1991). In addition, the evaluation of hyperlocomotion induced by morphine has also been used to assess the pharmacological mechanisms that underlie the abuse of morphine (Narita et al., 1993). A large and growing body of evidence has demonstrated that activation of the dopaminergic, especially mesolimbic, system plays a crucial role in the expression of both hyperlocomotion and the reinforcing/rewarding effects of opioids (Bonci and Williams, 1997; Johnson and North, 1992; Narita et al., 2001). In the present study, we clearly confirmed that morphine at a range of 5–10 mg/kg induced significant hyperlocomotion and conditioned place preference in mice, which reflected the abuse potential of morphine in rodents.

Although μ-opioids have potential for abuse and/or addiction, clinical studies have shown that abuse and addiction do not usually occur when μ-opioids are used appropriately to control pain (Eisenberg et al., 2005). We have proposed that the abuse potential of morphine is potently suppressed under chronic pain in rodents (Suzuki et al., 1996; Narita et al., 2005; Niikura et al., 2010). However, over the past decade, there has been some concern regarding the abuse of and addiction to μ-opioids caused by the inappropriate use, misuse, or overdose of prescription opioids (Woodcock, 2009). Dopamine receptor antagonists have been commonly used to reduce the adverse reactions to μ-opioids, including delusions and hallucinations (McNicol et al., 2003). Olanzapine is an atypical antipsychotic that is clinically indicated for schizophrenia and mania. It blocks multiple neurotransmitters (Glazer, 1997; Tohen and Grundy, 1999). In the present study, we found that olanzapine at 0.3 mg/kg, which did not induce motor coordination significantly suppressed the hyperlocomotion and rewarding effects induced by morphine. In our preliminary study, treatment with olanzapine at 0.3 mg/kg failed to reduce the antinociceptive effect of morphine. Furthermore, the delay in colonic expulsion induced by morphine was not affected by 0.3 mg/kg of olanzapine, which indicated that olanzapine did not exacerbate morphine-induced constipation. Taken together, these findings support the idea that olanzapine may have a wide margin of safety when used as an adjuvant for μ-opioids.

In a previous binding study in brain tissue, we found that olanzapine exhibited the highest affinity for muscarinic M_1 receptors and also showed affinity toward serotonin 5-$HT_{2A/2C}$, 5-HT_3, histamine H_1, dopamine D_2, dopamine D_4, and 5-HT_4 receptors. In light of this multiple binding property, we previously documented in an *in vivo* study that olanzapine dose-dependently decreased morphine-induced nausea and vomiting that are caused through various mechanisms (Torigoe et al., in press).

Muscarinic M_1 receptors have been suggested to be responsible for the enhancement of opioid-stimulated dopaminergic transmission related to the aggravation of drug addiction (Tanda et al., 2007). Since olanzapine showed the highest affinity toward muscarinic M_1 receptors, it is reasonable to wonder if olanzapine could aggravate the abuse potential of μ-opioids. This contention can be supported by the fact that the selective muscarinic M_1 receptor antagonist trihexyphenidyl significantly enhanced the morphine-induced increase in the release of dopamine in the nucleus accumbens (our preliminary study; data not shown), which indicates that M_1 receptors play an important role in opioid addiction. However, in the present study, olanzapine did not enhance either the hyperlocomotion or place preference produced by morphine. Although the exact mechanism by which olanzapine suppresses morphine's abuse profile remains unclear, this phenomenon may result from the fact that olanzapine acts not only on muscarinic M_1 receptors but also partly on serotonin 5-$HT_{2A/2C}$, 5-HT_3, histamine H_1, dopamine D_2, dopamine D_4, and 5-HT_4 receptors as an antagonist.

Morphine has subjective effects (e.g., "strength of drug effect," "sedated," "heavy or sluggish feeling," and "high") in healthy volunteers (Zacny et al., 1994). Furthermore, morphine can induce drowsiness, hallucination, and delirium, which have been considered to be important cues for the subjective effects of morphine (Adunsky et al., 2002; Maddocks et al., 1996). To assess the subjective effects in humans, animal models for studying the components of drug action that bear on the subjective effects have been developed. A methodology that has considerable potential in this regard is the drug discrimination procedure (Schuster and Johanson, 1988). However, little is known about the mechanism of the discriminative stimulus effects of morphine in animals. In the present study, olanzapine at 0.3 mg/kg significantly attenuated the discriminative stimulus effects of the training dose of morphine in rats. It should be emphasized that neither the D_1 receptor antagonist SCH23390, the D_2 receptor antagonist haloperidol nor their combination affected the discriminative stimulus effects of morphine in rats that had been trained to discriminate between 3.0 mg/kg morphine and saline (Suzuki et al., 1995). Therefore, we hypothesize that the blockade of neurotransmitters other than dopamine receptors by olanzapine at this dose may contribute to attenuate the discriminative stimulus effects of morphine.

In conclusion, we found that olanzapine at a dose that failed to induce motor dysfunction suppresses the hyperlocomotion, place preference, and discriminative stimulus effect induced by morphine. These results further provide evidence that cotreatment with olanzapine may be very useful as an adjuvant for pain control by μ-opioids.

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Effects of Dronabinol on Morphine-Induced Dopamine-Related Behavioral Effects in Animals

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KEY WORDS morphine; dronabinol; rewarding effects; emesis; dopamine

ABSTRACT The present study examined the effects of dronabinol, a United States FDA-approved synthetic cannabinoid receptor agonist, on morphine (a prototypic μ-opioid receptor agonist)-induced dopamine-related behaviors in animals. Dronabinol suppressed the rewarding effects of morphine in rats and its emetic effects in ferrets. Furthermore, the morphine-induced increase in dopamine release from the nucleus accumbens was significantly attenuated by dronabinol, which indicated that the suppressive effects of dronabinol on morphine-induced behaviors are at least in part mediated by regulation of the dopaminergic system. Since cannabinoid receptor agonists have been shown to enhance the antinociceptive effects of morphine, the use of dronabinol as an adjuvant could be useful for preventing the adverse effects of μ-opioid receptor agonists when used to control pain. **Synapse 66:931–937, 2012.** ©2012 Wiley Periodicals, Inc.

INTRODUCTION

Morphine exerts prominent antinociceptive effects, and is considered to be the "gold standard" for the treatment of patients with moderate to severe cancer pain (WHO, 1996) or chronic inflammatory or postoperative pain. Under pain control by morphine, it is important to control the adverse effects of opioids, such as emesis, constipation, drowsiness, hallucination, and delirium to improve the quality of life of the patient. Morphine, a μ-opioid receptor agonist, also induces reinforcing/rewarding effects in animals, and is abused in humans. A large growing body of evidence has demonstrated that activation of the dopaminergic, especially mesolimbic, system is involved in the expression of the reinforcing/rewarding effects and hyperlocomotion, as well as hallucination and delirium, produced by opioids.

Marijuana is the most commonly used illegal drug in the United States and throughout the world, and the psychoactive ingredient Δ^9-tetrahydrocannabinol (THC), which acts on cannabinoid (particularly CB1 and CB2) receptors, could trigger the reinforcing/rewarding effects of marijuana (Cooper and Haney, 2009). Animal studies have shown conflicting findings that THC and synthetic cannabinoid receptor agonist can both produce either aversive or rewarding effects depending on the test conditions (e.g., strains of animals used, duration of session, and methods) (Tanda and Goldberg, 2003). Interestingly, it has been suggested that there is cross-talk between μ-opioid and cannabinoid receptors. Cannabinoid receptor agonists enhance the antinociceptive effects and hyperlocomotion induced by morphine in rodents (Ayhan et al., 1979; Cichewicz, 2004). On the other hand, even though the activation of either μ-opioid or cannabinoid receptors activates mitogen-activated protein kinase (MAPK), which is important for acquisition of the reward process, these MAPK-activating effects of μ-opioid receptor agonist and cannabinoid receptor agonist reciprocally abolish each other when these agonists are combined (Rios et al., 2006). Thus, cannabinoid receptor agonists could differentially alter the behavioral effects induced by morphine depending on the conditions.

Dronabinol is the only United States FDA-approved synthetic cannabinoid that has little or no abuse potential, and dronabinol could be prescribed for the treatment of cachexia (weight loss) in patients due to nausea and vomiting associated with cancer chemo-

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therapy in patients who have failed to respond adequately to conventional antiemetic treatments (Plasse et al., 1991). Therefore, dronabinol could be co-administered with opioid in a clinical setting. Dronabinol enhances the antinociceptive effects of morphine in mice (Mori et al., submitted). Dopamine D_2-receptor antagonists have been used to reduce opioid-induced nausea and vomiting; however, the adverse effects of dopamine D_2-receptor antagonists, including extrapyramidal symptoms, could be a burden for the treatment of pain (Swegle and Logemann, 2006). A recent study showed that the cannabinoid receptor agonist WIN55,212-2 has prominent suppressive effects against morphine-induced emesis in ferrets (Simoneau et al., 2001). Thus, dronabinol could be a useful adjunct for the treatment of pain. However, limited information is available about the interaction between dronabinol and morphine, especially with regard to dopamine-related behaviors. Therefore, the present study was designed to investigate the effects of dronabinol on morphine-induced dopamine-related behaviors in animals.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (250–300 g) (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) and Institute of Cancer Research (ICR) mice (20–25 g) (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) were used. Food and water were available *ad libitum* for rats and mice in their home cages. Male ferrets weighing 1–1.5 kg were obtained from Marshall Research Labs (North Rose, NY) and housed individually in cages. They were given a standard cat diet (70–80 g/animal, Oriental Yeast Co. Ltd., Chiba, Japan) and allowed free access to water. Animals were housed in a room maintained at (22 ± 1)°C with a 12-h light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments.

Place conditioning

Place-conditioning studies were conducted using a shuttle box (30 × 60 × 30 cm^3: $w \times l \times h$) that was made of an acrylic resin board and divided into two equal-sized compartments (Suzuki et al., 1991). One compartment was white with a textured floor and the other was black with a smooth floor to create equally inviting compartments. The place-conditioning schedule consisted of three phases (pre-conditioning test, conditioning, and post-conditioning test). The pre-conditioning test was performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and mice that had not been treated with either drugs or saline were then placed on the platform. The time spent in each compartment during a 900-s session was recorded automatically using an infrared beam sensor (KN-80, Natsume Seisakusyo Co. Ltd, Tokyo, Japan). Conditioning sessions (three for morphine: three for saline) were started the day after the pre-conditioning test and conducted once daily for 6 days. Rats were pretreated with dronabinol (5.0 mg/kg, s.c.) or vehicle 30 min before morphine or saline injection. Immediately after the injection of morphine (10 mg/kg, s.c.), these animals were placed in the compartment opposite that in which they had spent the most time in the pre-conditioning test for 1 h. On alternate days, these animals were treated with saline after pretreatment with dronabinol or vehicle and placed in the other compartment for 1 h. On the day after the final conditioning session, a post-conditioning test that was identical to the pre-conditioning test was performed.

In vivo microdialysis study and quantification of dopamine and its metabolites

Before implantation of a cannula, all of the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for surgery as described previously. Briefly, the anesthetized animal was placed in a stereotaxic apparatus, the skull was exposed, and a small hole was made using a dental drill. A guide cannula (AG-8; Eicom, Kyoto, Japan) was implanted into the nucleus accumbens (NAc) (from the bregma:anterior, +4.0 mm; lateral, −0.8 mm; ventral, −6.8 mm; angle 16°) according to the atlas of Paxinos and Watson and fixed to the skull with cranioplastic cement. Three to five days after surgery, microdialysis probes (A-I- 8 – 02; 2 mm membrane length; Eicom) were slowly inserted into the nucleus accumbens through guide cannulas during anesthesia with diethyl ether, and the rats were placed in experimental cages (30 cm wide × 30 cm deep × 30 cm high). The probes were perfused continuously (2 μl/min) with artificial cerebrospinal fluid: 0.9 mM $MgCl_2$, 147.0 mM NaCl, 4.0 mM KCl, and 1.2 mM $CaCl_2$. Outflow fractions were collected every 20 min.

After three baseline fractions were collected from the rat nucleus accumbens, rats were given dronabinol (5 mg/ kg, i.p.) or saline (1 ml/kg) 30 min before treatment with morphine (10 mg/kg, i.p.). Dialysis samples were collected for 180 min after treatment and analyzed by high-performance liquid chromatography (Eicom) with electrochemical detection (Eicom). Dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and Homovanillic

acid (HVA), were separated by column chromatography and identified and quantified by the use of standards, as describe previously (Narita et al., 2006).

To measure the levels of dopamine and its metabolites under neuropathic pain, we produced a partial sciatic nerve injury under deep anesthesia with isoflurane by tying a tight ligature with a 8-0 silk suture around approximately one-third to one-half the diameter of the sciatic nerve on the right side (ipsilateral side) under a light microscope (SD30, Olympus, Tokyo, Japan) as described previously (Seltzer et al., 1990). In sham-operated animals, the nerve was exposed without ligation.

Evaluation of the emetic response

We previously reported that morphine (0.1, 0.3, and 0.6 mg/kg, s.c.) induced either of retching or vomiting in a dose-dependent manner in ferrets (Shiokawa et al., 2007). Therefore, in the present study, emesis in ferrets after the administration of morphine (0.6 mg/kg, s.c.) was evaluated by counting the number of retching and vomiting behaviors, as described elsewhere (Torigoe et al., 2012), where retching was defined as a rhythmic abdominal contraction without expulsion and vomiting was the oral expulsion of solid or liquid from the gastrointestinal tract. Emesis was assessed for 30 min after the injection of morphine. To determine the effect of dronabinol on morphine-induced emesis, groups of ferrets were pretreated with dronabinol 30 min before the injection of morphine.

An interval of at least 7 days was allowed between testing for each animal to allow for drug washout and to minimize the development of tolerance.

Locomotor activity

The locomotor activity of mice was measured by an ambulometer as described previously (Narita et al., 1993). Briefly, a mouse was placed in a tilting-type round activity cage 20 cm in diameter and 19 cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the mouse, was detected by three microswitches. Total activity counts in each 10-min segment were automatically recorded for 180 min following the administration of saline or morphine (20 mg/kg, s.c.). Dronabinol (0.3, 1.0, and 3.0 mg/kg, s.c.) was administered 15 min prior to morphine treatment.

Drugs

The drugs used in the present study were morphine hydrochloride (Daiichi-Sankyo, Tokyo, Japan) and dronabinol (Lipomed, Switzerland). Morphine and dronabinol were dissolved in saline. All drugs were administered in a volume of 0.25 ml/kg (ferret), 1.0 ml/kg (rats), or 10 ml/kg (mice).

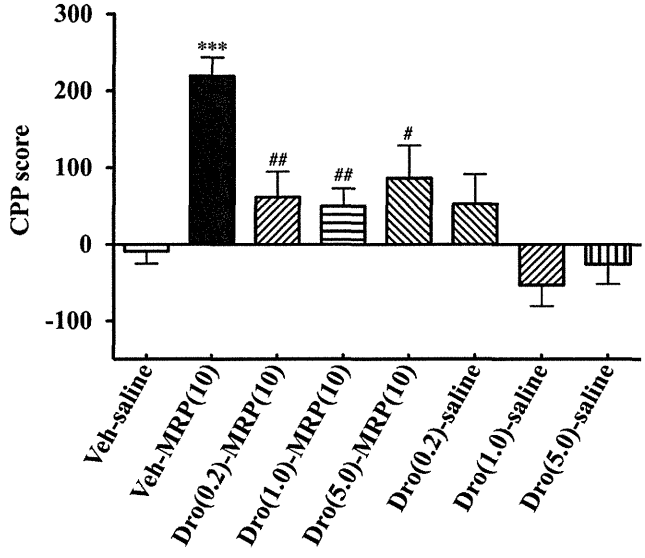

Fig. 1. Effects of dronabinol (DRO) on the morphine (MRP)-induced place-preference in mice. Ordinate: mean differences between time spent in the post-conditioning test and pre-conditioning test. Each column represents the mean ± SEM. of six rats. Statistical analyses were performed using one-way ANOVA followed by the Bonferroni multiple comparisons test: $F(7,62) = 19.7$, $P < 0.0001$. ***$P < 0.001$ vs. vehicle-saline. #$P < 0.05$, ##$P < 0.01$ vs. vehicle-morphine.

Statistical analysis

Data are expressed as the mean with SEM. The statistical significance of differences between groups was assessed by one-way and two-way ANOVA followed by the Bonferroni multiple comparisons test or Student t test (unpaired, two-tailed). All statistical analyses were performed using Prism software (version 5.0a, GraphPad Software). A P value of <0.05 was considered to reflect significance.

RESULTS

Consistent with our previous results with morphine using the same procedure (Suzuki et al., 1991), morphine (5.0 mg/kg, s.c.) produced a robust place-preference in rats. A previous report showed that 10 mg of dronabinol do not produce subjective effects, not even euphoria (Levin and Kleber, 2008). Dronabinol (0.2 – 5.0 mg/kg) did not produce either a place-preference or place-aversion in this study. Under these conditions, pretreatment with dronabinol (0.2–5.0 mg/kg, s.c.) significantly suppressed the rewarding effects induced by morphine (Fig. 1). To confirm that the mesolimbic dopaminergic system is involved in the suppression of morphine-induced rewarding effects by dronabinol, we also measured the release of dopamine from the nucleus accumbens, a terminal region of the mesolimbic dopaminergic system. Basal extracellular levels of DA, DOPAC, and HVA in the N.Acc were 2.98 ± 0.54 nM, 1.27 ± 0.21 μM, and 0.40 ± 0.09 μM/20min (mean with SEM of five samples), respectively.

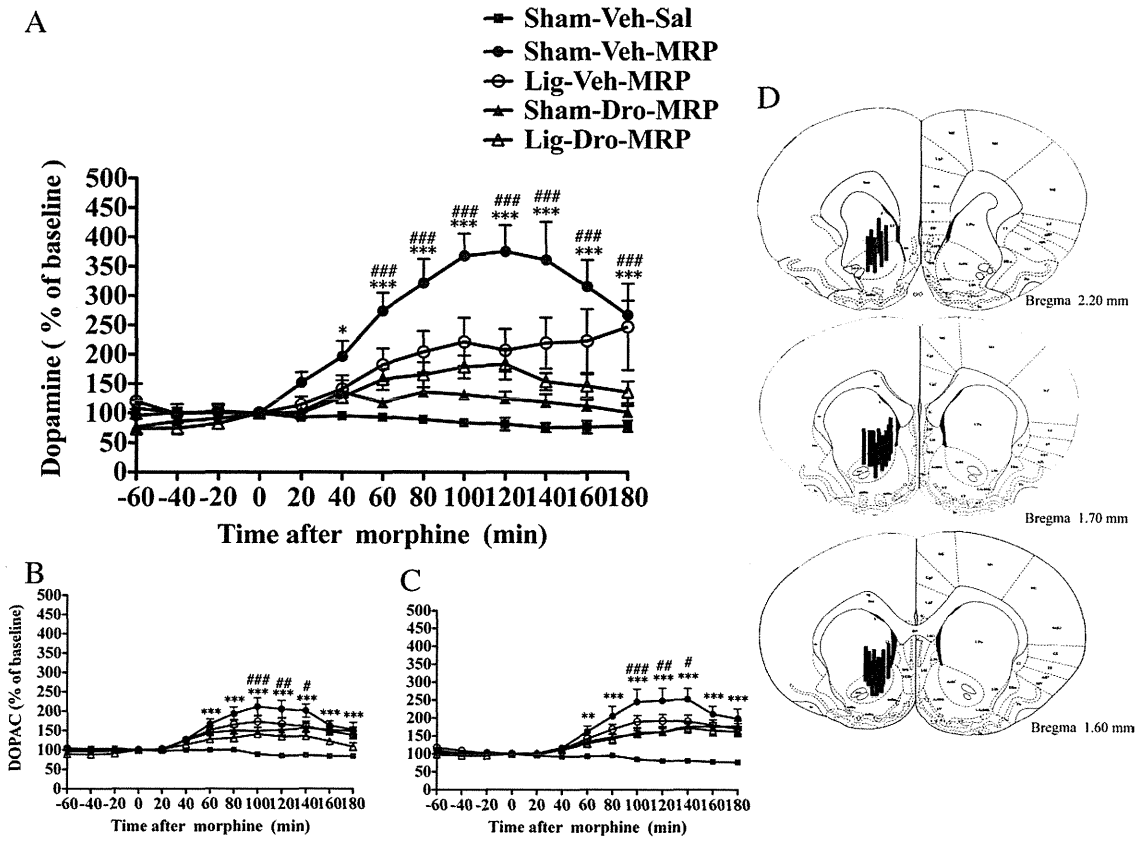

Fig. 2. Effect of dronabinol (DRO) on the influence of morphine (MRP) on the dialysate concentrations of dopamine (**A**) and its metabolites (**B**, **C**) in the nucleus accumbens. After baseline fractions were collected, rats were pretreated with dronabinol (0.3 mg/kg) or vehicle 30 min before the injection of morphine (10 mg/kg) at time 0 to evoke the release of dopamine. Data are expressed as percentages of the corresponding baseline levels with SEM for three to five rats (number of rats: dronabinol-morphine, $n = 5$; vehicle-morphine, dronabinol-saline, $n = 5$; vehicle-saline, $n = 4$). Statistical analyses were performed with two-way ANOVA followed by the Bonferroni multiple comparisons test: vehicle-saline vs. vehicle-morphine, $F(1,104) = 228.8$, $P < 0.0001$ vehicle-saline vs. vehicle-morphine (A), $F(1,91) = 222.2$, $P < 0.0001$ vehicle-saline vs. vehicle-morphine, $F(1,91) = 21.28$, $P < 0.0001$ dronabinol-morphine vs. vehicle-morphine (B), $F(1,91) = 194.5$, $P < 0.0001$ vehicle-saline vs. vehicle-morphine, $F(1,91) = 28.13$, $P < 0.0001$ dronabinol-morphine vs. vehicle-morphine (C). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham-vehicle-saline. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. sham-dronabinol-morphine. Histological representations of probe placement in the nucleus accumbens for experiment 1. Coronal sections are reproduced from Paxinos and Watson (1997) (**D**).

Morphine significantly increased the release of dopamine from the nucleus accumbens, as well as the DOPAC and HVA levels in the nucleus accumbens. Furthermore, we found that pretreatment with dronabinol significantly and almost completely attenuated the morphine-induced increase in dopamine release from the nucleus accumbens, and even reduced the increase in the levels of DOPAC and HVA by morphine (Fig. 2). These results may explain why the rewarding effects of morphine were attenuated by dronabinol.

We previously demonstrated that the morphine-induced rewarding effects and increase in the release of dopamine from the nucleus accumbens is not observed under neuropathic pain due to molecular adaptations (Niikura et al., 2010). To better understand the effects of the morphine-dronabinol combination, we initially examined the effects of co-treatment with morphine and dronabinol on the release of dopamine from the nucleus accumbens. In the present study, the combination of morphine and cannabinoid receptor agonist did not increase the release of dopamine or its metabolites from the nucleus accumbens under neuropathic pain, indicating that neuropathic pain might induce a molecular adaptation. However, the morphine–dronabinol combination does not activate the mesolimbic dopaminergic system. Therefore, the morphine–dronabinol combination is not likely to induce rewarding effects even under neuropathic pain conditions.

We have showed that morphine induces emetic responses (Shiokawa et al., 2007; Torigoe et al., 2012). In the present study, morphine (0.6 mg/kg) produced intense retching (more than 40 times in 30 min) and vomiting. Pretreatment with dronabinol (0.03–0.3 mg/kg, s.c.) 30 min before the injection of morphine (0.6 mg/kg, s.c.) slightly, but not significantly, reduced the number of retches, and significantly reduced the vom-

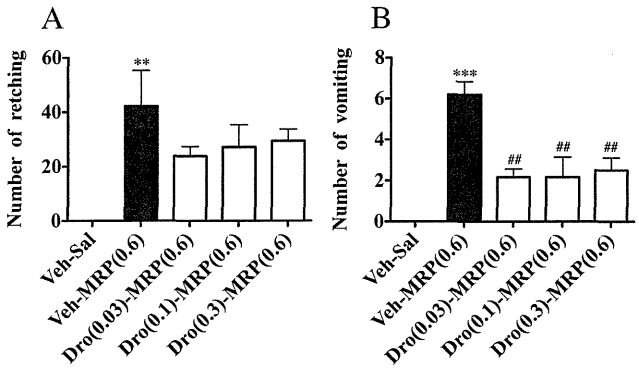

Fig. 3. Effects of dronabinol (DRO) on morphine (MRP) (s.c.)-induced retching (**A**) and vomiting (**B**) in ferrets. Groups of ferrets were pretreated with dronabinol (0.01 and 0.03 mg/kg, s.c.) or vehicle before the administration of morphine (0.6 mg/kg, s.c.). Animals were observed for 30 min after s.c. injection of morphine. Each column represents the mean ± SEM of six ferrets. Statistical analyses were performed using one-way ANOVA followed by the Bonferroni multiple comparisons test: $F(3,23) = 0.9574$, $P = 0.4320$ (A); $F(3,27) = 9.840$, $P = 0.0002$ (B). $^{***}P < 0.001$ vs. vehicle-saline; $^{\#\#}P < 0.01$ vs. vehicle-morphine.

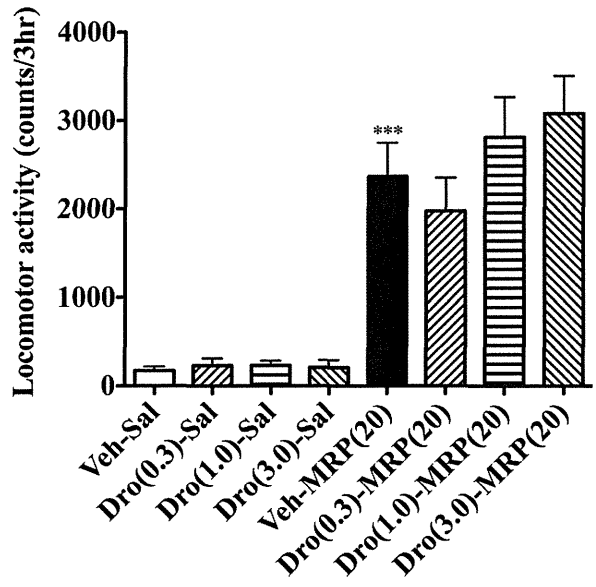

Fig. 4. Effects of dronabinol (DRO) on morphine (MRP)-induced hyperlocomotion in mice. Each column represents the mean total activity for 180 min with SEM of eight mice. $^{***}P < 0.001$ vs. vehicle-saline.

iting behaviors induced by morphine (Fig. 3). During the period of pretreatment with dronabinol, sedation was observed in all ferrets. These results indicate that dronabinol may be useful for the treatment of opioid-induced emesis.

It is well known that the hyperlocomotion and rewarding effects induced by morphine are mediated by activation of the dopaminergic (especially mesolimbic) system. Since the rewarding effects of morphine were significantly attenuated by dronabinol, we expected that morphine-induced hyperlocomotion would be attenuated by dronabinol. In this study, morphine significantly increased locomotor activity. On the other hand, dronabinol (0.3–3.0 mg/kg, s.c.) itself did not significantly affect locomotor activity. Most surprisingly, pretreatment with dronabinol (0.3, 1.0, and 3.0 mg/kg, s.c.) did not affect the hyperlocomotion induced by morphine (10 mg/kg, s.c.) (Fig. 4).

DISCUSSION

Morphine and cannabinoid receptor agonists can induce various pharmacological effects (e.g., antinociceptive effects, changes in locomotor activity, hypothermia, and reinforcing/rewarding effects) that are mediated by μ-opioid and cannabinoid CB1 receptors, respectively. μ-Opioid and cannabinoid receptors are co-distributed in the area of the mesolimbic dopaminergic system that controls their rewarding effects. It has been demonstrated that several behavioral effects induced by cannabinoid receptor agonists at least partially depend on the activation of endogenous opioid systems, indicating that there is cross-talk between the endogenous cannabinoid and opioid systems (Tanda and Goldberg, 2003). μ-Opioid and cannabinoid receptor agonists can positively regulate reinforcing effects and drug-seeking and discriminative stimulus effects in humans and animals, respectively (Tanda and Goldberg, 2003). Biological and molecular biological evidence suggests that cannabinoid receptors form a heterodimer with μ-opioid receptors. In the nucleus accumbens, μ-opioid receptor and cannabinoid CB1 receptors are colocalized with each other and synergistically regulate the release of N-methyl-D-aspartic acid (Pickel et al., 2004). In the present study, we found that dronabinol, which did not produce rewarding or aversive effects, abolished the rewarding effects of morphine in rats. The increase in the release of dopamine from the nucleus accumbens by morphine is thought to be associated with its dopamine-related side effects, such as reinforcing/rewarding effects. We found that the systemic administration of dronabinol alone did not affect the release of dopamine or its metabolites from the nucleus accumbens, and the morphine-induced increase in the release of dopamine and its metabolites from the nucleus accumbens was significantly attenuated by dronabinol. These results indicate that dronabinol may negatively regulate dopamine-related side effects by regulating the dopaminergic system.

A previous study showed that the cannabinoid receptor agonist WIN55,212-2 can attenuate the percentage of ferrets that exhibit morphine-induced vomiting, but not the percentage of ferrets that exhibit morphine-induced retching (Simoneau et al., 2001). In the present study, dronabinol significantly attenuated morphine-induced vomiting, but only slightly attenuated retching in ferrets. The suppression of vomiting could dramatically improve the quality of life of patients suffering from opioid-induced emesis. These

results may support our finding that dronabinol attenuates morphine-induced dopamine-related side effects, although there is some variation regarding each effect.

A growing body of evidence has demonstrated that the combination of μ-opioid receptor agonist and cannabinoid receptor agonist has at least two consequences; either the pharmacological effects of μ-opioid receptor agonist can be enhanced by cannabinoid receptor agonist, or they can be attenuated. However, the exact mechanism by which dronabinol attenuates the morphine-induced increase in dopamine release from nerve terminals of the mesolimbic dopaminergic system remains unclear. One possible mechanism is the inhibition of μ-opioid receptor-mediated function by the activation of cannabinoid receptors through receptor–receptor interactions. It is widely believed that the disinhibition of GABAnergic neurons in the mesolimbic dopaminergic system by morphine plays an important role in the expression of the reinforcing/rewarding effects of morphine. CB1 receptors are localized in the GABAnergic system, like μ-opioid receptors (Kano et al., 2009). The activation of G-protein by morphine was attenuated by cannabinoid receptor agonist in cells expressing μ- and CB1 receptors (Rios et al., 2006). Furthermore, the endogenous as well as exogenous co-expression of μ-opioid and cannabinoid receptors reciprocally inhibited G-protein-mediated activation of MAPK by regulating G-protein functional changes followed by inhibition of the MAPK signaling pathway (Rios et al., 2006). These changes in the signaling pathway might be involved in the suppression of the morphine-induced increase in dopamine release from the nucleus accumbens by dronabinol.

There has been some concern that the abuse of and addiction on opioids may be promoted by the inappropriate use, misuse, or overdose of prescription opioids (Woodcock, 2009). Even though μ-opioid receptor agonists have abuse and/or addictive potential, clinical studies have shown that when opioid receptor agonists are appropriately used to control pain, actual abuse or addiction does not usually occur (Eisenberg et al., 2005). We also showed that the abuse potential of morphine and the morphine-induced increase in the release of dopamine from the nucleus accumbens were suppressed under chronic pain states in rodents (Niikura et al., 2010; Suzuki et al., 1996). In the present study, dronabinol did not affect dopamine release from the nucleus accumbens in the presence of morphine under pain, indicating that activation of the mesolimbic dopaminergic system is also reduced by the co-administration of morphine and dronabinol under a pain state. On the other hand, a previous report showed that THC enhances the hyperlocomotion induced by morphine in mice (Ayhan et al., 1979). However, dronabinol did not affect the hyperlocomotion induced by morphine. These results indicate that dronabinol affects the locomotor activity induced by morphine through some mechanism that is different from that with THC. In addition, the effect of dronabinol on the hyperlocomotion induced by morphine also relied on a mechanism that was different from that for its effect on morphine's rewarding effects. With regard to this discrepant result, a high dose of morphine is required to induce robust hyperlocomotion, unlike other behavioral effects such as antinociceptive, rewarding, and emetic effects. Furthermore, an increase in locomotor activity was not observed (even sedation was observed), when dronabinol (2 mg/kg) was co-administered with morphine (3 mg/kg) (unpublished observation).

In conclusion, dopamine receptor antagonists have commonly been used to attenuate the adverse reactions to opioids such as emesis, delusions, and hallucinations; however, they also have adverse effects, like extrapyramidal symptoms. Dronabinol had attenuating effects (albeit with differences in potencies) on morphine-induced dopamine-related behaviors such as rewarding and emetic effects, and could enhance the antinociceptive effects of morphine (unpublished observation). These results provide evidence that dronabinol could be beneficial as an adjuvant for μ-opioid receptor agonists when used for pain control.

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SYNAPSE 66:174–179 (2012)

Olanzapine Suppresses the Rewarding and Discriminative Stimulus Effects Induced by Morphine

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KEY WORDS olanzapine; morphine; abuse potential; dopamine; discriminative stimulus effect

ABSTRACT Atypical antipsychotic medications are effective for treating both the positive and negative symptoms of schizophrenia. Olanzapine is an atypical antipsychotic that blocks dopaminergic, serotonergic, adrenergic, histaminergic, and muscarinic receptors. In this study, we used rodents to investigate whether olanzapine could suppress the hyperlocomotion, rewarding effect, and discriminative stimulus effect induced by the prototypic μ-opioid morphine, which are all considered to reflect the abuse potential or psychoactive effects of μ-opioids. Olanzapine at doses that failed to induce motor coordination produced a dose-dependent reduction in hyperlocomotion induced by morphine in mice. Olanzapine at a dose that did not produce motor dysfunction also inhibited the significant place preference induced by morphine in mice. Furthermore, the discriminative stimulus effect induced by morphine in rats was dose-dependently and significantly attenuated by olanzapine at the dose that did not induce the motor dysfunction. These results suggest that treatment with both μ-opioids and olanzapine at a dose lower than that at which it induces motor dysfunction could be very useful for preventing the abuse potential and/or psychoactive effects of μ-opioids. **Synapse 66:174–179, 2012.**

INTRODUCTION

Morphine has been used as a "gold standard" for the treatment of patients who suffer moderate to severe cancer pain (WHO, 1996). When morphine is used to treat pain, the adverse effects of opioids, such as emesis, constipation, drowsiness, hallucination, and delirium must be reduced to improve the quality of life of the patient. Against this background, antipsychotic drugs, many of which are dopamine receptor antagonists, have been used to reduce opioid-induced emesis and other dopamine-related side effects, including hallucination and delirium. However, the adverse effects of antipsychotic drugs, such as extrapyramidal symptoms, can be a disadvantage of their use (Swegle and Logemann, 2006).

Ever since chlorpromazine was shown to have an antipsychotic effect more than 50 years ago, the number of antipsychotic medications has continued to grow. Typical or first-generation antipsychotic drugs such as chlorpromazine and haloperidol are effective for the treatment of delusions and hallucinations, while such compounds have a high risk of adverse extrapyramidal side effects (Barnes and McPhillips, 1998). Atypical or second-generation antipsychotic drugs, especially chlorpromazine derivatives such as clozapine and olanzapine, have been developed and used for the treatment of dopamine-, serotonin-, acetylcholine-, and/or histamine-related symptoms and show a relative lack of extrapyramidal side effects (Kumar and Sachdev, 2009). Opioid-induced changes in emotionality and multiple physiological functions

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are likely to result from the regulation of multiple neurotransmitters.

Olanzapine is an atypical thienobenzodiazepine antipsychotic that blocks dopaminergic, serotonergic, adrenergic, histaminergic, and muscarinic receptors for multiple neurotransmitters. We previously demonstrated that olanzapine clearly showed affinities for these receptors in the rodent brain and was effective for the treatment of morphine-induced emesis, for reducing neuropathic pain, and for improving pain-related sleep disturbance (Torigoe et al., in press). We proposed that olanzapine could be used as a single adjunct agent against a background of increasing concern about "polypharmacy" and can be given in a state-dependent dose, which should improve the quality of life for patients while greatly reducing the side effects of opioids.

In this study, we used rodents to further investigate whether olanzapine could suppress the hyperlocomotion, rewarding effect, and discriminative stimulus effect induced by morphine, which are all considered to reflect the abuse potential or psychoactive effects of μ-opioids.

MATERIALS AND METHODS

Animals

In this study, male Institute of Cancer Research (ICR) mice (20–25 g) (Tokyo Laboratory Animals Science Co., Tokyo, Japan) and seven male Fischer 344 rats (Charles River Japan, Atsugi, Japan) maintained at 200–230 g (80% free-feeding weight) were used. Food and water were available *ad libitum* for mice, and water was available *ad libitum* for all of the rats in their individual home cages. Animals were housed in a room maintained at 22 ± 1°C with a 12-h light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments.

Rota-rod assay

To determine the doses of olanzapine to be tested, which would not affect animal behavior, we first used a rota-rod assay. The motor coordination produced by olanzapine in mice was evaluated in terms of the time until they fell from a rota-rod at 8 rpm (KN-95; Natsume Seisakusyo, Co., Tokyo, Japan). The apparatus consists of a rotating acrylic rod (3 cm in diameter) divided into four 8-cm sections by circular Plexiglas dividers (18-cm high). First, mice were trained to be able to walk on the rota-rod for 180 s. This training was performed for 3 days. On the day after the final training session, the time until the mouse fell from the rota-rod was measured for up to 180 s at 30 min after the administration of olanzapine (0.03–1 mg/kg, s.c.). In addition, any behavioral changes were also observed by comparison with the vehicle control.

Locomotor activity

The locomotor activity of mice was measured by an ambulometer as described previously (Narita et al., 1993). Briefly, a mouse was placed in a round tilting-type activity cage 20 cm in diameter and 19-cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the mouse, was detected by three microswitches. The total activity counts in each 10-min segment were automatically recorded for 180 min following the administration of saline or morphine (10 mg/kg, s.c.). Olanzapine (0.03, 0.1, and 0.3 mg/kg, s.c.) was administered 30 min prior to treatment with morphine.

Place conditioning

Place-conditioning studies were conducted using a shuttle box (15 cm × 30 cm × 15 cm: $w \times l \times h$) composed of an acrylic resin board divided into two equal-sized compartments (Suzuki et al., 1991). One compartment was white with a textured floor, and the other was black with a smooth floor to create equally inviting compartments. The place-conditioning schedule consisted of three phases (preconditioning test, conditioning, and postconditioning test). The preconditioning test was performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and mice that had not been treated with either drugs or saline were then placed on the platform. The time spent in each compartment during a 900-s session was then recorded automatically using an infrared beam sensor (KN-80, Natsume Seisakusyo Co., Tokyo, Japan). Conditioning sessions (three for morphine: three for saline) were started the day after the preconditioning test and conducted once daily for 6 days. Mice were pretreated with olanzapine (0.1 or 0.3 mg/kg, s.c.) or vehicle 30 min before the injection of morphine or saline. Immediately after the injection of morphine (5 mg/kg, s.c.), the animals were placed in the compartment opposite that in which they had spent the most time in the preconditioning test for 1 h. On alternate days, the animals were treated with saline after pretreatment with olanzapine or vehicle and placed in the other compartment for 1 h. On the day after the final conditioning session, a postconditioning test that was identical to the preconditioning test was performed.

Drug discrimination procedure

Experiments were conducted in operant-conditioning chambers (model ENV-008; Med Associates, St.

Albans, VT) equipped with two levers and a food cup mounted midway between the levers. White lamps were installed above each of the levers. White noise was used to mask extraneous sound. Reinforcement consisted of 45-mg food pellet (Bio-Serv, Frenchtown, NJ).

Discrimination training was performed as described previously (Mori et al., 2002). Briefly, before they were trained to discriminate between drugs and saline, all rats were trained to press a lever. Training began under a reinforcement schedule of fixed ratio 1 (FR 1), 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. The FR requirement for food reinforcement was gradually increased to a value of 10. After the response rates had stabilized under FR 10, rats were trained to discriminate between 3.0 mg/kg of morphine and saline. In the discrimination training, training morphine (D) and saline (S) were administered in a session-to-session sequence of DDSS (double alternation schedule), and the assignment of left and right levers to drug and saline states was counterbalanced. Rats were required to respond on the stimulus-appropriate lever to obtain reinforcement; there were no programmed consequences for responding on the incorrect lever. Substitution tests were only performed after the discrimination criterion described below had been satisfied for at least five consecutive daily discrimination-training sessions (accuracy of at least 83% and fewer than 12 responses to obtain the first reinforcement).

After the animals attained the criterion, a dose-response test was initiated; test sessions were performed only when FRF $\leq$ 12 for at least three consecutive discrimination training sessions. In the dose-response test, rats were placed in the operant box until they had made 10 responses on either lever or 5 min had elapsed. In the combination test, doses of olanzapine that produced <20% drug-lever responses and did not affect the response rates in the generalization tests were used in generalization tests. The pretreatment time and doses of drugs used were 30 min and 0.03–0.3 mg/kg of olanzapine. 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 morphine hydrochloride (Daiichi-Sankyo, Tokyo, Japan) and olanzapine (Toronto Research Chemicals, Toronto, Ontario, Canada). Morphine was dissolved in saline, and olanzapine was dissolved in DMSO: Tween80: saline (5:5:90). All drugs were administrated in a volume of 1.0 ml/kg (rats) or 10 ml/kg (mice).

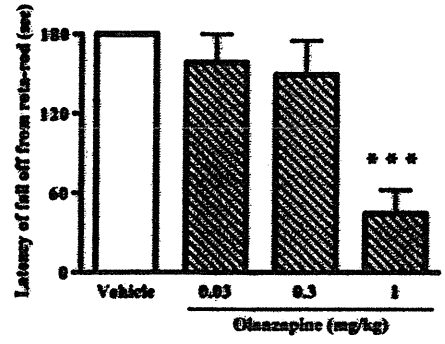

Fig. 1. Motor coordination produced by olanzapine (0.03, 0.3, and 1 mg/kg, s.c.) in mice. The motor coordination produced by olanzapine was evaluated in terms of the amount of time until the mouse fell from a rota-rod at 8 rpm. Each column represents the mean ± S.E.M. of 6 mice. ***$P < 0.001$ vs. vehicle.

Statistical analysis

Data are expressed as the mean with S.E.M. The statistical significant of differences between the groups was assessed by a one-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparisons test or two-tailed paired *t*-test. All statistical analyses were performed using Prism software (version 5.0a, GraphPad Software, La Jolla, CA). A P value of < 0.05 was considered to reflect significance.

RESULTS

Motor coordination produced by olanzapine in the mouse rota-rod assay

In the rota-rod test, olanzapine (1 mg/kg, s.c.) produced significant motor impairment in mice compared with vehicle-treated mice ($F(3,23) = 10.19$, $P < 0.0001$), whereas olanzapine at doses of 0.03–0.3 mg/kg did not induce motor dysfunction (Fig. 1).

Suppression of morphine-induced hyperlocomotion by pretreatment with olanzapine at doses that failed to induce motor dysfunction

A single injection of morphine at 10 mg/kg (s.c.) produced a significant increase in locomotion. Pretreatment with olanzapine at 0.03, 0.1, and 0.3 mg/kg (s.c.) induced a dose-dependent suppression of this morphine-induced hyperlocomotion, and these effects of olanzapine were significant at all of the doses tested ($P < 0.05$ for 0.03, $P < 0.01$ for 0.1, $P < 0.001$ for 0.3 vs. vehicle pretreatment) (Fig. 2A). In contrast, locomotion activity was not changed by olanzapine itself at dose of 0.3 mg/kg with saline.

Inhibition of morphine-induced place preference by pretreatment with olanzapine at a dose that did not induce motor impairment

Consistent with our previous results with morphine using the same procedure, morphine (5 mg/kg, s.c.)