Effects of Ginsenosides on $GABA_A$ and $GABA_B$ Receptors

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Introduction

Ginseng root has been used in Chinese medicine for thousands of years. The pharmacological effects of ginseng on various organs have been reviewed (Lee *et al.*, 1986). In 1959, Petkov demonstrated that ginseng played an important role in regulating the activity of the integrating nervous system. His study also showed that *Panax ginseng* may promote stimulation as well as inhibition of the cortex in the cerebral hemisphere. Oh *et al.* (1966) showed that saponin and oil fraction of ginseng extract at low dose (10 mg/kg) reduced the sedative effect of sodium pentobarbital and shortened sleeping time, whereas a higher dose (over 10 mg/kg) prolonged it. Their study also showed that a higher dose of ginseng saponin delayed the convulsion time and the death caused by cocaine and pentylenetetrazol. It was also reported that ginseng extract exhibited suppression of condition avoidance response (Nabata *et al.*, 1973; Saito *et al.*, 1973, 1977a; Takagi *et al.*, 1972) and reduced sound discrimination (Saito *et al.*, 1977b). Recently, Lee *et al.* (1990) showed that chronic intake of *Panax ginseng* extract stabilized sleep and wakefulenss in food-deprived rats.

Neurochemical studies have been performed by Tsang *et al.* (1983) on the effects of ginsenosides on the uptake of radioactive gamma-aminobutyric acid (GABA), glutamate, dopamine, norepinephrine and serotonin in rat brain synaptosomes. Their results showed that one of the ginsenosides, Rd, was the most effective in reducing the uptake of neurotransmitters and the inhibition of uptake was in the order of norepinephrine >GABA>dopamine>glutamate

> serotonin. Dainan *et al.* (1983) showed that a significant increase in the level of norepinephrine and dopamine was observed in the diencephalon and cerebellar cortex in animals which had been treated with ginseng, 100 mg/kg, s.c., for 10 days. Park *et al.* (1984) also showed the effects of several ginsenosides on the activity of adenylate cyclase and guanylate cyclase activities in the rat brain. Their results showed that addition of guanosine monophosphate activated adenylate cyclase inhibited by ginsenosides, Rb_2 and Rc. In contrast, Rc-activated guanylate cyclase was inhibited by the addition of adenosine monophosphate and guanosine monophosphate in a dose-dependent fashion.

Since the pharmacological and biochemical studies suggested that gamma–aminobutyric acid system may play a role in pharmacological effect induced by ginseng, it would be of interest to study the effects of ginseng extract and their constituents on the GABA system. GABA is an inhibitory neurotransmitter found throughout the nervous system (Cooper *et al.*, 1986). When GABA is released from the presynaptic site, it can bind to receptors or be taken up by cells and metabolized. There are two classes of GABA receptors, called $GABA_A$ and $GABA_B$. The $GABA_A$ receptor activates a chloride channel (Turner and Whittle, 1983) and the $GABA_B$ receptor activates a second messenger system (Andrade *et al.*, 1986). The $GABA_A$ receptor activates a second messenger system (Andrade *et al.*, 1986). The $GABA_A$ receptor is a membrane-bound protein complex with binding sites for GABA, benzodiazepines, barbiturates, and channel blocking convulsants. The four units of the $GABA_A$ receptor com-

plex form chloride channels which open in response to GABA (Bormann *et al.*, 1987).

Strong evidence has suggested that GABA receptors play an important role in the action of CNS depressants. Therefore, it is the purpose of this study to investigate the effects of ginsenosides extracted from Korean ginseng on both $GABA_A$ and $GABA_B$ receptors.

Materials and Methods

Materials

[^{3}H]Muscimol, [^{3}H]flunitrazepam, [^{3}H]SR-95531, [^{3}H]baclofen and t-[^{35}S]butylbiclophosphorothionate ([^{35}S]TBPS) were purchased from New England Nuclear (Boston, MA, USA). Clonazepam was provided by Hoffmann-La Roche (Nutley, NJ, USA). All other chemicals used for receptor binding studies were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 180-200 g at the start of experiments were maintained at constant temperature, four to a cage with free access to food and water, in a room illuminated for 14 h and kept dark for 10 h.

The animals were decapitated and their brains were removed and frontal cortices were dissected on ice according to the procedure described by Glowinski and Iversen (1966) and Segal and Kuczenski (1974). Crude synaptic membrane fractions were prepared according to the method of Zukin *et al.* (1974) with slight modification (Ito and Kuriyama, 1982). Frontal cortices were homogenized in 10 volumes of 0.32 M sucrose with a Polytron (setting of 3 for 30 s). The homogenate was centrifuted at 1,000 g for 10 min. The pellet was discarded, and the supernatant was centrifuged at 20,000 g for 20 min. The crude mitochondrial pellet was resuspended in ice cold distilled water and dispersed with a Polytron (setting of 6 for 30 s). The suspension was centrifuged at 8,000 g for 20 min. The supernatant,

with the soft buffy uppercoat layer, was collected carefully, and the combined supernatant fractions were centrifuged at 48,000 g for 20 min. The resulting pellet was suspended in 50 mM Tris-citrate buffer (pH 7.1), centrifuged at 48,000 g for 20 min, and then stored at $-70\,°C$ for at least 24 h. The frozen pellet was thawed, suspended in 50 mM Tris-citrate buffer (pH 7.1), and centrifuged at 48,000 g for 20 min. The pellet obtained was resuspended with 50 volumes of 50 mM Tris-citrate buffer and incubated at 37 °C for 30 min to remove endogenous inhibitors and GABA itself. After centrifugation at 48,000 g for 20 min, the pellet was then suspended in the buffer for binding assays.

Procedures for binding assay

[^{3}H]Muscimol and [^{3}H] flunitrazepam binding assays were performed as previously described (Ito and Kuriyama, 1982). The [^{3}H]SR-95531 binding assay was carried out according to the method of Heaulme *et al.* (1987). Binding assays were carried out with 1.0 ml of the buffer containing 0.10-0.25 mg of membrane. The incubation conditions were set at 0 °C for 30 min for [^{3}H]muscimol and [^{3}H]SR-95531 and at 0 °C for 60 min for [^{3}H]flunitrazepam. Specifically bound [^{3}H]muscimol, [^{3}H]SR-95531 and [^{3}H]flunitrazepam were defined as radioactivity displaceable by 1 mM GABA, 100 μM SR-95531 and 1 μM clonazepam, respectively. The specific binding of [^{35}S]-TBPS was carried out according to the method of Ito *et al.* (1986), with 0.5 ml of 50 mM Tris-citrate buffer (pH 7.4) containing 0.20-0.25 mg of protein. The reaction mixture also contained 200 mM KCl. Incubation was at 24 °C for 100 min. Specifically bound [^{35}S]TBPS was defined as that displaceable by 100 μM picrotoxinin.

The effects of ginsenosides on $GABA_B$ receptor binding assay was done using baclofen as a ligand. The incubation conditions were [^{3}H]baclofen (10 nM), $CaCl_2$ (2.5 mM) and Tris-HCl buffer (50 mM, pH 7.4). Nonradioactive baclofen (200 μM) was used for nonspecific binding. In this assay, about 0.2 mg of rat synaptic membrane protein were added to the reaction mixture for a final volume of 0.5 ml and

incubated at 4 °C for 10 min.

After incubation, the bindings of [^{3}H]muscimol, [^{3}H]SR-95531 and [^{35}S]TBPS were terminated by rapid filtration through Whatman GF/B glass fiber filters using cell hervesters (model M-24; Brandel). The filters were washed twice with ice-cold buffer. The washing buffer for [^{35}S]TBPS binding also contained 200 mM KCl. Then, filters were transferred to scintillation counting vials containing 10 ml of Safety Solve (Research Products International Corp., Mount Prospect, IL, USA). Radioactivity trapped on the filter was measured by conventional scintillation techniques.

[^{3}H]Baclofen binding was terminated by centrifugation at 42,000 × g for 10 min. The pellets were superficially washed twice with 1.5 ml Tris-citrate buffer and dissolved overnight with 0.2 ml of Protosol. The liberated radioactivity was counted in 10 ml of scintillation cocktail.

Protein concentration was measured according to the method of Lowry *et al.* (1951).

Statistics

Data were analyzed using analyses of variance. When significant effects were observed, the Newman-Keul's multiple range test was applied for the degrees of significance.

Results and Discussion

The effects of ginseng total saponin fraction on GABA_A receptor were carried out by using both GABA_A agonist and antagonist ligand, muscimol, and SR-95531, respectively. The results indicated that ginseng total saponin fraction (1 mg/ml) inhibited specific [^{3}H]muscimol binding at the concentration of both 2.5 nM and 50 nM of [^{3}H]muscimol.

Purified ginsenosides, Rb$_1$, Rb$_2$, Rc, Re, Rf and Rg$_1$ inhibited the specific [^{3}H]muscimol binding at 2.5 nM of [^{3}H]muscimol, but not 50 nM of [^{3}H]muscimol. The ginseng total saponin fraction also reduced the specific [^{3}H]SR-95531 binding at a concentration of 5 nM and 100 nM of [^{3}H]SR-95531.

The *in vitro* addition of ginseng total saponin fraction (1 mg/ml) inhibited specific [^{35}S]TBPS binding at the concentration of 55 nM of [^{35}S]TBPS. However, the ginsenosides, e.g., Rb$_1$, Rb$_2$, Rc, Re, Rf and Rg$_1$, at 100 μM, did not affect [^{35}S]TBPS binding. On the hand, the *in vitro* addition of ginseng total saponin fraction (1 mg/ml) enhanced the specific binding of [^{3}H]flunitrazepam binding. The purified ginsenosides (Re and Rf) also increased [^{3}H]flunitrazepam binding.

The effect of ginsenosides on GABA_B receptor binding assay was also done by using baclofen (an agonist) as a ligand. The specific [^{3}H]baclofen binding was reduced by ginseng total saponin fraction and Rc.

In summary, our results showed that total saponin fraction inhibited specific [^{3}H]muscimol (high and low affinity sites), [^{3}H]SR-95531 (high and low affinity sites), [^{35}S]TBPS and [^{3}H]baclofen binding. The results also showed that purified ginsenosides, Rb$_1$, Rb$_2$, Rc, Re, Rf and Rg$_1$ also inhibited high affinity binding of [^{3}H]muscimol binding. A ginsenoside, Rc, also inhibited specific [^{3}H]baclofen binding. A ginsenoside, Rc, also inhibited specific [^{3}H]baclofen binding. On the other hand, total saponin fraction, Re and Rf, enhanced specific [^{3}H]flunitrazepam binding. These results suggest that certain ginsenosides from ginseng extract may interact with both GABA_A and GABA_B receptors.

Experiments are in progress to further characterize the effects of ginsenosides on the GABA-benzodiazepine chloride channel complex and the GABA_B receptor. Future studies are planned to investigate the effects of ginseng on GABA systems, the pharmacological properties of the ginsenosides relating to GABA systems and the correlation of pharmacologic responses with biochemical findings.

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