Gastrodia Elata Bl Attenuates Cocaine-Induced Conditioned Place Preference and Convulsion, but not Behavioral Sensitization in Mice: Importance of $GABA_A$ Receptors

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Abstract: It has been suggested that GABAergic neurotransmission can modulate cocaine dependence and seizure activity. Since *Gastrodia elata* Bl (GE), an oriental herb agent, has been shown to enhance GABAergic transmission, we examined whether GE affects cocaine-induced seizures, conditioned place preference (CPP), and behavioral sensitization in mice. Treatment with GE (500 or 1000 mg/kg, p.o.) significantly delayed seizure onset time and significantly shortened seizure duration induced by cocaine (90 mg/kg, i.p.). In addition, cocaine (15 mg/kg, i.p.)-induced CPP was significantly attenuated by GE in a dose-dependent manner. However, GE did not significantly alter behavioral sensitization induced by cocaine (15 mg/kg, i.p.). In order to understand whether GABAergic receptors are implicated in GE-mediated pharmacological action in response to cocaine, $GABA_A$ receptor antagonist bicuculline and $GABA_B$ receptor antagonist SCH 50911 were employed in the present study. GE-mediated attenuations on the cocaine-induced seizures and CPP were significantly reversed by bicuculline (0.25 or 0.5 mg/kg, i.p.), but not by SCH 50911 (1.5 or 3.0 mg/kg, i.p.). Therefore, our results suggest that GE attenuates cocaine-induced seizures and CPP via, at least in part, $GABA_A$ receptor activation.

Keywords: *Gastrodia elata* Bl, cocaine, seizure, conditioned place preference, $GABA_A$ receptors.

INTRODUCTION

Cocaine is a widely used psychostimulant. According to the United Nations Office on Drugs and Crime (UNODC), the total number of people who used cocaine at least once in 2007 is estimated maximally 21 million [1]. It has been suggested that multiple neurotransmitter systems are involved in the cocaine addiction [2] and cocaine-elicited seizures [3], one of the consequences of cocaine intoxication. For instance, extensive findings indicated that cocaine dependence is primarily mediated by enhanced dopaminergic transmission, especially in the mesocorticolimbic pathway [4], but cocaine-induced dopaminergic neurotransmission and behavioral changes can be modulated by GABAergic [5] or glutamatergic [6] innervations to the mesocorticolimbic area. Previous studies also have shown that cocaine-induced seizures can be mediated by enhanced dopaminergic and glutamatergic transmissions [7] and reduced GABAergic transmission [8].

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Gastrodia elata Blume (GE), a traditional oriental herbal agent, has long been used for epilepsy, stroke, and other neurological disorder in Asian countries, and its major components are gastrodin, p-hydroxybenzylaldehyde, p-hydroxybenzylalcohol, vanillyl alcohol, vanillin, and etc. [9]. A number of *in vitro* and *in vivo* studies have suggested therapeutic potentials of GE and its individual components against epileptic seizure/convulsion [10], cerebral ischemia [11], anxiety [12], and depression [13]. GE or its active components could exert pharmacological effects via anti-oxidation [14], anti-inflammation [15], and modulation of monoaminergic [13] and amino acid [16] neurotransmitter systems. Especially, GE has been shown to increase the extracellular GABA levels in *in vivo* microdialysis study, and to consequently enhance the GABAergic neurotransmission [17]. Earlier study suggested that activation of $GABA_A$ receptor is important for GE-mediated anxiolytic effect in mice [12].

Since GABA-mimetic drugs and GABAergic agonists have shown to attenuate behavioral sensitization or seizures induced by cocaine [18, 19], we examined whether GE affects cocaine-induced seizures, CPP, and behavioral sensitization in mice. In addition, we examined whether GABAergic receptors are involved in GE-mediated pharmacological action in response to cocaine.

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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 (Bio Genomic Inc., Charles River Technology, Gapyung-Gun, Gyeonggi-Do, South Korea) weighing 25 ± 3 g were maintained on a 12 h:12 h light:dark cycle and fed *ad libitum*.

Cocaine hydrochloride (Hansaem Pharmaceutical Company, Seoul, South Korea) and SCH 50911 (Tocris bioscience, Ellisville, MO, USA) were dissolved in 0.9 % sterile saline. Methanol extract of GE was obtained from Samsung Herb Medicine, Co. (Chunchon, South Korea) and suspended in 0.5 % carboxymethylcellulose. (+)-Bicuculline (Tocris bioscience, Ellisville, MO, USA) was dissolved in saline acidified to pH 3 using 0.1 N HCl. All solutions were immediately prepared before use. Experimental schedules are shown in Fig. (**1**).

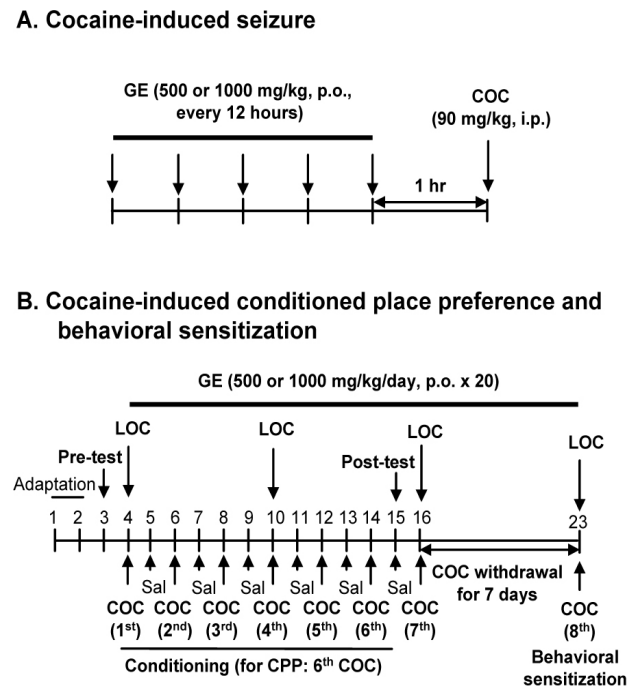

Fig. (1). Experimental schedules for evaluating the effect of GE on the cocaine-induced seizures (**A**), CPP (**B**), and behavioral sensitization (**C**). COC = cocaine, LOC = locomotor activity. For CPP, mice received cocaine (15 mg/kg/day, i.p.) once every 2 days for 12 days. For behavioral sensitization, cocaine (15 mg/kg, i.p.) was administered after 7 days of withdrawal from cocaine. Bicuculline or SCH 50911 was administered 15 min before every cocaine injection.

CPP and behavioral sensitization were performed as described previously [20]. An automated video-tracking system (Noldus Information Technology, Wageningen, The Netherlands) was employed to record and analyze the movements of mice.

Statistical analyses were performed using one-way analysis of variance (ANOVA) or *Chi* square test. A *post-hoc* Fisher's PLSD test was followed. A *P*-value <0.05 was accepted as statistically significant.

RESULTS AND DISCUSSION

GE Attenuated Cocaine-Induced Seizures

All mice receiving saline plus cocaine (90 mg/kg, i.p.) showed strong seizure behaviors with a latency of 188.3 ± 22.1 seconds. Treatment with GE significantly delayed cocaine-induced seizure onset, and significantly shortened seizure duration [for both of seizure latency and seizure duration: GE (500 mg/kg) + cocaine vs. Saline + cocaine, $P < 0.05$; GE (1000 mg/kg) + cocaine vs. Saline + cocaine, $P < 0.01$] in a dose-dependent manner. In addition, GE significantly increased survival rate [GE (1000 mg/kg) + cocaine vs. Saline + cocaine, $P < 0.05$] after cocaine-induced seizures, however GE did not affect significantly convulsing rate (Fig. **2A** and **B**). Since GE has been reported to enhance GABAergic neurotransmission, we applied $GABA_A$ receptor antagonist bicuculline and $GABA_B$ receptor antagonist SCH 50911 to understand whether GABAergic receptors are involved in the anti-convulsive effect of GE against cocaine toxicity. Bicuculline (0.25 or 0.5 mg/kg, i.p.) or SCH 50911 (1.5 or 3.0 mg/kg, i.p.), at the doses we used here, did not induce any seizure behavior, or affect seizure activity. GE-mediated anticonvulsant effect was significantly reversed by bicuculline [for both of seizure latency and seizure duration: bicuculline (0.5 mg/kg) + GE (1000 mg/kg) + cocaine vs. saline + GE (1000 mg/kg) + cocaine, $P < 0.01$] in a dose-related manner, but not by SCH 50911, suggesting that GE attenuates cocaine-induced seizures, at least in part, via $GABA_A$ receptors activation (Fig. **3A** and **B**). Our results were consistent with previous report [7], which has shown that $GABA_A$ receptor-positive modulator inhibits cocaine-induced seizures. In addition, another finding [24] suggested that cocaine suppresses the hippocampal inhibitory $GABA_A$ current, and that this suppression may contribute to cocaine-induced seizures, which are in line with our results.

GE Attenuated Cocaine-Induced CPP, but not Behavioral Sensitization

Although acute treatment with GE (1000 mg/kg, p.o.) significantly decreased locomotor activity (vs. Saline, $P < 0.05$, data not shown), mice received GE repeatedly did not show any significant difference in basal place preference (Fig. **2C**) or locomotor activity (Fig. **2D**) as compared with saline-treated mice. Cocaine (15 mg/kg, i.p.)-induced CPP was significantly blocked by GE [GE (500 mg/kg) or GE (1000 mg/kg) + cocaine vs. Saline + cocaine, $P < 0.01$] (Fig. **2C**), whereas GE failed to attenuate behavioral sensitization induced by cocaine (Fig. **2D**).

Similarly, Filip *et al.* [18, 21] showed that some GABA-mimetic agents, which increase synaptic GABA transmission, have showed different effects on cocaine-induced behavioral sensitization and self-administration, suggesting that unknown neuropharmacological mechanisms are also involved in the anti-cocaine effects of these GABA-mimetic agents. Since GE has been reported to modulate not only GABAergic system, but also other neurotransmitter systems [13, 16], it remains to be explored on this discrepancy using precise pharmacological tools/parameters. In addition, it has

been demonstrated that the expression of cocaine sensitization requires GABAergic modulation, which showing decreased GABAergic responses [22] in the striatum and nucleus accumbens, while increased GABAergic responses in the medial prefrontal cortex [23].

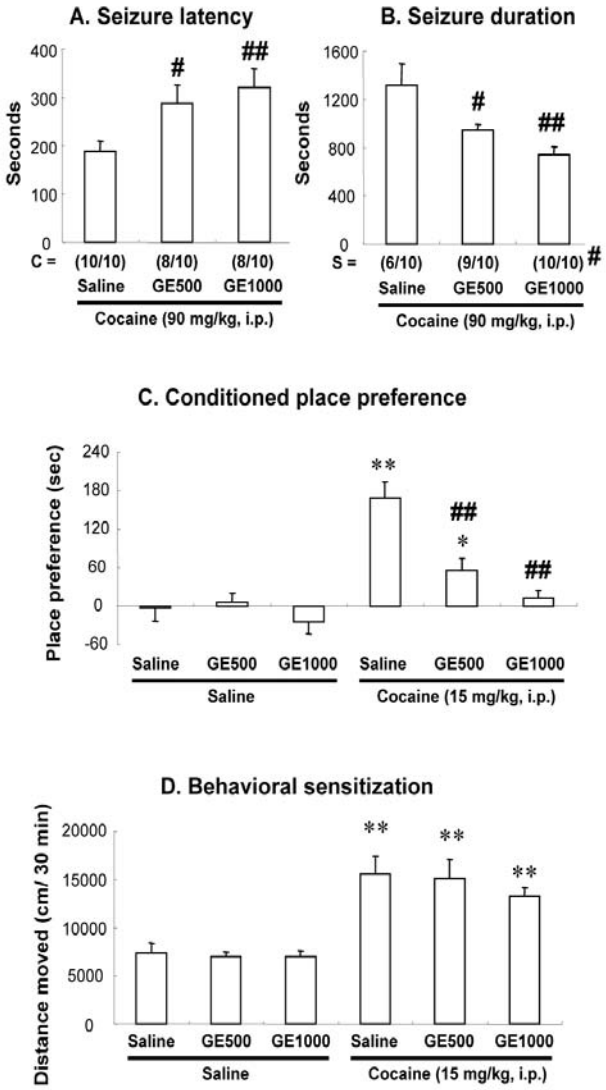

Fig. (2). Effect of GE on the cocaine-induced seizure latency (**A**), seizure duration (**B**), CPP (**C**), and behavioral sensitization (**D**). GE 500 or GE 1000 = GE 500 or 1000 mg/kg, C = convulsing ratio, S = survival ratio. Each value is the mean ± S.E.M. of 10 (A and B) or 12 mice (C and D). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Saline + Saline; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Saline + Cocaine (one-way ANOVA followed by Fisher's PLSD test; *Chi* square test was done for convulsing and survival ratio).

In order to examine whether specific GABAergic receptors are involved in the GE-mediated pharmacological action in response to cocaine-induced CPP, $GABA_A$ receptor antagonist bicuculline and $GABA_B$ receptor antagonist SCH 50911 were used in the present study. Bicuculline (0.25 or 0.5 mg/kg, i.p.) or SCH 50911 (1.5 or 3.0 mg/kg, i.p.), at the doses we used, did not affect cocaine-elicited CPP. GE-

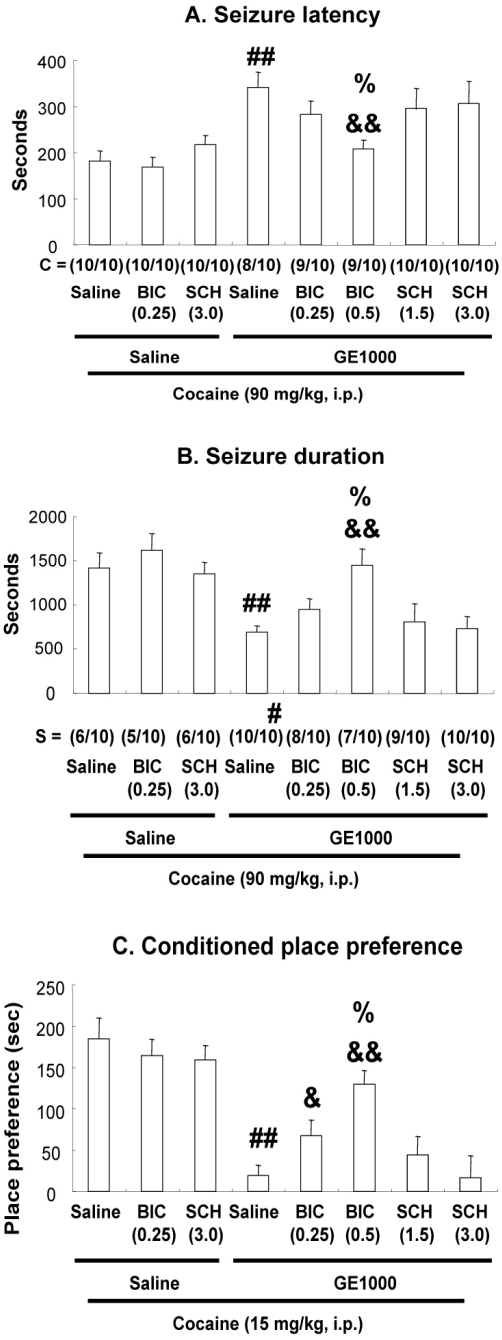

Fig. (3). Effect of bicuculline or SCH 50911 on the GE-mediated pharmacological actions in response cocaine-induced seizure latency (**A**), seizure duration (**B**), and CPP (**C**). GE 1000 = GE 1000 mg/kg, BIC (0.25) or BIC (0.5) = bicuculline 0.25 or 0.5 mg/kg, SCH (1.5) or SCH (3.0) = SCH 50911 1.5 or 3.0 mg/kg, C = convulsing ratio, S = survival ratio. Each value is the mean ± S.E.M. of 10 (A and B) or 12 mice (C). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Saline + Saline + Cocaine; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ vs. Saline + GE 1000 + Cocaine; $^{\%}P < 0.05$ vs. BIC 0.25 + GE 1000 + Cocaine (one-way ANOVA followed by Fisher's PLSD test; *Chi* square test was done for convulsing and survival ratio).

mediated attenuation on cocaine-induced CPP was significantly reversed by bicuculline [bicuculline (0.25 mg/kg) or

bicuculline (0.5 mg/kg) + GE (1000 mg/kg) + cocaine vs. saline + GE (1000 mg/kg) + cocaine, $P < 0.05$ or $P < 0.01$] in a dose-related manner, but not by SCH 50911, suggesting that stimulation of $GABA_A$ receptors may be essential for the pharmacological action of GE (Fig. **3C**). Thus, our results may be in line with the previous finding, which has shown that $GABA_A$ receptor positive-modulators inhibit rewarding properties of cocaine [24]. In addition, it has been reported that intra-hippocampal injection of muscimol, a $GABA_A$ receptor agonist, inhibits context acquisition and expression of CPP paradigm after cocaine injection [25], suggesting that modulation of hippocampal GABAergic system is important for context-cocaine associations. Thus, it remains to be explored whether this area is involved in the GE-mediated attenuation of CPP induced by cocaine.

Taken together, our finding suggests that treatment with GE attenuates cocaine-induced seizures and CPP via, at least in part, $GABA_A$ receptor activation.

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