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Effects of osemozotan, ritanserin and azasetron on cocaine-induced behavioral sensitization in mice

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Abstract

Repeated intermittent administration of psychostimulants causes behavioral sensitization in rodents. Previous studies using serotonin (5-HT) receptor ligands show that the 5-HT system is involved in cocaine-induced behavioral sensitization in rats, but the role of the 5-HT system has not been studied in mice. The present study examined the effects of the 5-HT_{1A} receptor agonist osemozotan, the 5-HT_2 receptor antagonist ritanserin and the 5-HT_3 receptor antagonist azasetron on cocaine-induced behavioral sensitization in male ddY mice. Repeated administration of cocaine for 7 days enhanced cocaine-induced locomotor activity, and this sensitization was observed even after withdrawal for 7–14 days. Cocaine-induced behavioral sensitization after a 7-day withdrawal was significantly reduced with the coadministration of osemozotan, ritanserin or azasetron with cocaine repeatedly for 7 days. A single injection of osemozotan or ritanserin before cocaine challenge also reduced repeated cocaine-induced behavioral sensitization. However, none of these ligands inhibited cocaine-induced behavioral sensitization, when each drug was administered for 7 days after repeated cocaine administration. These results suggest that the central 5-HT system plays a role in the development and expression, but not maintenance, of behavioral sensitization in cocaine-treated mice.

Keywords: Cocaine; Behavioral sensitization; Serotonin (5-HT) receptor subtypes; Osemozotan; Ritanserin; Azasetron; Mouse

1. Introduction

Repeated intermittent administration of cocaine can enhance the stimulating effect on locomotor activity, a phenomenon called behavioral sensitization. This behavioral model has been used extensively to analyze the neural modification associated with repeated cocaine exposure and withdrawal (White and Kalivas, 1998). The central dopamine (DA) system plays a crucial role in the stimulating effect of cocaine, and dopaminergic neurons have been evaluated as a neural substrate mediating behavioral sensitization to psychostimulants (Kalivas and Stewart, 1991; Vanderschuren and Kalivas, 2000). However, the serotonin (5-HT) system has also been shown to be involved in the processes that underlie behavioral sensitization. Parsons and Justice (1993) reported that repeated cocaine administration enhances the capacity of a subsequent cocaine injection to elevate extracellular 5-HT in the accumbens. In addition, cocaine-induced locomotor sensitization is modulated by a 5-HT_{1A} receptor agonist (De La Garza and Cunningham, 2000; Szumlinski et al., 2004), 5-HT_{1B} receptor ligands (Przegalinski et al., 2002, 2004), a 5-HT_2 receptor antagonist (Davidson et al., 2002; Filip et al., 2004) and 5-HT_3 receptor antagonist (King et al., 1997, 2000). However, these studies were carried out in rats. With respect to the evidence that there is a species difference pertaining to the behavioral effects that the 5-HT_{1A} receptor agonist has on rats and mice (Blanchard et al., 1997; Dulawa and Geyer, 2000; Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995), it seems important to confirm the roles of central 5-HT system in cocaine-induced behavioral sensitization in mice, specially.

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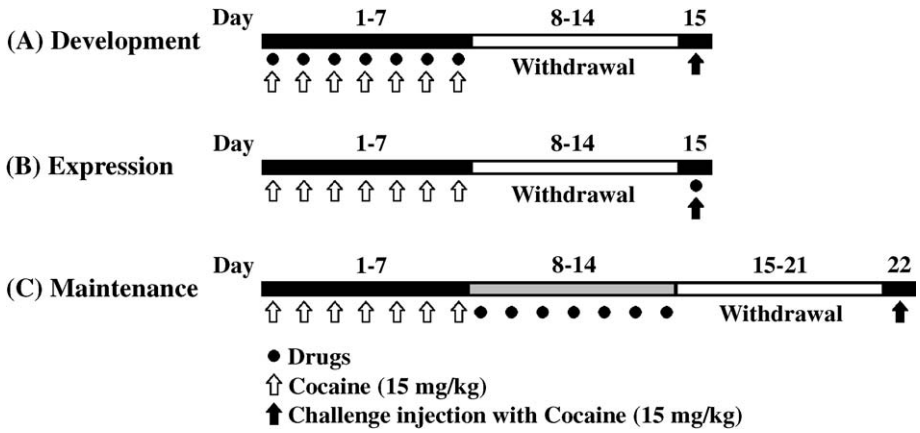

Fig. 1. Experimental design for the development (A), expression (B), and maintenance (C) of cocaine-induced locomotor sensitization in mice. For the development of the sensitization, mice were coadministered drugs and cocaine (15 mg/kg) repeatedly for 7 days and then challenged with cocaine (15 mg/kg) after a 7-day withdrawal period (on day 15). For the expression of the sensitization, mice were administered cocaine (15 mg/kg) repeatedly for 7 days and then challenged with cocaine (15 mg/kg) on day 15. Drugs were injected 30 min before its challenge dose on day 15. For the maintenance of the sensitization, mice were treated with cocaine (15 mg/kg) repeatedly for 7 days, administered drugs twice daily for 7 days, and challenged with cocaine (15 mg/kg) after a 7-day withdrawal period (on day 22).

The present study examines the effects of some 5-HT receptor ligands on cocaine-induced behavioral sensitization in mice. In this study, we used osemozotan (previously called MKC-242) as a selective 5-HT_{1A} receptor agonist. This compound is about 500-fold and more than 1000-fold more active at the 5-HT_{1A} site than at the 5-HT_{2A} site, and at the 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, α_2-adrenoceptor and DA-D_1 receptor sites, respectively (Matsuda et al., 1995a,b; Abe et al., 1996). This agonist has inhibitory effects on isolation rearing-induced abnormal behaviors such as aggression and prepulse inhibition deficits besides the antidepressant-like and anxiolytic-like effects, but it at the doses used here (0.1–1.0 mg/kg) does not affect locomotion (Matsuda et al., 1995b; Sakaue et al., 2001; Sakaue et al., 2003a,b). In addition, the non-selective 5-HT_2 receptor antagonist ritanserin (Baxter et al., 1995) and the selective 5-HT_3 receptor antagonist azasetron (previously called Y-25130) (Kelley and Hodge, 2003; Szumlinski et al., 2003) were used. Azasetron inhibits the specific [^{3}H]granisetron binding with a Ki value of 0.33 nM (Katayama et al., 1997), suggesting a high affinity for 5-HT_3 receptors, and shows the potent and selective 5-HT_3-receptor antagonistic activity in vitro (Sato et al., 1992) and in vivo (Miyata et al., 1991).

2. Method

2.1. Animals and drugs

Male ddY mice, aged 3 weeks, were housed in cages (24×17×12 cm) in groups of 5 to 6 animals under controlled environmental conditions (22±1 °C; 12:12-h light–dark cycle, lighting at 08:00 h; food and water *ad libitum*) for at least 1 week before use in the experiments. The handling procedures for the animals and their care were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. The following drugs were used: cocaine hydrochloride (Shionogi and Co., Ltd., Osaka, Japan); osemozotan, azasetron and ondansetron (Mitsubishi Pharma Co., Yokohama, Japan); ritanserin (Sigma, St Louis,

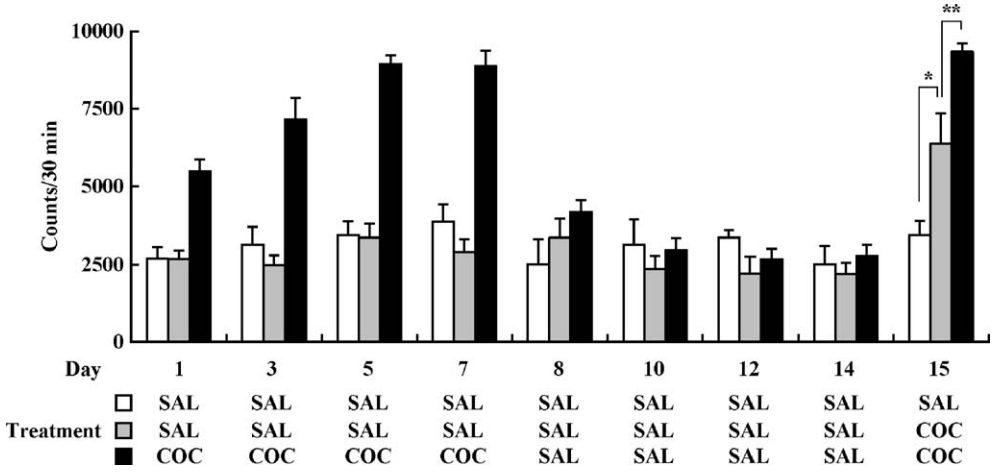

Fig. 2. Effects of single and repeated administration of cocaine on spontaneous locomotor activity in mice. Mice were treated with saline (SAL) or cocaine 15 mg/kg (COC) daily for 7 days (day 1 to day 7), administered SAL daily for 7 days (day 8 to day 14), and challenged with SAL or COC after a 7-day withdrawal period (on day 15). Values are expressed as the mean±S.E.M. of 12 mice. *P<0.05, **P<0.01.

MO, USA). All other commercially available chemicals used in the experiments were of exceptional quality. Cocaine and azasetron were dissolved in saline (0.9% solution of NaCl). Osemozotan and ritanserin were suspended in 0.5% w/v carboxymethylcellulose. The drugs were injected intraperitoneally at 10 ml/kg.

2.2. Induction of behavioral sensitization

Previous studies showed that repeated administration of psychostimulants causes behavioral sensitization in rats when the administration is started at the age of at least 3 weeks (Tirelli et al., 2003; Tsuchida et al., 1994). To induce behavioral sensitization, 4-week-old mice were administered i.p. 15 mg/kg of cocaine once daily for 7 days. The protocol for the experiments to examine the effects of drugs on the development, expression, and maintenance of behavioral sensitization is shown in Fig. 1. At the development period of sensitization, mice were coadministered drugs and cocaine repeatedly for 7 days and were challenged with 15 mg/kg of cocaine on day 15. At the expression period of sensitization, mice were administered i.p. 15 mg/kg of cocaine repeatedly for 7 days and were challenged i.p. with 15 mg/kg of cocaine after a 7-day withdrawal period. Drugs were injected i.p. 30 min before a challenge dose of 15 mg/kg of cocaine on day 15. At the maintenance period of sensitization, mice were treated with 15 mg/kg of cocaine repeatedly for 7 days, administered drugs twice daily for 7 days, and challenged with 15 mg/kg of cocaine after a 7-day withdrawal period (on day 22). We used 649 mice in total experiments, and used different mice for examining the effects of 5-HT receptor ligands on cocaine-induced hyperactivity and locomotor sensitization.

2.3. Measurement of spontaneous locomotor activity

The locomotor activity (including walking and rearing) of mice was measured using a digital counter system with an infrared sensor (Supermex®, Muromachi Kikai, Tokyo, Japan). Each mouse was housed individually in a clear plastic cage (24×17×12 cm) for a 30-min habituation period, was taken out to be injected with the drug, and was returned to the same cage. Locomotor activity was recorded for 30 min immediately after the injection.

2.4. Statistics

In the induction of behavioral sensitization, analysis was made using two-way analysis of variance (ANOVA) for treatment as the intersubject factor and repeated measures with time as the intrasubject factor. In another experiments, values were analyzed using one-way ANOVA followed by Tukey–Kramer test. Statistical analyses were made using the software package Statview 5.0J for Apple Macintosh computer (SAS Institute Inc., Cary, NC, USA). A value of $P<0.05$ was considered statistically significant.

3. Results

Fig. 2 shows that cocaine-induced hyperlocomotion is augmented by repeated administration of cocaine (15 mg/kg, i.p., once daily for 7 days) to mice. Sensitization persisted even

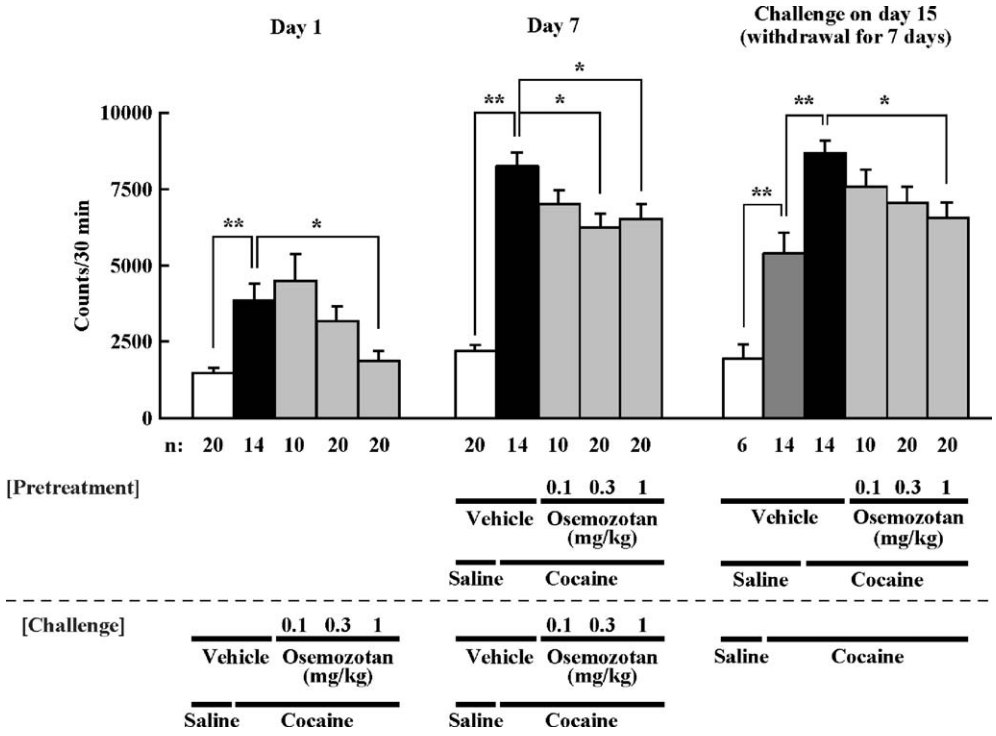

Fig. 3. Effect of osemozotan on the development of cocaine-induced locomotor sensitization in mice. Mice were coadministered vehicle or osemozotan at the indicated doses and saline or cocaine (15 mg/kg) repeatedly for 7 days, and then challenged with cocaine (15 mg/kg) after a 7-day withdrawal period. *n*: the number of mice used. *$P<0.05$, **$P<0.01$.

after a 7-day withdrawal period (day 15 in Fig. 2). Cocaine challenge-induced hyperactivity was significantly increased (day 1 to 7) [F (6, 63)=3.891, P=0.0023], suggesting behavioral sensitization. Mice pretreated with 15 mg/kg of cocaine for 7 days also showed behavioral sensitization to cocaine even after cocaine withdrawal for 7 days (day 15) [F (2, 20)=17.777, P<0.0001], while saline treatment during cocaine withdrawal did not affect the locomotor activity in cocaine-pretreated mice (day 8 to day 14) [F (6, 63)=2.122, *n.s.*].

Fig. 3 shows the effect of osemozotan on the development of behavioral sensitization induced by repeated cocaine administration to mice. A challenge dose of 15 mg/kg of cocaine caused a significant increase in locomotor activity in mice, and this hyperactivity was significantly inhibited by single injection of 1 mg/kg of osemozotan (day 1) [F (3, 63)=4.565, P=0.0060]. Mice were coadministered cocaine and osemozotan repeatedly for 7 days and were challenged with 15 mg/kg of cocaine after a 7-day withdrawal period (Fig. 1A). 1 mg/kg of osemozotan blocked cocaine-induced locomotor responses up to the seventh injection [F (3, 63)=3.360, P=0.0245 for day 7], and behavioral sensitization was also inhibited on the challenge day after a 7-day withdrawal period [F (3, 63)=2.928, P=0.0408]. Fig. 4 shows the effect of osemozotan on the expression of behavioral sensitization induced by repeated cocaine administration to mice. A single injection of osemozotan at doses of 0.1 to 1 mg/kg on the day of challenge (Fig. 1B) inhibited the expression of cocaine-induced locomotor sensitization [F (3, 42)=4.804, P=0.0061]. Fig. 5 shows the effect of osemozotan on the maintenance of behavioral sensitization induced by repeated cocaine administration to mice. Mice were treated first with cocaine for 7 days, then

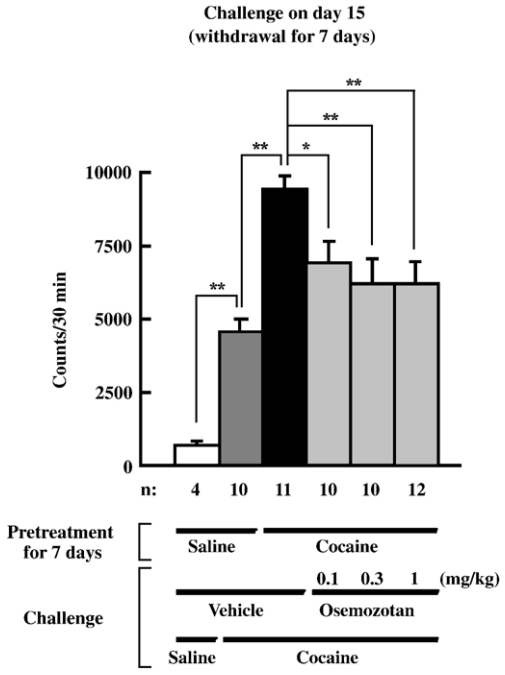

Fig. 4. Effect of osemozotan on the expression of cocaine-induced locomotor sensitization in mice. Mice were administered saline or cocaine (15 mg/kg) repeatedly for 7 days and then challenged with cocaine after a 7-day withdrawal period. Vehicle or osemozotan at the indicated doses was injected 30 min before a challenge dose (15 mg/kg) of cocaine. *n*: the number of mice used. *P<0.05, **P<0.01.

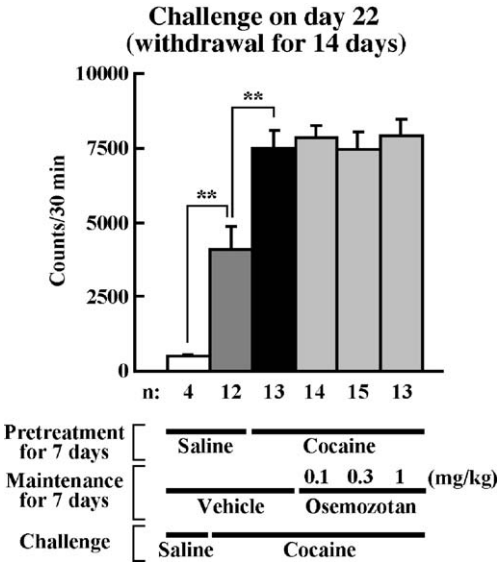

Fig. 5. Effect of osemozotan on the maintenance of cocaine-induced locomotor sensitization in mice. Mice were treated with saline or cocaine (15 mg/kg) repeatedly for 7 days, administered vehicle or osemozotan at the indicated doses twice daily for 7 days, and then challenged with cocaine (15 mg/kg) after a 7-day withdrawal period. *n*: the number of mice used. **P<0.01.

treated with osemozotan twice daily for 7 days, and finally challenged with cocaine after a 7-day withdrawal period (Fig. 1C). Mice pretreated with 15 mg/kg of cocaine for 7 days also showed behavioral sensitization to cocaine even after a 14 day withdrawal of the drug (day 22), whereas osemozotan did not affect cocaine-induced behavioral sensitization [F (3, 54)=0.190, *n.s.*].

To investigate the effects of other 5-HT receptor ligands, we examined the effects of ritanserin, a 5-HT_2 receptor antagonist, and azasetron, a selective 5-HT_3 receptor antagonist, on cocaine-induced locomotor sensitization in mice. Fig. 6 shows the effect of ritanserin on the development, expression and maintenance of behavioral sensitization. Ritanserin at doses of 1 and/or 3 mg/kg inhibited the development [F (3, 63)=2.784, P=0.0485] and expression [F (3, 40)=5.175, P=0.0044], but not maintenance [F (3, 57)=0.921, *n.s.*], of cocaine-induced behavioral sensitization in mice. Fig. 7 shows the effect of azasetron on the development, expression and maintenance of behavioral sensitization. 3 mg/kg of azasetron inhibited the development of behavioral sensitization [F (2, 62)=8.792, P=0.0004], but did not affect the expression [F (2, 55)=0.506, *n.s.*] and maintenance [F (2, 42)=0.390, *n.s.*] of cocaine sensitization. The lack of the effect of 5-HT_3 receptor antagonist on the expression of cocaine-induced behavioral sensitization in mice was also observed using the typical 5-HT_3 receptor antagonist ondansetron (3 mg/kg) (data not shown). Single cocaine-induced hyperactivity was significantly inhibited by 3 mg/kg of ritanserin [F (3, 63)=5.643, P=0.0018] or 3 mg/kg of azasetron [F (2, 62)=10.000, P=0.0002] in mice (data not shown).

4. Discussion

In this study, we did not observe any general depressant effects of osemozotan on behavior in agreement with the previous report

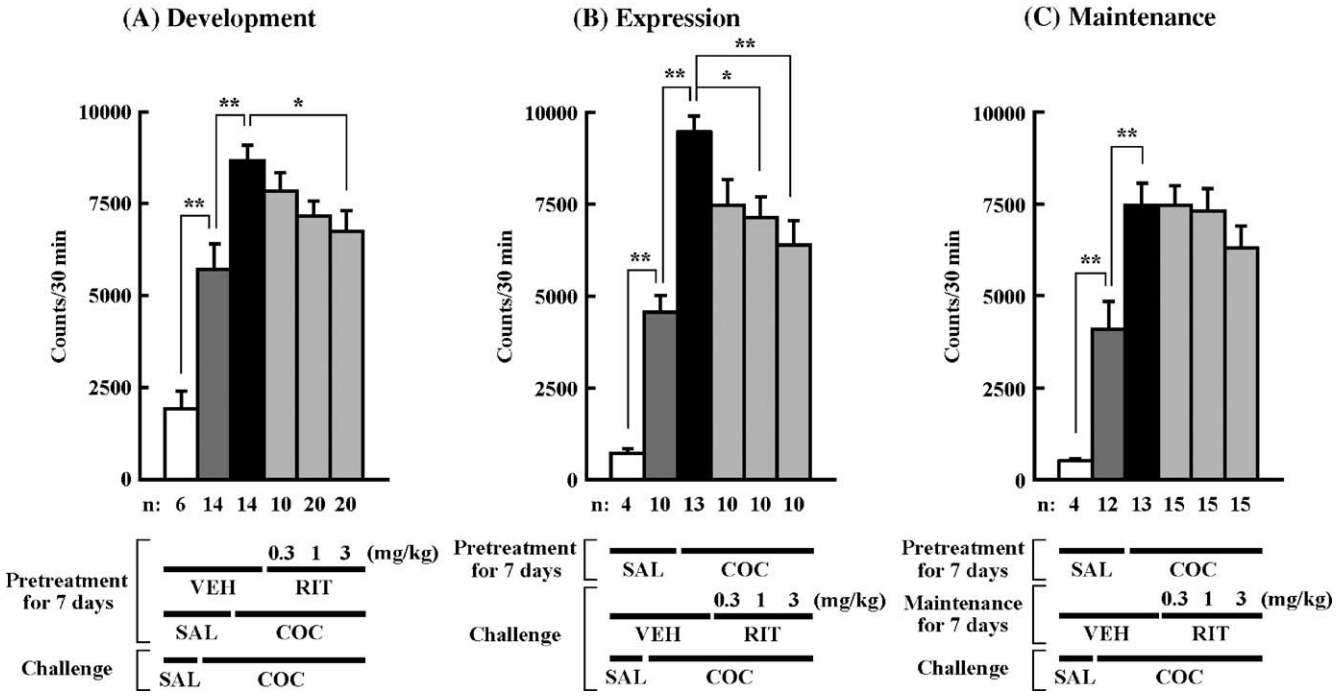

Fig. 6. Effect of ritanserin on the development, expression, and maintenance of cocaine-induced locomotor sensitization in mice. (A) For the development of the sensitization, mice were coadministered vehicle (VEH) or ritanserin (RIT) at the indicated doses and saline (SAL) or cocaine (15 mg/kg) (COC) repeatedly for 7 days, and then challenged with 15 mg/kg of cocaine after a 7-day withdrawal period. (B) For the expression of the sensitization, mice were administered saline (SAL) or cocaine (15 mg/kg) (COC) repeatedly for 7 days and then challenged with cocaine after a 7-day withdrawal period. Vehicle (VEH) or ritanserin (RIT) at the indicated doses was injected 30 min before a challenge dose (15 mg/kg) of cocaine. (C) For the maintenance of the sensitization, mice were treated with saline (SAL) or cocaine (15 mg/kg) (COC) repeatedly for 7 days, administered vehicle (VEH) or ritanserin (RIT) at the indicated doses twice daily for 7 days, and then challenged with 15 mg/kg of cocaine after a 7-day withdrawal period. *n*: the number of mice used. **P*<0.05, ***P*<0.01.

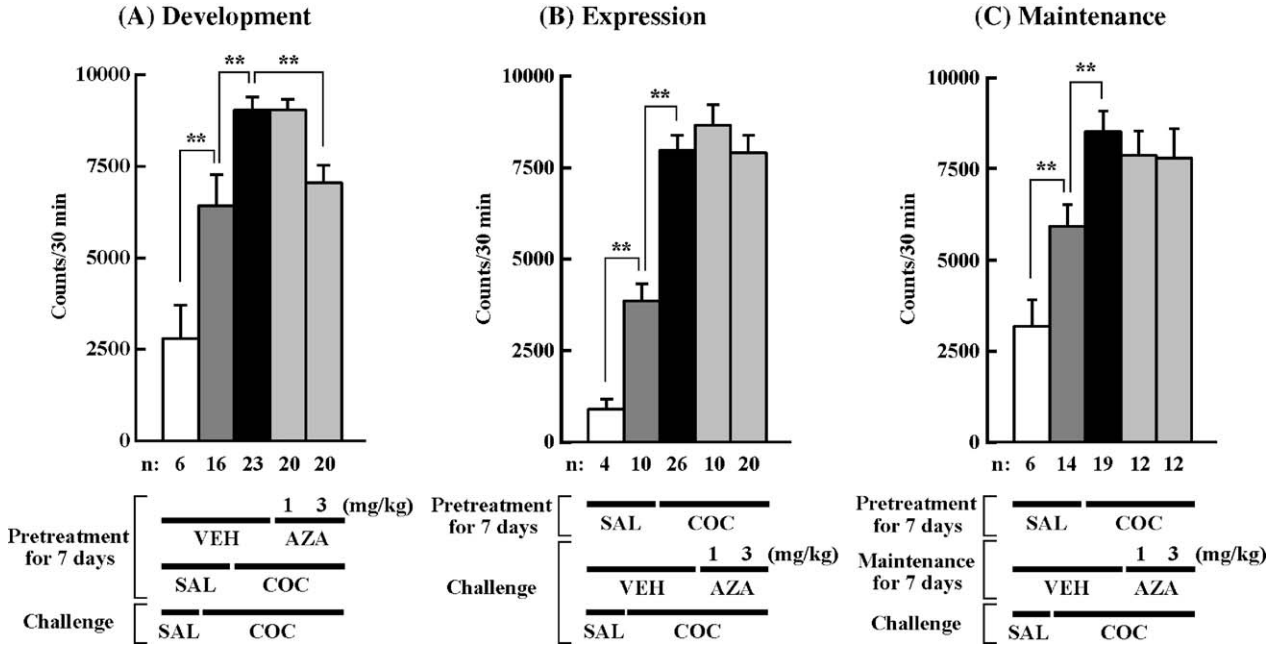

Fig. 7. Effect of azasetron on the development, expression, and maintenance of cocaine-induced locomotor sensitization in mice. (A) For the development of the sensitization, mice were coadministered vehicle (VEH) or azasetron (AZA) at the indicated doses and saline (SAL) or cocaine (15 mg/kg) (COC) repeatedly for 7 days, and then challenged with 15 mg/kg of cocaine after a 7-day withdrawal period. (B) For the expression of the sensitization, mice were administered saline (SAL) or cocaine (15 mg/kg) (COC) repeatedly for 7 days and then challenged with cocaine after a 7-day withdrawal period. Vehicle (VEH) or azasetron (AZA) at the indicated doses was injected 30 min before a challenge dose (15 mg/kg) of cocaine. (C) For the maintenance of the sensitization, mice were treated with saline (SAL) or cocaine (15 mg/kg) (COC) repeatedly for 7 days, administered vehicle (VEH) or azasetron (AZA) at the indicated doses twice daily for 7 days, and then challenged with 15 mg/kg of cocaine after a 7-day withdrawal period. *n*: the number of mice used. ***P*<0.01.

(Abe et al., 1996). Osemozotan (0.1–1 mg/kg) does not cause any behavioral change or motor dysfunction, although a higher dose (2–5 mg/kg) of osemozotan induces typical 5-HT syndrome behaviors. These postsynaptic 5-HT_{1A} receptor-mediated 5-HT syndrome behaviors induced by osemozotan seem to be smaller than those by 8-hydroxy-2(di-*n*-propylamino)tetraline (8-OH-DPAT) (Matsuda et al., 1995a). The present results demonstrate that the selective 5-HT_{1A} receptor agonist osemozotan inhibits the development and expression of cocaine-induced behavioral sensitization in mice. This finding appears to contrast with the previous observation that 8-OH-DPAT, a 5-HT_{1A} receptor agonist, enhances the development and expression of behavioral sensitization in rats (De La Garza and Cunningham, 2000). This discrepancy may be due to the difference in method for analysis of behavior. We measured a global horizontal activity, while the previous study analyzed a horizontal activity in the periphery and center of the open field test enclosure, as well as vertical activity. However, they did not observe any inhibitory effect of 8-OH-DPAT on cocaine sensitization in any measurements. In addition, Szumlinski et al. (2004), using a global horizontal movement analysis, have recently found that the intraraphe microinjection of 8-OH-DPAT augmented cocaine sensitization in rats. Therefore, the inconsistent result we have determined is likely due to species difference in the role of 5-HT_{1A} receptor activation in cocaine-induced behavioral sensitization between rats and mice. Alternatively, possible differences in pharmacological selectivity beside 5-HT_{1A} receptors between osemozotan and 8-OH-DPAT may contribute to the discrepant results between the previous and present studies. Although both compounds are highly selective 5-HT_{1A} receptor agonist, they differ in efficacy at postsynaptic 5-HT_{1A} receptors. We showed that osemozotan and 8-OH-DPAT had similar efficacy at presynaptic 5-HT_{1A} receptors, whereas the former had less efficacy than the latter at postsynaptic 5-HT_{1A} receptors (Matsuda et al., 1995a). That is, the postsynaptic 5-HT_{1A} receptor-mediated 5-HT syndrome behaviors induced by osemozotan are smaller than those by 8-OH-DPAT. This suggests that osemozotan acts as a full and partial agonist at pre- and postsynaptic 5-HT_{1A} receptors, respectively. However, it does not appear that the partial activity at postsynaptic 5-HT_{1A} receptors may explain for the opposite effect of osemozotan observed in mice. The species difference was also observed in the effects of the 5-HT_{1A} receptor agonists on the acute locomotor stimulant effects of cocaine. The present study shows that osemozotan inhibits cocaine-induced hyperactivity in mice, while the previous studies show that 8-OH-DPAT enhances the locomotor stimulant effects of cocaine in rats (Carey et al., 2002, 2004; De La Garza and Cunningham, 2000; Müller et al., 2003).

The present study also shows that the 5-HT_2 and 5-HT_3 receptors play a role in cocaine-induced behavioral sensitization in mice, and their antagonists inhibit the acute locomotor stimulant effect of cocaine in mice. Davidson et al. (2002) reported that ketanserin, a relatively non-selective 5-$HT_{2A/2C}$ receptor antagonist, inhibited the development of cocaine-induced behavioral sensitization in rats. In contrast, Filip et al. (2001) reported that ketanserin did not inhibit the development of cocaine-induced behavioral sensitization in rats, although it inhibited the expression of such sensitization. This apparent discrepancy may be due to the differences in procedure for induction of cocaine sensitization. In addition, the use of non-selective 5-HT_2 receptor antagonist may be related to the discrepant results between the studies by Davidson et al. (2002) and Filip et al. (2001), since the functions of 5-HT_{2A} and 5-HT_{2C} receptors are considered to be opposite (Filip et al., 2004). We found that ritanserin inhibited the development and expression of cocaine-induced behavioral sensitization in mice. In view of the evidence that ritanserin is a non-selective 5-HT_2 receptor antagonist, it appears that the involvement of 5-HT_2 receptors in cocaine-induced behavioral sensitization is similar between the rats and mice. Concerning 5-HT_3 receptors, King et al. (1997, 1998, 2000) reported that ondansetron, a 5-HT_3 receptor antagonist, inhibited the development, expression and maintenance of cocaine-induced behavioral sensitization in rats. We also observed the inhibitory effect of azasetron on the development of cocaine-induced behavioral sensitization in mice, but we did not observe any inhibitory effect of the 5-HT_3 receptor antagonist on the expression and maintenance. Concerning the effects of 5-HT receptor ligands on the maintenance (Fig. 1C), the experiments were carried out 7 days after treatment with 5-HT receptor ligands, to examine whether the possible improvement by the ligands is observed after the withdrawal. However, none of 5-HT receptor ligands affected the maintenance of cocaine-behavioral sensitization under the conditions. The lack of the effect may be related to the withdrawal period. Further studies under the different conditions are necessary to clarify the role of 5-HT system in the maintenance of the sensitization.

The behavioral sensitization associated with repeated cocaine administration is most frequently associated with neuroadaptation in mesocorticolimbic DA and corticofugal glutamate transmission (Everitt and Wolf, 2002; Vanderschuren and Kalivas, 2000; Wolf, 1998). In addition, previous studies in rats show that repeated cocaine-induced behavioral sensitization is modulated by the 5-HT_{1A} receptor agonist (De La Garza and Cunningham, 2000; Szumlinski et al., 2004), 5-HT_{1B} receptor ligands (Przegalinski et al., 2002, 2004), 5-HT_2 receptor antagonist (Davidson et al., 2002; Filip et al., 2001, 2004) and 5-HT_3 receptor antagonist (King et al., 1997, 1998, 2000). These results suggest that the central 5-HT system plays a role in cocaine-induced behavioral sensitization. The present finding is consistent with this hypothesis, although the effect of the 5-HT_{1A} receptor agonist on the behavioral sensitization differs from the result observed in rats. The important point is that the modulation of cocaine-induced sensitization by 5-HT receptor ligands is observed in mice. 5-HT and DA systems are anatomically and functionally linked in the brain (Jacobs and Azmitia, 1992; Le Moal and Simon, 1991; Van Bockstaele and Pickel, 1993), and cocaine-induced increases in DA in the nucleus accumbens may have a critical role in the mediation of cocaine stimulant and reward effects (Koob, 1992; Woolverton and Johnson, 1992). In addition, repeated cocaine-administration enhances the capacity of a subsequent cocaine injection to elevate extracellular 5-HT in the accumbens (Carey and Damianopoulos, 1994; Parsons and Justice, 1993; Parsons et al., 1996), and it also attenuates the increase in extracellular DA levels elicited by a systemic cocaine injection (Chefer et al., 2000; Sorg et al., 1997). The adaptation of serotonergic and

dopaminergic neurons to repeated cocaine administration may result in alteration of the balance of the central dopaminergic and serotonergic activities. Previous neurochemical studies show that 5-HT_{1A} receptor agonists and 5-HT_2 receptor antagonists increase DA release in the prefrontal cortex (Sakaue et al., 2000; Tanda et al., 1994; Pehek, 1996; Pehek and Bi, 1997) and nucleus accumbens (Di Matteo et al., 1998) while 5-HT_3 receptor antagonists inhibit DA release in the nucleus accumbens (De Deurwaerdere et al., 1998; Kankaanpää et al., 1996; McNeish et al., 1993). The difference in the effects on DA release between the 5-HT receptor ligands suggest that the effects of the 5-HT receptor ligands observed in this study can not be explained simply by the effects on DA release. Further neurochemical studies are required to clarify the mechanism for neuroadaptation pertaining to cocaine sensitization.

In conclusion, the present study demonstrates that the 5-HT_{1A} receptor agonist osemozotan and the 5-HT_2 receptor antagonist ritanserin inhibit the development and expression of cocaine-induced behavioral sensitization in mice. In addition, this study shows that the 5-HT_3 receptor antagonist azasetron inhibits the development of cocaine-induced behavioral sensitization in mice. These results suggest that the central 5-HT system plays a role in cocaine-induced behavioral sensitization in mice.

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