Anxiolytic-Like Effects of *Gastrodia elata* and Its Phenolic Constituents in Mice

Ji Wook JUNG, a Byung Hoon YOON, a Hye Rim OH, a Jae-Hyeong AHN, a Sun Yeou KIM, b Sang-Yong PARK, a and Jong Hoon RYU a,b

a Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University; and b Department of Herbal Pharmacology, Graduate School of East-West Medical Science, Kyung Hee University; 1 Hoeki-dong, Dongdaemoon-ku, Seoul 130–701, Korea. Received August 4, 2005; accepted November 18, 2005

The purpose of this study was to characterize the putative anxiolytic-like effects of the aqueous extract of the rhizome of *Gastrodia elata* along with its phenolic constituents, 4-hydroxybenzyl alcohol (HA) and 4-hydroxybenzaldehyde (HD), using an elevated plus maze (EPM) in mice. The mice were administered either the aqueous *G. elata* extract orally or received an intraperitoneal injection of the phenolic constituents, 1 h before the behavioral evaluation in the EPM. A single treatment of the aqueous *G. elata* extract significantly increased the percentage of time spent and arm entries into the open arms of the EPM versus the saline controls. Among the phenolic constituents of *G. elata*, HA and HD significantly increased the percentage of time spent and arm entries into the open arms of the EPM versus saline controls (p<0.05). Moreover, there were no changes in the locomotor activity and myorelaxant effects in any group compared with the saline controls. In addition, the anxiolytic-like effects of *G. elata* extract were blocked by both WAY 100635 (0.3 mg/kg, i.p.), a 5-HT1A receptor antagonist, and flumazenil (10 mg/kg, i.p.), a GABA receptor antagonist. The anxiolytic-like effects of HA were inhibited by WAY 100635 and the effects of HD were antagonized by flumazenil. These results indicate that *G. elata* is an effective anxiolytic agent, and suggests that the anxiolytic-like effects of *G. elata* via the serotonergic nervous system depends on HA and those effects of *G. elata* via the GABAergic nervous system depends on HD.

Key words: anxiety; *Gastrodia elata*; 4-hydroxybenzyl alcohol; 4-hydroxybenzaldehyde; WAY 100635; flumazenil

Anxiety affects one eighth of the total population and has become a very important area of research in psychopharmacology in the current decade. Since the introduction of benzodiazepines in the 1960s, they have remained the most commonly prescribed treatment for anxiety. Although these compounds are the mainstay of drug treatment for anxiety disorders, they have many side-effects such as sedation, myorelaxation, ataxia, amnesia, and pharmacological dependence. Recently, research has been conducted to identify safer, more specific, and perhaps lower cost therapies.

*Gastrodia rhizome*, the steamed and dried tuber of *Gastrodia elata* (Blume) (Orchidaceae) is a very important traditional Chinese herbal medicine used to treat headache, paralysis, and other nervous disorders. Phytochemical studies of this plant have revealed the presence of several phenolic compounds, including 4-hydroxybenzyl alcohol (HA), 4-hydroxybenzaldehyde (HD), vanillin, vanillyl alcohol, β-sitosterol, and gadinor. Recently Hsieh et al. reported the anticonvulsant effect of *G. elata* in kainic acid-treated rats. In addition, the ether fraction of the methanol extracts of *G. elata* attenuates the decrease in the GABA level and the increase in the glutamate content showing anti-convulsant effects on pentylenetetrazole-induced seizures. However, there were no reports on the anxiolytic effects of *G. elata*.

The purpose of this study was to characterize the anxiolytic-like activity of the aqueous extract of *G. elata* (AEGE) and to show which constituent of *G. elata* exhibits such activity. Its anxiolytic and myorelaxant effects were examined using the elevated plus-maze (EPM) and a horizontal wire test in mice, respectively. In addition, this study also investigated which nervous systems are involved in the anxiolytic-like effects of the AEGE through the co-administration of AEGE and either flumazenil or WAY 100635.
270 nm. The column was a Capcell Pak C18 UG120 (4.6 mm x 250 mm, 5 μm, Shiseido, Tokyo, Japan) with a flow rate of 1 ml/min. The injection volume was 100 μl. The mobile phase was a 30% acetonitrile with 1% acetic acid. HA and HD contents in the AEGE were 9.46±0.07% and 3.48±0.03%, respectively. AEGE was freshly dissolved in saline and orally administered. HD and flumazenil were suspended with a 10% aqueous solution of Tween-80 for the intraperitoneal injection. Buspirone, HA, and WAY 100635 were dissolved in saline, respectively.

**Spontaneous Behavior in the Open Field Test** Testing was carried out in clear black Plexiglas boxes (40x40x40 cm) equipped with the video-based Ethovision System (Noldus, Wageningen, The Netherlands). The mice were placed in the center of the apparatus to evaluate horizontal locomotor activity 1 h after being treated with AEGE (50, 100, 200, 400 mg/kg), HA (5, 10, 25, 50, 100 mg/kg), or HD (5, 10, 25, 50, 100 mg/kg) and video-recorded for 5 min. The horizontal locomotor activity is expressed in terms of the total ambulatory distance and the frequency of rearing.

**Elevated Plus-Maze Test** The EPM for mice consisted of two perpendicular open arms (30x7 cm) and two enclosed arms (30x7 cm) with 20 cm high walls, extending from the central platform (7x7 cm). The open and closed arms were connected by a central square, 7x7 cm, to give an apparatus of a plus sign appearance. The floor and walls of the maze were constructed from the dark opaque polyvinylplastic. The maze was raised to a height of 50 cm above the floor level in a dimly lit room (20 lux) and a video camera was suspended above the maze to record the movements for analysis. Each mouse was placed at the center of the platform, its head facing an open arm. The animals were tested individually and only once for 5 min. The maze was cleaned after each trial so as to remove any residue or odors. The following measurements were taken and analyzed using the video-based Ethovision System: the number of entries into the open or closed arms, the time spent in each arm, and the total distance moved in the EPM. All the experiments were carried out between 10:00 and 16:00 o'clock.

One hour after the AEGE treatment (50, 100, 200, 400 mg/kg, p.o.), the mice were placed in the EPM. For the pharmacological constituents of *G. elata*, the mice were administered HA and HD (5, 10, 25, 50, 100 mg/kg, i.p.). The mice in the control group were given the vehicle solvent only, and the animals were tested individually once only for 5 min. In a separate antagonism study, the mice were subjected to the co-administration of AEGE (400 mg/kg, p.o.) and either WAY 100635 (0.3 mg/kg, i.p.) or flumazenil (10 mg/kg, i.p.) 1 h and 30 min prior to testing, respectively. In addition, 30 min after the HA or HD (100 mg/kg, i.p.) treatment, the mice were injected with either WAY 100635 (0.3 mg/kg, i.p.) or flumazenil (10 mg/kg, i.p.). The mice were treated with buspirone (2 mg/kg, i.p.) 1 h before EPM test and used as the positive controls.

**Horizontal Wire Test** A horizontal wire test was carried out by treating the mice with AEGE (50, 100, 200, 400 mg/kg, p.o.), HA (5, 10, 25, 50, 100 mg/kg, i.p.), or HD (5, 10, 25, 50, 100 mg/kg, i.p.) according to a slight modification of the method reported by Bonetti et al. Briefly, the mice were lifted by the tail and allowed to grasp a horizontally strung wire (1 mm diameter, 15 cm long and placed 20 cm above the table) with their forepaws, after which they were then released. The number of mice from each treatment group that did not grasp the wire with their forepaws within a 10 s period was recorded. A myorelaxant drug would impair the ability of the mice to grasp the wire, and muscle relaxation is commonly associated with sedation.

**Statistics** The values are expressed as means±S.E.M. The percentage of time spent in the open arms was calculated using the formula as follows: time spent in the open arms/(time spent in the open arms+time spent in the closed arms)×100. The percentage of the number of entries into the open arms was calculated by the same method. The data was analyzed by a one-way analysis of variance (ANOVA) followed by Student-Newman–Keuls test for the multiple comparisons. Statistical significance was set at *p*<0.05.

**RESULTS**

**Effect of AEGE and Its Constituents Treatment in the EPM** The mice in the saline-treated group typically avoided spending time on or entering into the open arms. The vehicle-treated mice remained for 73.7±4.35 s in the open arms. The percentage of time spent in the open arms was significantly increased in the AEGE-treated mice (400 mg/kg) compared with saline treated group (Fig. 1; *p*<0.05). In addition, there was also significantly increased in the percentage of open arm entries in the AEGE-treated mice (400 mg/kg) compared with saline treated group (Fig. 1; *p*<0.05). However, no significant change was observed in terms of the percentage of time spent or the open arm entries at doses of 50, 100 and 200 mg/kg of AEGE. Among the *G. elata* constituents, HA (50, 100 mg/kg) and HD (100 mg/kg) significantly increased the percentage of time spent and arm entries into the open arms (Figs. 2, 3; *p*<0.05). In the buspirone-treated (2 mg/kg) group, as a positive control, the percentages of time spent and arm entries into the open arms were significantly increased compared with the saline-treated
Effect of WAY 100635 and Flumazenil on the Anxiolytic-Like Activity of AEGE, HA, or HD

In order to determine if the anxiolytic effect of AEGE is exerted via the serotonergic or GABAergic nervous system, the AEGE (400 mg/kg) treated mice were subjected to a co-treatment with either WAY 100635, a 5-HT1A receptor antagonist, or flumazenil, a GABA_A receptor antagonist. As shown in Fig. 4, the anxiolytic-like effects of AEGE were antagonized by both WAY 100635 (0.3 mg/kg) and flumazenil (10 mg/kg). Among the constituents of *G. elata*, the anxiolytic-like effects of HA (100 mg/kg) were blocked by WAY 100635 (0.3 mg/kg) but not by flumazenil (10 mg/kg). In contrast, the anxiolytic-like effects of HD (100 mg/kg) were inhibited by flumazenil but not by WAY 100635 (Figs. 5, 6; \( p < 0.05 \)).

Effect of AEGE, HA, or HD on the Locomotor Activity Test and Horizontal Wire Test

A locomotor activity test was performed to differentiate between the possible stimulatory effects of the tested drugs on the modulation of exploratory behavior. AEGE (50, 100, 200, 400 mg/kg), HA (5, 10, 25, 50, 100 mg/kg), or HD (5, 10, 25, 50, 100 mg/kg) caused no significant changes in the total ambulatory distances or rearing frequencies compared with the saline control group (data not shown).

Moreover, AEGE (50, 100, 200, 400 mg/kg), HA (5, 10, 25, 50, 100 mg/kg), or HD (5, 10, 25, 50, 100 mg/kg) did not
compromise the mice grasping the wire compared with saline control group, indicating a lack of myorelaxation at these doses.

DISCUSSION

AEGE significantly increased the percentage of time spent in the open arms and the percentage of the open arm entries in a mouse using the EPM test, and these effects were antagonized by both WAY 100635 and flumazenil. This demonstrated the involvement of both the 5-HT1A and GABA_A receptors. Moreover, the anxiolytic-like effects of HA and HD, which are the main ingredients of AEGE, were blocked by WAY 100635 and flumazenil, respectively. Therefore, both HA and HD play roles in the anxiolytic-like effects of AEGE. However, there were no changes in locomotor activities or myorelaxant effects. Accordingly, AEGE has an anxiolytic-like effect that is mediated by 5-HT1A and GABA_A receptor activation, which is dependent on the HA and HD, and has no adverse effect, such as myorelaxant effects.

The dried rhizome of G. elata is used in traditional Chinese, Japanese, and Korean medicine as an anticonvulsant, an analgesic, and a sedative against general paralysis, epilepsy, vertigo, and tetanus. G. elata is always included as an ingredient in prescriptions issued by traditional Chinese medicine practitioners for the treatment of convulsion, epilepsy, and cerebral apoplexy. Ha et al. reported that the ether fraction of the methanol extracts of G. elata inhibited the GABA transaminase activity and on the radioligands to the GABA_A receptor complexes of rat cerebral cortices. Wu et al. reported that HA can act by suppressing the dopaminergic and serotonergic activities thereby improving learning. However, to our knowledge no study has examined the anxiolytic-like effects of AEGE and its constituents or determined what neuronal system is primarily involved in.

The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly lit open space and a fear of balancing on a relatively narrow raised surface. An anxiolytic agent increases the frequency of entries into the open arms and increases the time spent in open arms of the EPM. In this study, the buspirone treatment prolonged the percentage of time spent in the open arms and the percentage of open arm entries (Fig. 1). The AEGE treatment also prolonged the percentage of time spent in the open arms as well as the percentage of open arm entries without altering the spontaneous behavior at the chosen dose regimen. The total distances of movement on the EPM were also unchanged by the AEGE treatment versus the saline controls (data not shown). In the horizontal wire test, no significant myorelaxant effect was observed after administering the AEGE. These observations suggest that the anxiolytic-like effect of AEGE is selective, and not the result of either a general stimulation of the locomotor activity or an exploratory behavior consequent to exposure to a novel environment. In addition, after treating the mice with AEGE at 400 mg/kg, the anxiolytic-like effects were antagonized by both WAY 100635, a 5-HT1A receptor antagonist and flumazenil, a GABA_A receptor antagonist (Fig. 4). Therefore, it is believed that the 5-HT1A and GABA_A receptors primarily mediate the anxiolytic-like effects of AEGE.

HA and HD are major phenolic compounds isolated from G. elata. Previously, it was reported that HD and its derivative, 4-hydroxy-3-methoxybenzaldehyde inhibited the [3H]Ro 15-1788 and [3H]flunitrazepam binding to the benzodiazepine receptor on the GABA_A receptor complex without a concentration–response relationship. The present results showed that HD (100 mg/kg) has anxiolytic-like effects and, probably works via the activation of the benzodiazepine site of the GABA_A receptors in the central nervous system because those effects were blocked by flumazenil (Fig. 6). Many clinical and preclinical studies have confirmed the anxiolytic properties of the 5-HT1A receptor agonists, such as buspirone. EPM test showed that the HA (50, 100 mg/kg) treatment produced good anxiolytic-like activities (Fig. 2). Furthermore, these anxiolytic-like behaviors were completely blocked by WAY 100635, a specific 5-HT1A receptor antagonist (Fig. 5). Therefore, the anxiolytic-like activity of HA may be mediated via the activation of the 5-HT1A receptor. However, vanillin which is also a main ingredient of G. elata did not exhibit any anxiolytic-like effects (data not shown). Collectively, these results suggested that both HA and HD are responsible for the anxiolytic-like effect of AEGE because the anxiolytic-like effect of AEGE is mediated by the activation of the both 5-HT1A and GABA_A receptors. However, considering the contents of HA and HD from AEGE as described in the Materials and Methods part (400 mg of AEGE contained 38 mg of HA and 14 mg of HD), they are not the only compounds showing the anxiolytic-like effect because these effects were observed at slightly high doses (50 or 100 mg/kg). It is speculative that the other components may co-act with HA and HD on the anxiolytic-like effects of AEGE. Otherwise, the other unidentified compounds may have the anxiolytic properties. Further studies are required to elucidate a major compound of AEGE.
In conclusion, an extract of *G. elata* has a significant anxiolytic-like effect, and HA and HD are most likely responsible for this effect. These results also suggest that the anxiolytic-like effect of AEGE might involve the serotonergic and GABAergic nervous systems.

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REFERENCES