Calcium-Channel Antagonists Inhibit Marble-Burying Behavior in Mice

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Abstract. In the present study, we examined the effects of calcium-channel antagonists on marble-burying behavior, which is an animal model of obsessive-compulsive disorder. Amlodipine (10 mg/kg, i.p.), cilnidipine (10 mg/kg, i.p.), nilvadipine (3 and 10 mg/kg, i.p.), and flunarizine (30 mg/kg, i.p.) significantly inhibited marble-burying behavior in mice. These results suggest that calcium channels may be involved in the marble-burying behavior.

Keywords: marble-burying behavior, calcium-channel antagonist, obsessive-compulsive disorder

Obsessive-compulsive disorder is a psychiatric condition with a lifetime prevalence of 1% – 3% that is characterized by recurrent and persistent thoughts, impulses or images (obsessions), and/or repetitive, seemingly purposeful behaviors (compulsions) (for example, doubting, checking, and washing) (1, 2). Although classified as an anxiety disorder, patients with obsessive-compulsive disorder demonstrate a high incidence of comorbid depression (2). Currently, serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SRIs) and selective 5-HT reuptake inhibitors (SSRIs) are considered to be the first choice agents for the pharmacological treatment of obsessive-compulsive disorder (3).

Some clinical evidence indicates that glutamatergic abnormalities are associated with obsessive-compulsive disorder symptoms (4, 5). Recently, the uncompetitive \(N\)-methyl-\(d\)-aspartate (NMDA)-receptor antagonist memantine was reported to exhibit augmentation effects in treatment-resistant obsessive-compulsive disorder (6, 7). The activation of NMDA receptors especially leads to excessive elevation in intracellular \(Ca^{2+}\) levels. NMDA-receptor blockade attenuates intracellular \(Ca^{2+}\) influx. Calcium-channel antagonists also inhibit the intracellular \(Ca^{2+}\) influx by blocking voltage-gated calcium channels. Interestingly, dihydropyridine and diphenylpiperazine calcium-channel antagonists display an antidepressant- or anxiolytic-like property in mice and rats (8, 9).

Marble-burying behavior is considered to be a potential model of obsessive-compulsive disorder on the basis of behavioral similarity (10, 11). Indeed, SSRIs such as fluvoxamine and paroxetine, which have been used to treat the symptoms of human obsessive-compulsive disorder (3), inhibit marble-burying behavior without affecting locomotor activity (10, 12). Moreover, uncompetitive NMDA-receptor antagonists such as memantine and amantadine inhibit marble-burying behavior without affecting locomotor activity (13). However, the effects of calcium-channel antagonists on marble-burying behavior have not been reported for this model. Therefore, we investigated the effects of calcium-channel antagonists on marble-burying behavior in mice.

Five-week-old male ICR mice (Nihon SLC, Shizuoka) were used in each experiment. The mice were housed in groups of five per cage. For at least 7 days before the behavioral tests, the mice were housed in a room under controlled temperature (23 ± 2°C), 60 ± 10% relative humidity, and a cycle of 12 h light and 12 h dark, with the period of light starting at 07:00 h. The animals had free access to food (CE-2; Clea Japan, Tokyo) and water in their home cages. All procedures regarding animal care and use were performed in compliance with the...
regulations established by the Experimental Animal Care and Use Committee of Fukuoka University. Amlodipine and nilvadipine were generous gifts from Astellas Pharma, Inc. (Tokyo). Cilnidipine was a generous gift from Ajinomoto Co., Inc. (Kawasaki). Flunarizine was purchased from Sigma–Aldrich (St. Louis, MO, USA). Amlodipine, nilvadipine, and cilnidipine were dissolved in 25% polyethylene glycol. Flunarizine was suspended in 1% Tween 80 solution. All drugs were administered intraperitoneally (i.p.) 30 min before the test.

The marble-burying behavior test was performed as described previously (11). All experiments were conducted between 10:00 and 17:00 h. Mice were placed individually in clear plastic boxes (30 × 30 × 28 cm), containing 25 glass marbles (1.5 cm in diameter) evenly spaced on 5-cm-deep sawdust, without food and water. At the same time, the locomotor activity of mice was measured using an automated activity counter (NS-AS01; Neuroscience, Tokyo) placed 15-cm-above the same plastic boxes. Activity was measured under the illumination of a 100-W bulb. The results of marble-burying behavior were expressed as the number of marbles buried to at least two-thirds of the depth, within 30 min. The observer did not know which agent was being tested.

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 ± S.E.M.

The L- and N-type dihydropyridine calcium-channel antagonists amlodipine (10 mg/kg, i.p.) and cilnidipine (10 mg/kg, i.p.) significantly reduced the number of buried marbles [amlodipine: F(2,24) = 10.949, P<0.001 by one-way ANOVA; P<0.01; cilnidipine: F(2,22) = 6.650, P<0.01 by one-way ANOVA; P<0.01] (Fig. 1: A and C). However, these drugs significantly did not affect locomotor activity (Fig. 2: A and C). Similarly, the L- and T-type dihydropyridine calcium-channel antagonist nilvadipine (3 and 10 mg/kg, i.p.) significantly reduced the number of buried marbles [nilvadipine: F(3,32) = 7.007, P<0.001 by one-way ANOVA; 3 mg/kg: P<0.05, 10 mg/kg: P<0.01] (Fig. 1B). Moreover, the L- and T-type diphenylpiperazine calcium-channel antagonist flunarizine (30 mg/kg, i.p.) significantly reduced the number of buried marbles [flunarizine: F(3,36) = 5.916, P<0.01 by one-way ANOVA; P<0.05] (Fig. 1D). However, these drugs also significantly did not affect locomotor activity (Fig. 2: B and D).

In the present study, we found that the calcium-channel antagonists amlodipine, cilnidipine, nilvadipine, and flunarizine inhibited marble-burying behavior, which is considered to be an animal model of obsessive-compulsive disorder (10, 11), without affecting locomotor activity in mice. Therefore, these effects were not attributable to non-specific sedative effects or a deficit of motor function. These findings suggest that the reduced number of marbles buried by calcium-channel antagonists might reflect an inhibitory effect on obsessive-compulsive disorder.

Alternatively, the interpretation that mice have anxiety when they are burying foreign objects is based on the assumption that the animals would consider marbles startling by virtue of their novelty. Therefore, the reduced number of marbles buried by the mice administered calcium-channel antagonists might reflect an anxiolytic effect of these drugs. This idea is supported by the previous report that the calcium-channel antagonists displayed an anxiolytic-like property in the Vogel-type conflict test in rats (9).

Recently, we have reported that uncompetitive NMDA-receptor antagonists inhibited marble-burying behavior without affecting locomotor activity (13). NMDA-receptor blockade attenuates intracellular Ca\(^{2+}\) influx. The calcium-channel antagonists also inhibit the intracellular Ca\(^{2+}\) influx by blocking voltage-gated calcium channels because the voltage-dependent calcium channels are important for calcium influx and the ensuing intracellular calcium signal in various excitable membranes. Several studies have demonstrated the importance of voltage-dependent calcium channels and Ca\(^{2+}/\)calmodulin-stimulated adenylyl cyclases in emotional behavior (14, 15). Therefore, the calcium-channel antagonists amlodipine, cilnidipine, nilvadipine, and flunarizine may inhibit the marble-burying behavior by inhibiting the intracellular Ca\(^{2+}\) influx. Taken together with these findings, the present results suggest that intracellular Ca\(^{2+}\) mobilization might play an important role in the marble-burying behavior.

Among the calcium-channel antagonists, cilnidipine tended to reduce the locomotor activity in this study. Cilnidipine reduces electrically stimulated noradrenaline release from the sympathetic nerve endings of vascular tissues in spontaneously hypertensive rats (16). Therefore, inhibition of the noradrenergic nervous system may be involved in the reduction of locomotor activity by cilnidipine.

In conclusion, the study presented here demonstrates, for the first time, that calcium-channel antagonists inhibit marble-burying behavior without affecting locomotor activity in mice. These findings suggest that calcium channels may be involved in the marble-burying behavior.
Fig. 1. Effects of calcium-channel antagonists on number of buried marbles in mice. A: amlodipine, B: nilvadipine, C: cilnidipine, and D: flunarizine. All drugs were administered i.p. 30 min prior to the test. Values are expressed as means ± S.E.M. (n = 8 – 10). *P<0.05, **P<0.01, compared with the vehicle-treated group (Tukey-Kramer post-hoc test).

Fig. 2. Effects of calcium-channel antagonists on locomotor activity in mice. A: amlodipine, B: nilvadipine, C: cilnidipine, and D: flunarizine. All drugs were administered i.p. 30 min prior to the test. Values are expressed as means ± S.E.M. (n = 8 – 10).
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References