Effects of *Gastrodia Elata* Bl on Phencyclidine-Induced Schizophrenia-Like Psychosis in Mice

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Abstract: It has been demonstrated that 5-HT_{1A} receptors play an important role in the pathophysiology of schizophrenia. Because *Gastrodia elata* Bl (GE) modulates the serotonergic system, we examined whether GE could affect phencyclidine (PCP)-induced abnormal behavior in mice. Repeated treatment with PCP increased immobility time, while it decreased social interaction time and recognition memory. PCP-induced abnormal behaviors were significantly attenuated by GE, and these effects were comparable to those of 8-OH-DPAT, a 5-HT_{1A} receptor agonist. Furthermore, GE-mediated effects were counteracted by WAY 100635, a 5-HT_{1A} receptor antagonist. Our results suggest that the antipsychotic effects of GE are, at least in part, mediated *via* activation of 5-HT_{1A} in mice.

Keywords: *Gastrodia elata* Bl, phencyclidine, schizophrenia, 5-HT_{1A} receptors.

INTRODUCTION

Schizophrenia is a chronic, devastating, and costly mental illness, affecting about 1% of the world population. It develops progressively, and is often undetected during childhood and adolescence in a premorbid phase, leading to the onset of psychosis in early adulthood [1].

Phencyclidine [1-(1-phenylcyclohexyl)piperidine hydrochloride (PCP)], a non-competitive N-methyl-D-aspartate (NMDA) antagonist, has been shown to induce schizophrenia-like psychosis, with positive symptoms, negative symptoms, and cognitive deficits in humans [2], which persist for several weeks after withdrawal from chronic PCP use [3].

To understand the pathophysiology of schizophrenia, an animal model of schizophrenia was established using PCP [3]. Nabeshima and colleagues previously demonstrated that repeated treatment with PCP induces several behavioral abnormalities, such as increased immobility in a forced swimming test, social deficits on a social interaction test, impairment of latent learning in a water finding test, and associative learning impairment in cue and contextual fear conditional tests in mice [3]. Thus, PCP-treated mice might be a useful animal model of schizophrenia.

Several lines of evidence have suggested that serotonin 5-HT_{1A} receptors may play a role in the pathophysiology of psychiatric diseases, including schizophrenia, and that 5-HT_{1A} receptors might be an important target for emotion and cognition [4].

Gastrodia elata Blume (GE) is a well-known herbal agent that has long been used to treat headache, paralysis, migraine, and other neurological disorders in traditional oriental medicine. Major components of GE include gastrodin, p-hydroxybenzyl aldehyde, p-hydroxybenzyl alcohol, vanillyl alcohol, and vanillin. Earlier reports indicated that GE has various biological properties, including anti-convulsant, anti-oxidant, cognitive enhancing, anxiolytic, and anti-depressant effects [5]. Recently, it was demonstrated that GE significantly decreased immobility duration in a forced-swimming test in rats, primarily by modulating the serotonergic system [6].

Thus, to extend the pharmacological investigation of GE, we examined whether GE affected PCP-induced changes in immobility, social interaction, and cognitive function in mice. We also examined whether the 5-HT_{1A} receptor is involved in GE-mediated pharmacological actions in response to PCP.

METHODS

All animals were treated in accordance with the NIH *Guide for the care and use of laboratory animals* (NIH Publication No. 85-23, 1985; www.dels.nas.edu/ila). This study was performed in accordance with the Institute for Laboratory Animal Research (ILAR) guidelines for the care and use of laboratory animals.

Male C57BL/6J mice or male ICR mice (Bio Genomic Inc., Charles River Technology, Gapyung-Gun, Gyeonggi-

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Do, South Korea), weighing 25±3 g, were maintained on a 12:12 h light:dark cycle and fed *ad libitum*. Male ICR mice were only used as the "target" in the social interaction test, with no drug treatment.

N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride (WAY 100635; Sigma-Aldrich, St. Louis, MO, USA), PCP hydrochloride (Tocris Bioscience, Ellisville, MO, USA), and (+)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; Sigma-Aldrich) were dissolved in 0.9% sterile saline. The GE was obtained from Samsung Herb Medicine, Co. (Chunchon, South Korea) and was suspended in 0.5% carboxymethylcellulose. All solutions were prepared immediately before use. Experimental schedules are shown in Fig. (**1**).

The novel object recognition, forced swimming, and social interaction tests were performed as described previously [6,7,8]. An automated video-tracking system (Noldus Information Technology, Wageningen, The Netherlands) was used to record and analyze the movements of mice in all three tests.

Statistical analyses were performed using one-way analysis of variance (ANOVA) or repeated measure one-way ANOVA. A *post-hoc* Fisher's PLSD test was then applied. A P value < 0.05 was deemed to indicate statistical significance.

RESULTS AND DISCUSSION

Repeated treatment with PCP resulted in significant increases ($P < 0.01$) in immobility time in the forced swimming test (Fig. **2A**), while PCP resulted in significant decreases ($P < 0.01$) in the interaction time in the social interaction test (Fig. **2B**) and exploration rate for a novel object ($P < 0.01$) in the novel object recognition test (Fig. **2C**). GE treatment significantly attenuated PCP-induced increase in immobility time [GE (500 mg/kg) + PCP vs. saline + PCP, $P < 0.05$; GE (1000 mg/kg) + PCP vs. saline + PCP, $P < 0.01$] (Fig. **2A**), sociability deficit [GE (1000 mg/kg) + PCP vs. saline + PCP, $P < 0.01$] (Fig. **2B**), and impaired visual recognition memory [GE (1000 mg/kg) + PCP vs. saline + PCP, $P < 0.01$] (Fig. **2C**), in a dose-dependent manner. The effects of GE (8-OH-DPAT + PCP vs. saline + PCP, $P < 0.05$ in all behaviors) were comparable to those of 8-OH-DPAT (0.05 mg/kg, i.p.), a 5-HT_{1A} receptor agonist. Consistently, WAY 100635 (0.5 mg/kg, i.p.), a 5-HT_{1A} receptor antagonist, significantly inhibited GE-mediated pharmacological actions [forced swimming test: WAY 100635 + GE (1000 mg/kg) + PCP vs. saline + GE (1000 mg/kg) + PCP, $P < 0.05$; social interaction test and novel object recognition test: WAY 100635 + GE (1000 mg/kg) + PCP vs. saline + GE (1000 mg/kg) + PCP, $P < 0.01$] in response to PCP (Fig. **2**). These results suggest that GE attenuated PCP-induced changes in immobility time, social interaction, and cognitive function *via* modulation of 5-HT_{1A} receptors.

Our results are consistent with earlier findings that repeated treatment with PCP showed significant increases in immobility time and significant decreases in social interaction and recognition memory in mice [3]. Prolonged exposure to GE significantly blocked PCP-induced behavioral effects, in a dose-related manner. The protective effects of GE in response to PCP were about equipotent to those of the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.05 mg/kg, i.p.). Furthermore, the 5-HT_{1A} receptor antagonist WAY 100635 (WAY; 0.5 mg/kg, i.p.) significantly counteracted GE-mediated pharmacological effects in response to PCP. Thus, we believe that GE-mediated activation of 5-HT_{1A} receptors may be important in antipsychotic effects in response to PCP, although this remains to be explored further in other GE-mediated neuropharmacological activities.

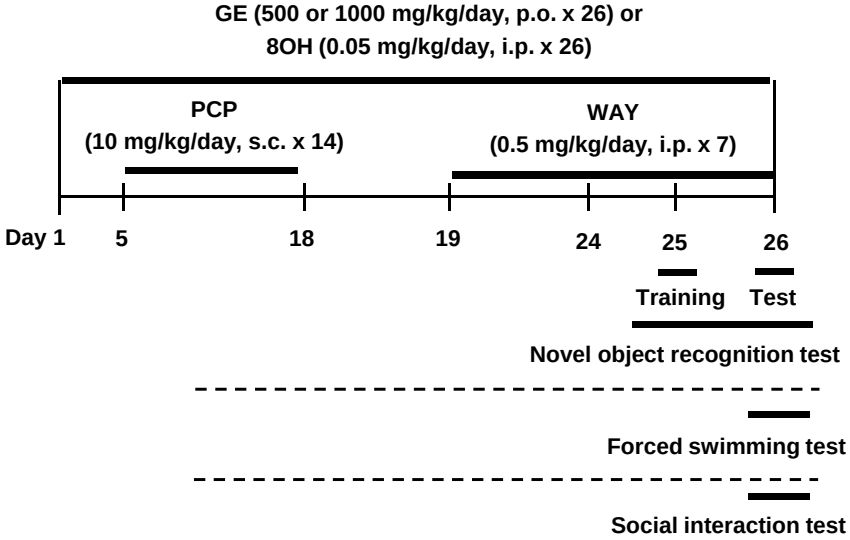

Fig. (1). Experimental schedules. PCP = phencyclidine, GE = *Gastrodia elata* Blume, 8OH = 8-OH-DPAT, WAY = WAY 100635. 8-OH-DPAT was used as a reference drug. Mice were treated with PCP (10 mg/kg/day, s.c.) for 14 consecutive days. After a 7- or 8-day withdrawal period, the novel object recognition, forced swimming, and social interaction tests were performed using independent sets of mice; each set of mice was used for one of the three behavioral tests. Treatment with GE (500 or 1000 mg/kg/day, p.o.) or 8-OH-DPAT (0.05 mg/kg/day, i.p.) was started from 4 days before the first PCP injection, and continued throughout the experimental period. WAY 100635 (0.5 mg/kg/day, i.p.) was administered during the PCP withdrawal period. GE was injected 1 h prior to PCP or WAY 100635, and WAY 100635 was injected 30 min prior to the behavior test.

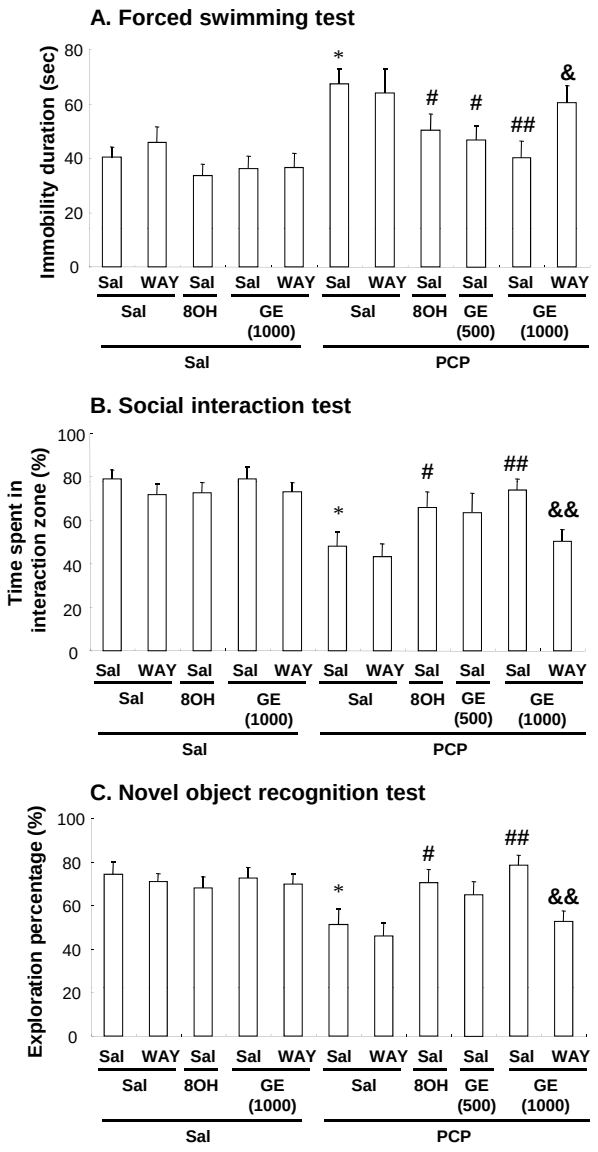

Fig. (2). Effect of WAY 100635 (WAY) on the GE-mediated pharmacological actions in response to PCP-induced changes in the immobility time (A), social interaction time (B), and recognition memory (C). Sal = saline, GE (500) = GE 500 mg/kg, p.o., GE (1000) = GE 1000 mg/kg, p.o., 8OH = 8-OH-DPAT 0.5 mg/kg. i.p., WAY = WAY 100635 0.5 mg/kg, i.p. Each value is the mean ± S.E.M. of 12 mice. * $P < 0.01$ vs. Saline + Saline + Saline, $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ vs. Saline + Saline + PCP, $^{\&}$ $P < 0.05$, $^{\&\&}$ $P < 0.01$ vs. Saline + GE (1000) + PCP (One-way ANOVA followed by Fisher's PLSD test).

Recent finding have suggested that repeated PCP treatment significantly decreases the density of 5-HT_{1A} receptors in the mouse brain [9]. 5-HT_{1A} agonist properties are thought to improve negative symptoms and cognitive deficits by stimulating the release of dopamine in the prefrontal cortex [10]. Consistent with this, Sumiyoshi *et al.* [11] reported that the 5-HT_{1A} agonists exert anti-depressant-like effects [12], of particular interest for schizophrenic patients suffering from depression. Recent reports have indicated that potential antipsychotic effects on PCP require combined modulation of 5-HT_{1A} and dopamine receptors [8]. Interestingly, the GE-mediated anti-depressant effects are exerted, at least in part, by dopaminergic modulation in the rat brain [6]; however, the interaction between 5-HT_{1A} and specific dopamine receptors remains to be determined.

Similar to GE, various $5HT_{1A}$ receptor agonists, such as buspirone, 8-OH-DPAT, and ipsapirione, have been shown to enhance social interaction [13]. Furthermore, various preclinical data strengthen the notion that targeting the $5HT_{1A}$ receptor system should result in beneficial effects on dysfunctional social behavior, possibly not only in schizophrenic patients but also in the population suffering from social withdrawal of other etiologies.

Hagiwara *et al.* [9] demonstrated that the hippocampal density of 5-HT1A receptor is much higher than the frontal cortical density of 5-HT receptor in mice, and that repeated treatment with PCP did not significantly alter the frontal cortical density of 5-HT, but did change the hippocampal density of 5-HT receptors, and that perospirone, a 5-HT_{1A} receptor agonist, ameliorated PCP-induced cognitive deficits, as measured by a novel object recognition test. Thus, the cognitive enhancing effect of GE or 8-OH-DPAT may be similar to that of perospirone. It remains to be determined whether GE also modulates hippocampal 5-HT_{1A} receptors in our experimental system.

Atypical antipsychotic drugs, such as clozapine, ziprasidone, aripiprazole, and quetiapine, are all 5-HT_{1A} receptor (partial) agonists, which may be relevant for their actions in treating schizophrenia [14]. While current antipsychotic treatments are effective against positive symptoms, they have significant side effects and have little effect on negative or cognitive symptoms [15].

In conclusion, our finding suggests that 5-HT_{1A} receptor agonistic properties of GE offer potential therapeutic advantages in response to PCP-induced schizophrenia-like psychosis, although many details of the GE-mediated effect(s) remain to be determined.

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