Role of 5-Hydroxytryptamine$_{2C}$ Receptors in Marble-Burying Behavior in Mice

Nobuaki Egashira,$^{a,b}$ Ryoko Okuno,$^a$ Atsunori Shirakawa,$^a$ Masaki Nagao,$^a$ Kenichi Mishima,$^a$ Katsunori Iwasaki,$^a$ Ryozo Oishi,$^a$ and Michihiro Fujiwara$^a$

$^a$Department of Neuropharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University; Fukuoka 814–0180, Japan; and $^b$Department of Pharmacy, Kyushu University Hospital; Fukuoka 812–8582, Japan.

Received October 6, 2011; accepted December 12, 2011; published online December 15, 2011

We examined the role of 5-hydroxytryptamine$_{2C}$ (5-HT$_{2C}$) receptors in marble-burying behavior in mice. When administered alone, the selective 5-HT$_{2C}$ agonist WAY161503 (3 mg/kg) inhibited marble-burying behavior. Moreover, the selective 5-HT$_{2C}$ antagonist SB242084 (3 mg/kg) reversed the inhibition of marble-burying behavior by 2,5-dimethoxy-4-iodoamphetamine (DOI) (1 mg/kg) or WAY161503 (3 mg/kg). Similarly, SB242084 (1 mg/kg) reversed the inhibition of marble-burying behavior by fluvoxamine (30 mg/kg) or paroxetine (3 mg/kg). These results suggest that 5-HT$_{2C}$ receptors play a role in marble-burying behavior in mice.

Key words marble-burying behavior; 5-hydroxytryptamine$_{2C}$ receptor; WAY161503; obsessive-compulsive disorder; fluvoxamine; paroxetine

Obsessive-compulsive disorder (OCD) is a psychiatric condition with a lifetime prevalence of 1—3%, and it is characterized by recurrent and persistent thoughts, impulses, or images (obsessions), and/or repetitive, seemingly purposeful behaviors (compulsions). Currently, 5-hydroxytryptamine (5-HT) reuptake inhibitors and especially selective 5-HT reuptake inhibitors (SSRIs) are the first-line agents for the pharmacological treatment of OCD. Despite the strong evidence for the role of 5-HT in the treatment of OCD, the specific receptors involved in mediating its effects have not been clearly identified. Identifying the receptor systems involved in OCD could provide novel targets for future therapeutic interventions.

Marble-burying behavior is considered to be a potential model of OCD on the basis of behavioral similarity. Indeed, SSRIs such as fluvoxamine and paroxetine are used to treat human OCD symptoms, and they inhibit marble-burying behavior in mice. Similarly, the 5-HT$_{2A/C}$ agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) has also been reported to inhibit marble-burying behavior in mice. The 5-HT$_{2C}$ receptor agonists Ro 60-0175 and Ro 60-0332 also inhibit marble-burying behavior in mice. Furthermore, 5-HT$_{2C}$ receptor knockout mice display compulsive-like behaviors. As such, it is possible that 5-HT$_{2C}$ receptor activity influences marble-burying behavior. However, whether 5-HT$_{2C}$ receptors are involved in the inhibition of marble-burying behavior by DOI or SSRIs remains unconfirmed. In the present study, we investigated the role of 5-HT$_{2C}$ receptors in marble-burying behavior in mice. First, we examined the effects of the selective 5-HT$_{2C}$ agonist WAY161503 on this behavior. Moreover, through drug interaction experiments, we investigated the role of 5-HT$_{2C}$ receptors in the inhibitory effects of DOI, WAY161503 and SSRIs on marble-burying behavior in mice.

MATERIALS AND METHODS

Animals Five-week-old male ICR mice (Nihon SLC, Shizuoka, Japan) were used in each experiment. The mice were housed in groups of five per cage. The total number of animals used was 255. For at least 7 d before the behavioral tests, the mice were housed in a room under controlled temperature (23±2°C), relative humidity (60±10%), and ambient lighting (cycle of 12 h light and 12 h dark, with the period of light starting at 07:00 h) conditions. The animals had free access to food (CE-2, Clea Japan, Tokyo, Japan) and water in their home cages. All animal care and use procedures were performed in compliance with the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University followed the Guidelines of the Science Council of Japan (approved No. 1007410 of institutional review board).

Drugs DOI hydrochloride and the selective 5-HT$_{2C}$ antagonist SB242084 dihydrochloride hydrate were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). WAY161503 was purchased from Tocris Bioscience (Ellisville, MO, U.S.A.). Fluvoxamine maleate and paroxetine hydrochloride hemihydrate were generous gifts from Solvay Pharmaceutical (Tokyo, Japan) and GlaxoSmithKline (West Sussex, U.K.), respectively. DOI and WAY161503 were dissolved in saline. SB242084 was dissolved in saline containing drops of Tween 80. Fluvoxamine and paroxetine were dissolved in distilled water. All drugs were administered at a volume of 0.1 mL/10 g of body weight. Doses are in terms of the base.

Marble-Burying Behavior Test The marble-burying behavior test was performed as described previously. Experiments were conducted between 10:00 and 17:00 h. Mice were placed individually, without food and water, in clear plastic boxes (30×30×28 cm) that containing 25 glass marbles (1.5 cm in diameter) that were evenly spaced on 5 cm deep sawdust. Concurrently, the locomotor activity patterns of the mice were measured using an automated activity counter (NAS101; Neuroscience, Tokyo, Japan) placed 15 cm above the same plastic boxes. Activity was measured under the illumination of a 100 W bulb. The dependent measure of marble-burying behavior was the number of marbles buried to at least two-thirds of the depth of the sawdust, within 30 min. On the first day, we selected mice that buried more than 20 glass marbles, which were in all except nine mice. We carried out the drug evaluations on the next day. DOI, SB242084, and WAY161503 were administered intraperitoneally (i.p.) 30 min before the test. Fluvoxamine and paroxetine were administered 60 min before the test. DOI and WAY161503 were administered intraperitoneally (i.p.) 30 min before the test.
paroxetine were administered orally 60 min before the test. The observers did not know what agent was being tested.

**Statistical Analysis** The results obtained in the marble-burying behavior test were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey–Kramer post-hoc test to determine whether there were differences between the groups. A probability level of $p<0.05$ was accepted as statistically significant. Values are expressed as means±standard error of the mean (S.E.M.).

**RESULTS**

The selective 5-HT$_{2C}$ agonist WAY161503 (3 mg/kg) significantly reduced the number of buried marbles $[F(2,28)=7.512, p<0.01$ by one-way ANOVA; $p<0.01$ by the Tukey–Kramer post-hoc test, Fig. 1a]. No significant change in total locomotor activity during the marble-burying behavior was observed at this dose (Fig. 1b).

The 5-HT$_{3A/C}$ agonist DOI dose-dependently reduced the number of buried marbles $[F(3,41)=9.163, p<0.0001$ by one-way ANOVA]. DOI (0.3, 1 mg/kg) significantly reduced the number of buried marbles, without significantly affecting total locomotor activity (vehicle: 22.0±0.8, DOI 0.1 mg/kg: 22.1±1.3, DOI 0.3 mg/kg: 14.5±1.9, DOI 1 mg/kg: 10.2±2.2; 0.3 mg/kg: $p<0.05$, 1 mg/kg: $p<0.01$ by the Tukey–Kramer post-hoc test). The capacity of the selective 5-HT$_{2C}$ antagonist SB242084 to reverse the inhibition of marble-burying behavior by DOI (1 mg/kg) or WAY161503 (3 mg/kg) was also examined. SB242084 (3 mg/kg) significantly reversed the inhibition of marble-burying behavior by DOI $[F(4,48)=12.647, p<0.0001$ by one-way ANOVA; $p<0.01$ by the Tukey–Kramer post-hoc test, Fig. 2a]. No significant change in total locomotor activity during the marble-burying behavior was observed at this dose (Fig. 2c). Similarly, SB242084 (3 mg/kg) significantly reversed the inhibition of marble-burying behavior by WAY161503 $[F(5,38)=3.734, p<0.01$ by one-way ANOVA; $p<0.05$ by the Tukey–Kramer post-hoc test, Fig. 2b]. Moreover, SB242084 (3 mg/kg) significantly increased total locomotor activity in mice treated with WAY161503 $[F(5,38)=3.045, p<0.05$ by one-way ANOVA; $p<0.05$ by the Tukey–Kramer post-hoc test, Fig. 2d].

The capacity of the selective 5-HT$_{2C}$ antagonist SB242084 to reverse the inhibition of marble-burying behavior by fluvoxamine (30 mg/kg) or paroxetine (3 mg/kg) was then examined. SB242084 (1 mg/kg) significantly reversed the inhibition of marble-burying behavior by fluvoxamine $[F(4,60)=5.762, p<0.0005$ by one-way ANOVA; 0.3 mg/kg: $p<0.01$, 1 mg/kg: $p<0.05$ by the Tukey–Kramer post-hoc test, Fig. 3a]. Similarly, SB242084 (1 mg/kg) significantly reversed the inhibition of marble-burying behavior by paroxetine $[F(4,63)=15.683, p<0.0001$ by one-way ANOVA; $p<0.01$ by the Tukey–Kramer post-hoc test, Fig. 3b]. No significant change in total locomotor activity during the marble-burying behavior was observed at this dose (Fig. 3d).

**DISCUSSION**

The present pharmacological study showed that activation of 5-HT$_{2C}$ receptors is a necessary component of the inhibitory effects of DOI, fluvoxamine and paroxetine on marble-burying behavior. In this regard, the selective 5-HT$_{2C}$ antagonist SB242084 reversed the inhibition of marble-burying behavior by DOI. Similarly, SB242084 reversed the inhibition of marble-burying behavior by fluvoxamine and paroxetine. These results suggest that activation of 5-HT$_{2C}$ receptors inhibits this behavior in mice. Moreover, the selective 5-HT$_{2C}$ agonist WAY161503 inhibited marble-burying behavior, which is essentially consistent with previous findings.8) Furthermore, SB242084 reversed the inhibition of marble-burying behavior by WAY161503. These findings support the involvement of 5-HT$_{2C}$ receptors in the inhibition of marble-burying behavior by DOI and SSRIs.

Recently, it has been reported that pretreatment with the selective 5-HT$_{1A}$ antagonist WAY100635 (3 mg/kg) prevents the effects of paroxetine on marble-burying behavior,10) suggesting that the involvement of 5-HT$_{1A}$ receptor. Furthermore, it has been reported that the effects of fluvoxamine is attenuated by WAY100635 at 1 mg/kg, while it is enhanced by WAY100635 at 0.1 mg/kg.11) These findings suggest that the effects of fluvoxamine and paroxetine may be mediated, at least in part, by 5-HT$_{1A}$ receptors.

In the present study, SB242084 significantly increased total locomotor activity in mice treated with fluvoxamine. Since SB242084 alone had no effect on total locomotor activity, we suggest that this effect is due to an interaction between these drugs. Furthermore, based on these findings, we suggest that this enhancement of locomotor activity is mediated by 5-HT receptors other than the 5-HT$_{2C}$ receptor. We base this as-

---

Fig. 1. Effects of WAY161503 on Marble-Burying Behavior in Mice

The data are presented as the (a) number of buried marbles and (b) locomotor activity. WAY161503 (1, 3 mg/kg) was administered i.p. 30 min before the test. Values are expressed as the mean±S.E.M. **$p<0.01$ compared with the vehicle-treated group (Tukey–Kramer post-hoc test). The number of mice is shown at the bottom of each column.
Fig. 2. Effects of SB242084 on Inhibition of Marble-Burying Behavior by DOI or WAY161503 in Mice

The data are presented as the (a, b) number of buried marbles and (c, d) total locomotor activity. DOI (1 mg/kg) WAY161503 (3 mg/kg) and SB242084 (0.3, 1, 3 mg/kg) were administered i.p. 30 min before the test. Values are expressed as the mean±S.E.M. **p<0.01 compared with the vehicle-treated group, #p<0.05, ##p<0.01 compared with the group treated with DOI alone or WAY161503 alone (Tukey–Kramer post-hoc test). The number of mice is shown at the bottom of each column.

Fig. 3. Effects of SB242084 on Inhibition of Marble-Burying Behavior by Fluvoxamine (a, c) or Paroxetine (b, d) in Mice

The data are presented as the (a, b) number of buried marbles and (c, d) total locomotor activity. SB242084 (0.3, 1 mg/kg) was administered i.p. 30 min before the test. Fluvoxamine (30 mg/kg) and paroxetine (3 mg/kg) were administered orally 60 min before the test. Values are expressed as the mean±S.E.M. **p<0.01 compared with the vehicle-treated group, #p<0.05, ##p<0.01 compared with the group treated with fluvoxamine or paroxetine alone (Tukey–Kramer post-hoc test). The number of mice is shown at the bottom of each column.
sertion on the notion that fluvoxamine retains its capacity to indirectly activate other 5-HT receptors in the presence of a 5-HT2C receptor antagonist as it can elevate extracellular 5-HT levels by inhibiting the 5-HT transporter.

In addition, WAY106635 tended to reduce total locomotor activity, whereas SB242084 significantly increased total locomotor activity in mice treated with WAY106635. Martin et al.9 reported that a high dose of each of the 5-HT2C receptor agonists Ro 60-0175 and Ro 60-0332 markedly reduced spontaneous motor activity in rats. 5-HT2C receptors are enriched in the ventral tegmental area, which contain the perikarya of corticolimbic dopaminergic pathways.12,13) Accordingly, 5-HT2C receptors may exert an inhibitory influence upon the activity of dopaminergic pathways projecting to the frontal cortex and of mesolimbic and nigrostriatal pathways innervating the nucleus accumbens and striatum, respectively.12—15)

5-HT2C receptors are concentrated in corticolimbic regions such as the frontal cortex, parietal cortex, hippocampus, nucleus accumbens, and amygdala.16,17) Dysfunction in cortico-thalamic-striatal circuits is an integral component of OCD-like behavior. Patients with OCD show orbitofrontal cortex disruption.18) Joel et al.19 reported that lesions to the rat orbital frontal cortex led to an increase in compulsive lever-pressing that was prevented by systemic administration of paroxetine in rat model of OCD. Since marble burying also reflects a repetitive and perseverative behavior more than novelty-induced anxiety,20) the orbital frontal cortex might be involved in the marble-burying behavior.

In conclusion, the results of the present study directly show, for the first time, that activation of the 5-HT2C receptors is involved in the inhibitory effects of fluvoxamine and paroxetine on marble-burying behavior. Therefore, these findings support the possibility that 5-HT2C receptor agonists may be a potential treatment for OCD.

Acknowledgments We thank Solvay Pharmaceutical and GlaxoSmithKline for generously donating the fluvoxamine and paroxetine used in this study.

REFERENCES


