Perospirone, a Novel Antipsychotic Drug, Inhibits Marble-Burying Behavior via 5-HT\textsubscript{1A} Receptor in Mice: Implications for Obsessive-Compulsive Disorder

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Abstract. Perospirone is a novel atypical antipsychotic drug with dopamine (DA) D\textsubscript{2} and serotonin (5-hydroxytryptamine, 5-HT) 5-HT\textsubscript{2A}-receptor antagonist, and 5-HT\textsubscript{1A}-receptor agonist properties. In the present study, we examined the effect of perospirone on marble-burying behavior, which has been considered an animal model of obsessive-compulsive disorder (OCD), compared with the effects of other antipsychotics such as haloperidol and risperidone. Perospirone at a dose of 10 mg/kg (p.o.) inhibited marble-burying behavior without affecting the locomotor activity in mice. On the other hand, haloperidol (0.1 mg/kg, i.p.) and risperidone (1 mg/kg, p.o.) showed significant suppression of locomotor activity at the dose that inhibited marble-burying behavior. Furthermore, the inhibition of marble-burying behavior by perospirone was antagonized by WAY100135 (10 mg/kg, i.p.), a selective 5-HT\textsubscript{1A}-receptor antagonist. WAY100135 at the same dose also antagonized the inhibition of marble-burying behavior by 8-OH-DPAT (3 mg/kg, i.p.), a selective 5-HT\textsubscript{1A}-receptor agonist. These findings suggest that perospirone may exhibit anti-OCD activity in clinical use and that 5-HT\textsubscript{1A}-receptor agonistic activity may be involved in the inhibition of marble-burying behavior by perospirone.

Keywords: marble-burying behavior, perospirone, antipsychotic drug, 5-HT\textsubscript{1A} receptor, obsessive-compulsive disorder

Introduction

Obsessive-compulsive disorder (OCD) is characterized by recurrent and persistent thoughts, impulses, or images (obsessions) and/or repetitive, seemingly purposeful behaviors (compulsions) \textsuperscript{(1)}. Currently, the serotonin (5-hydroxytryptamine, 5-HT)-reuptake inhibitor (SSRI) and selective 5-HT\textsubscript{1A}-reuptake inhibitor (SSRI) are considered the “first choice” agents for pharmacological treatment of OCD \textsuperscript{(2)}. However, up to 50\% of patients with OCD fail to respond to a SSRI trial \textsuperscript{(3)}. Recently, antipsychotics, including haloperidol and risperidone, have reportedly augmented the effect of SSRI therapy in OCD \textsuperscript{(4 – 6)}.

In general, marble-burying behavior has been considered an animal model of OCD \textsuperscript{(7, 8)}. In fact, SRI and SSRI, which have been used to treat human OCD symptoms, inhibit marble-burying behavior without affecting locomotor activity \textsuperscript{(7 – 9)}. Moreover, the inhibition of marble-burying behavior by fluvoxamine, a SSRI, was antagonized by NAN-190, a 5-HT\textsubscript{1A}-receptor antagonist \textsuperscript{(7)}. These findings suggest that the 5-HT\textsubscript{1A} receptor may be involved in the inhibition of marble-burying behavior by SSRI.

Perospirone is a novel atypical antipsychotic drug. Like other atypical antipsychotics, perospirone antagonizes the 5-HT\textsubscript{2A} receptor more potently than the dopamine (DA) D\textsubscript{2} receptor. In addition, perospirone acts as a 5-HT\textsubscript{1A}-receptor agonist \textsuperscript{(10 – 13)}. The 5-HT\textsubscript{1A}-
receptor agonists commonly cause anxiolytic and antidepressant effects (14–16). Perospirone has also been reported to exert anxiolytic-like effects in conditioned defensive burying and social interaction tests (17). Based on the unique profile of perospirone’s affinity to the 5-HT$_{1A}$, 5-HT$_{2A}$, and D$_2$ receptors, perospirone has been postulated to be of possible benefit in treating OCD. However, the effect of perospirone on marble-burying behavior has not been studied. In the present study, we examined the effect of perospirone on marble-burying behavior compared with the effects of other antipsychotics such as haloperidol and risperidone. We also examined the effect of WAY100135 on the inhibition of marble-burying behavior by perospirone to investigate the involvement of the 5-HT$_{1A}$ receptor.

Materials and Methods

Animals

Five-week old male ICR mice (Nihon SLC Co., Shizuoka) were used in each experiment. For at least seven days before the behavioral tests, the mice were housed in a room under controlled temperature (23 ± 2°C), relative humidity (60 ± 10%), and a cycle of 12 h of light and 12 h of darkness, with the period of light starting at 7:00 A.M. Food and water were available ad libitum. The experiments were conducted in compliance with guidelines stipulated by the Animal Care and Use Committee of Fukuoka University.

Marble-burying behavior test

The marble-burying behavior test is based on the method of Ichimaru et al. (7). All experiments were conducted between 10:00 and 17:00. The mice were placed individually in clear plastic boxes (30 × 30 × 28 cm) containing 25 glass marbles (1.5 cm in diameter) evenly spaced on sawdust 5-cm-deep without food or water. At the same time, the locomotor activity of mice was measured using an automated activity counter (NS-AS01; Neuroscience, Inc., Tokyo) placed 15-cm above the same plastic boxes. This counter has a pyroelectric sensor, which can generate a voltage-change by altering the amount of infrared radiation emitted from the sensor window. The activity was measured with the illumination of a 100-W bulb. The results of marble-burying behavior were expressed as the number of marbles buried at least two-thirds deep in this paradigm within 30 min. The average of total counts of locomotor activity for 30 min. in the group treated with vehicle was expressed as 100%; values of the group treated with drug were expressed as a percentage of variations from values of the group treated with vehicle.

Drugs

Haloperidol solution (5 mg/ml, Serenade Injection; Dainippon Pharmaceutical Co., Ltd., Osaka) was diluted with saline. Risperidone (Janssen Research Foundation, Beerse, Belgium), (-)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT; Sigma-Aldrich, Inc., St. Louis, MO, USA) and WAY100135 (Tocris Cookson, Inc., Ellisville, MO, USA) were dissolved in saline. Perospirone (Sumitomo Pharmaceutical Co., Osaka) was suspended in 0.5% methylcellulose solution. Risperidone or perospirone was administered orally 60 min before the test. Haloperidol, 8-OH-DPAT, or WAY100135 was administered intraperitoneally (i.p.) 30 min before the test. All drugs were administered at a volume of 0.1 ml/10 g of body weight.

Statistical analyses

The values were expressed as means ± S.E.M. The Kruskal-Wallis test followed by the Mann-Whitney U-test was used for statistical analysis of the data on the number of buried marbles. The results of locomotor activity were analyzed by a one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn test.

Results

Haloperidol at a dose of 0.1 mg/kg (i.p.) significantly reduced the number of buried marbles ($H(3) = 7.939$, $P<0.05$, 0.1 mg/kg: $P<0.05$, Fig. 1A). At this dose, it also significantly reduced locomotor activity during the marble-burying behavior ($F(3, 30) = 6.834$, $P<0.01$, 0.1 mg/kg: $P<0.05$, Fig. 1B). Risperidone also significantly reduced the number of buried marbles at a dose of 1 mg/kg (p.o.) ($H(3) = 11.676$, $P<0.01$, 1 mg/kg: $P<0.01$, Fig. 2A). At the same dose, it also significantly reduced locomotor activity during the marble-burying behavior ($F(3, 36) = 6.961$, $P<0.01$, 1 mg/kg: $P<0.01$, Fig. 2B). Conversely, perospirone significantly reduced the number of buried marbles without affecting locomotor activity at a dose of 10 mg/kg (p.o.) ($H(2) = 6.518$, $P<0.05$, 10 mg/kg: $P<0.01$, Fig. 3). Moreover, the inhibition of marble-burying behavior by perospirone was antagonized by WAY100135, a 5-HT$_{1A}$-receptor antagonist ($H(2) = 8.823$, $P<0.05$, perospirone + WAY100135: $P<0.05$, Fig. 4A). No change in locomotor activity during the marble-burying behavior was observed at this time (Fig. 4B). Similarly, a 5-HT$_{1A}$-receptor agonist 8-OH-DPAT significantly reduced the number of buried marbles without affecting locomotor activity at a dose of 3 mg/kg (p.o.) ($H(3) = 10.001$, $P<0.05$, 3 mg/kg: $P<0.05$, Fig. 5). WAY100135 also antagonized the inhibition of marble-burying behavior by 8-OH-DPAT ($H(2) = 12.801$, $P<0.01$, 8-OH-DPAT +
WAY100135: \( P < 0.01 \), Fig. 6). In addition, WAY100135 alone at a dose of 10 mg/kg (i.p.) had no effect on the number of buried marbles or locomotor activity (data not shown).

**Discussion**

In the present study, we demonstrated that perospirone inhibited marble-burying behavior, which has been considered an animal model of OCD (7, 8), without affecting the locomotor activity in mice. These effects were therefore not attributable to non-specific sedative effects or a deficit of motor function. Similarly, SRI and SSRI, which have been used to treat human OCD symptoms (2), have also been reported to inhibit marble-burying behavior without affecting locomotor activity (7 – 9). These findings suggest that perospirone, as well as SRI and SSRI, may exhibit anti-OCD activity. Conversely, haloperidol and risperidone showed significant suppression of locomotor activity at the dose that inhibited marble-burying behavior. Therefore, these inhibitions of marble-burying behavior are thought to be due to the suppression of locomotor activity.

Perospirone has an agonistic effect on 5-HT\(_{1A}\) receptors as well as an antagonistic effect on 5-HT\(_{2A}\) and D\(_2\) receptors (10 – 13). In the present study, the inhibition of marble-burying behavior by perospirone was antagonized by WAY100135, the selective 5-HT\(_{1A}\)-receptor antagonist. Therefore, the 5-HT\(_{1A}\)-receptor agonistic activity may be involved in the inhibition of marble-burying behavior by perospirone. On the other hand, the inhibition of marble-burying behavior by fluvoxamine was antagonized by NAN-190, the 5-HT\(_{1A}\)-receptor antagonist (7). Moreover, the 5-HT\(_{1A}\)-receptor agonist 8-OH-DPAT has reportedly inhibited marble-burying behavior without affecting locomotor activity (7). We also found that 8-OH-DPAT inhibited the marble-burying behavior without affecting the locomotor activity, and this inhibition was clearly antagonized by
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WAY100135. These findings suggest that the 5-HT$_{1A}$ receptor may play an important role in marble-burying behavior.

Several studies suggested that 5-HT$_{2A}$-receptor antagonistic activity also contributes to the anti-OCD effects. Some drugs with 5-HT$_{2A}$-receptor antagonistic activity potentiated the effect of zimeldine (a SSRI) on marble-burying behavior (9). Moreover, the co-administration of risperidone, which exhibits 5-HT$_{2A}$- and D$_2$-receptor antagonistic activities, with SSRI has been reported to be effective in SSRI-refractory OCD patients (4, 6). However, the pharmacological mechanism responsible for this augmentation remains unclear. The combination of risperidone and fluoxetine (a SSRI) has been reported to produce remarkable increases in DA release in the medial prefrontal cortex that are greater than the increases produced by either alone (18). Similarly, the combination of perospirone and fluoxetine markedly increases DA release, and these increases in DA release were suppressed by WAY100635, the selective 5-HT$_{1A}$-receptor antagonist (18). Moreover, the increases in DA release were greater for a combination of perospirone and fluoxetine than for a combination of risperidone and fluoxetine (18). These findings may be attributable to the fact that perospirone, unlike risperidone, has an agonistic effect on 5-HT$_{1A}$ receptors as well as an antagonistic effect on 5-HT$_{2A}$ receptors. Based on perospirone’s affinity to not only 5-HT$_{2A}$ receptors but 5-HT$_{1A}$ receptors as well, perospirone may prove effective in SSRI-refractory OCD patients.

In conclusion, perospirone significantly inhibited marble-burying behavior, which is a model for evaluating clinical potential in the treatment of OCD, without affecting the locomotor activity in mice. Moreover, it was thought that 5-HT$_{1A}$-receptor agonistic activity is involved in the inhibition of marble-burying behavior by perospirone. These findings suggest that perospirone may exhibit anti-OCD activity in clinical use.
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References

4 McDougle CJ, Epperson CN, Pelton GH, Wasylink S, Price LH.

Fig. 5. Effect of 8-OH-DPAT on the marble-burying behavior in mice. A: Marble-burying behavior, B: locomotor activity. The values are expressed as the means ± S.E.M. of n = 8 – 12 mice per group. *P<0.05, compared to the group treated with vehicle.

Fig. 6. Effect of WAY100135 on the inhibition of marble-burying behavior by 8-OH-DPAT in mice. A: Marble-burying behavior, B: locomotor activity. The values are expressed as the means ± S.E.M. of n = 8 – 9 mice per group. **P<0.01, compared to the group treated with vehicle; ††P<0.01, compared to the group treated with 8-OH-DPAT alone.
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