Studies on hypnotic effects and GABAergic mechanism of kajime (Ecklonia cava) and licorice (Glycyrrhiza glabra)

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In Asia, there are many medicinal plants with potential hypnotic activity; however, scientific evidence for their effects and mechanisms of action have been not widely investigated relative to Western countries. Therefore, the objectives of this study were to identify novel hypnotic plants and their active compounds as a source of new sleep drugs or aids from plant resources, and to investigate their sleep-promoting effects and action mechanisms. In the present study, 30 marine and 30 land plants distributed mainly in Japan and Korea were screened.

In the screening stage, the most active plant extracts were ECE (Ecklonia cava ethanol extract; kajime) in the marine plants and GGE (Glycyrrhiza glabra ethanol extract; licorice) in the terrestrial plants, and their binding activities were 0.392 and 0.093 mg/mL, respectively. The oral administration of ECE and GGE (1000 mg/kg) significantly decreased sleep latency and increased sleep duration in mice treated with pentobarbital. Their hypnotic effects were similar with the positive controls diazepam (DZP; 2 mg/kg) and valerian extract (1000 mg/kg). ECE and GGE were considered as subjects for further investigations.

To better understand the hypnotic activity of ECE and GGE, their effects on sleep architecture and profile were evaluated by analyzing electroencephalogram (EEG) and electromyogram recordings in mice. The optimized ECE and GGE increased the amounts of non-rapid eye movement sleep (NREMS) in a dose-dependent manner (100-500 mg/kg), and their rate of increase in the amount of NREMS at 500 mg/kg was 71.4% and 63.4%, respectively (the positive control DZP at 2 mg/kg: 103.8%). ECE and GGE effectively induced NREMS during the first 2 h after their administration, and there was no further disruption of sleep architecture during the subsequent period. DZP, ECE, and GGE did not significantly change rapid eye movement sleep (REMS). The BZD agent DZP
significantly decreased delta (0.5-4 Hz) activity, which is an indicator of the quality or intensity of NREMS. However, ECE and GGE induced NREMS that was very similar to physiological sleep without changing delta activity.

ECE and GGE demonstrated binding activity and in vivo hypnotic effects; therefore, they may have the potential to induce sleep via the positive allosteric modulation of GABAA-BZD receptors. To verify their GABAergic mechanism, the effects of the well-known GABAA-BZD receptor antagonist flumazenil (FLU) on the hypnotic effects of ECE and GGE were investigated. As expected, the hypnotic effect of the GABAA-BZD receptor agonist DZP was fully inhibited by FLU. Similar to DZP, a significant inhibition of the hypnotic effects of ECE and GGE by FLU was also observed. Therefore, they were found to act as the GABAA-BZD receptor agonists like DZP.

During the isolation of the active compounds, 6 phlorotannins and 3 flavonoids were obtained from ECE and GGE, respectively. The active EC phlorotannins (binding affinity, µM) were eckstolonol (ETN; 1.49), triphlorethol A (TPRA; 4.42), eckol (1.07), fucodiphlorethol G (2.97), 6,6’-bieckol (3.07), and dieckol (3.36). The active GG flavonoids were glabridin (GBD; 0.84), glabrol (1.63), and isoliquiritigenin (ILTG; 1.07).

All EC phlorotannins and GG flavonoids potentiated pentobarbital-induced sleep in mice in a dose-dependent manner (5-50 mg/kg), and their hypnotic effects were significantly inhibited by FLU. ETN and TPRA of EC and GBD and ILTG of GG, which exerted strong hypnotic activity and are of biological interest were further investigated by analyzing sleep architecture and profile. All active EC and GG compounds (50 mg/kg) significantly increased the amount of NREMS during the first 3 h after their administration, and did not change REMS, similar to the positive control ZPD (10 mg/kg). ZPD significantly decreased delta activity compared with vehicle; however, none of the EC and GG compounds produced a significant change in delta activity.

To generate more evidence for the GABAergic mechanism of the active EC and GG compounds, their potentiation on GABA-induced currents in dorsal raphe (DR) neurons was evaluated. The maximum potentiation (Pmax) values of ETN, TPRA, and ILTG were 145% (relative efficacy to DZP: 27.1%), 171% (42.8%), and 151% (30.7%), respectively. They were found to act as partial agonists of GABAA-BZD receptors relative to the well-known full agonist DZP (100%). Surprisingly, GBD showed a Pmax value of 581% (3-fold higher than the Pmax of DZP).

In the present study, it was demonstrated for the first time that EC and GG have hypnotic effects that originate from their phlorotannins and flavonoids, which have the characteristics of GABAA-BZD receptor agonists. Considering their in vitro and in vivo hypnotic effects, ECE and GGE should prove to be useful for developing natural sleep aids.