Psychotropic Effects of Ginseng Saponins on Agonistic Behavior in Male and Female Mice

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Abstract: Psychotropic actions of crude ginseng saponins(CGS), pure ginsenoside Rb1(GS-b1) and ginsenoside Rg1(GS-g1) isolated from the root of *Panax ginseng*, were evaluated by determining their effects on agonistic behavior in male(Experiment 1) and female(Experiment 2) mice, using a biologically relevant method. The results of experiment 1 demonstrated that CGS and GS-b1 significantly suppressed aggressive episodes(offensive sideways posture and attack bite) in a dose-dependent manner when the resident was drugged, whereas GS-g1 was ineffective. However, when the intruder was treated with one of three ginseng saponins, agonistic behavior between resident and intruder males was not altered. In experiment 2, acute administration of CGS and GS-b1 significantly suppressed maternal aggression, whereas GS-g1 was ineffective. As compared with the vehicle-treated group, chronic treatment with CGS and GS-b1 significantly suppressed maternal aggression, while GS-g1 showed a tendency to increase the frequency of attack bite by females. These findings clearly indicate that the root of *Panax ginseng* contains psychoactive ingredient which can suppress both intermale and maternal aggression in mice. We suggest that the present results have important implications for the clinical usefulness of ginseng saponins in psychiatric medicine.

Introduction

The root of *Panax ginseng* has served as an important component of the Chinese prescription "Kan-Pou" for thousands of years in the Far East. Although the therapeutic efficacy of ginseng in a wide variety of illnesses has been demonstrated through its long history of use in Oriental medicine, experimental proof supporting these clinical applications, especially psychotropic actions, is limited.

Ever since the classic finding that benzodiazepine anxiolytics have a "taming" effect on savage zoo animals without producing excessive sedation and sleep, various experimental models of aggression have been developed and used for evaluating the psychotropic action of drugs. Most of the testing procedures require, however, potentially aversive experimental manipulations such as electrical footshock or physical provocation. The topography of fighting episodes under these conditions differs from the species-specific patterns of aggressive behavior seen ferally.

Recently, new approaches based on ethological considerations have been introduced for the behavioral analysis of drug action(for review see Miczek and Barry, 1976; Miczek and Krsiak, 1979), under the general heading of "ethopharmacology". Such procedures make an interdisciplinary approach possible for the study of both species-typical and atypical, disordered behavior(for review see Miczek, 1983). In this context, agonistic behavior, which is a system of behavioral patterns, proven adaptive in conflict situations, is the focus rather than aggression *per se*. Consequently, a resident-intruder paradigm which does not employ aversive experimental manipulations has been introduced into the preclinical evaluation of psychotropic drugs(Miczek and Yoshimura, 1982; Yoshimura and Ogawa, 1982; 1983; Yo-

shimura *et al.*, 1987). Resident male mice which have cohabited with a female for several weeks will reliably show species-specific agonistic behavior when confronting an unfamiliar intruder male in the resident's home cage.

On the other hand, in preclinical psychopharmacology, little attention has been paid to female aggression in laboratory animals. Increasing evidence, however, indicate that lactating animals of a variety of species manifest vigorous attack toward an intruder(for review see Svare, 1981). Among the variables which affect the incidence and intensity of maternal aggression in mice, a sociopsychological factor during pregnancy and early lactation is of importance: females which have been cohabited with their partner males rarely display attack behavior toward an intruder, while females which have been housed alone after the mating manifest intense attack bites. Our systematic behavioral studies on maternal aggression showed that female ICR mice displayed intense aggressiveness toward intruders during early(between 0 and 12 days after parturition) lactation(Yoshimura *et al.*, 1986; Yoshimura, 1987), Because of the stable aggression level and because of the constancy of its behavioral structure it appears that maternal aggression in mice serves as useful model for evaluationg the psychotropic action of a drug on female aggression.

The present study was conducted to investigate the psychotropic effects of various ginseng saponins on agonistic behavior in male and female mice, using the resident-intruder paradigm. The following saponins were administered intraperitoneally : crude ginseng saponin(CGS), pure ginsenoside Rb1(GS-b1) and ginsenoside Rg1(GS-g1).

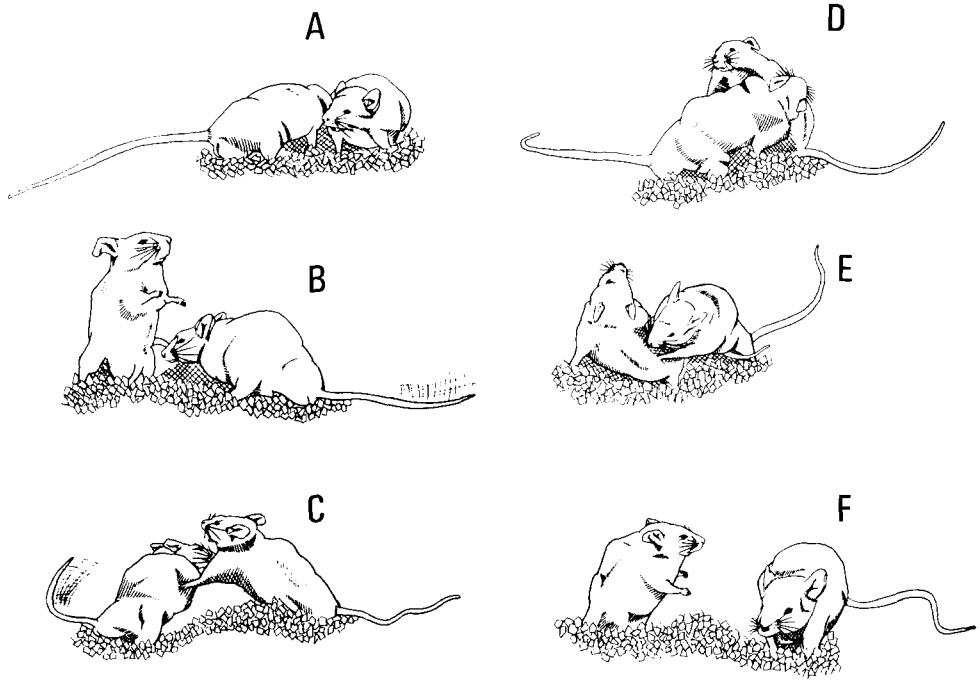

Fig.1. *Agonistic acts and postures shown by resident and intruder mice. A: Anogenital contact by the resident mouse(left). B: Mincing with tail rattling by the resident mouse(right) and defensive upright posture by the intruder mouse(left). C and D: Offensive sideways posture by the resident mouse(left). E: Attack bite by the resident mouse(right). F: Defensive upright posture by the intruder mouse(left).*

General Methods

Subjects: All animals employed were ICR albino mice obtained originally from Clea Inc. (Osaka, Japan) and inbred in this laboratory. All subjects had free access to food and water, and were handled once per week for cage cleaning. Their cage floors were covered with wood shavings. The temperature in the vivarium was maintained at 23±1℃, and a 12hr light-dark cycle was automatically controlled(lights on at 7:00 a.m., off at 7:00 p.m.).

Behavioral testing: Agonistic behavior was observed in the resident's home cage, mode from clear polycarbonate(21×32×14cm). The test was started immediately after the introduction of an intruder, and lasted for 5 min from the time of the first attack bite. The test was terminated if the resident mouse did not attack an intruder within 5 min. Each test was recorded using a video monitor system; at a later time, the following behavioral elements were scored, with the aid of a computerized event recorder(TORAY CO. Ltd., Japan): resident's behavior(offensive sideways posture, tail rattle, attack bite, locomotion, and rearing); intruder's behavior(defensive upright posture, escape, locomotion, and rearing). The observer, who did not know the drug condition, depressed one key on a console for as long as each behavioral element occurred. The pattern of species-specific agonistic behavior between resident and intruder males is illustrated in Fig.1, and the typical pattern of maternal aggression by a lactating female resident on an unfamiliar male intruder is shown in Fig.2. Maternal and inter-male aggression in mice differ with respect to the sequence and the frequency of occurrence of components of agonistic behavior. In aggressive male mice, attack bite is usually preceded by offensive sideways posture and frequently followed by rapid pursuing the intruder. However, aggressive female mice infrequently displayed these elements. The frequency of each behavioral item was analyzed. Agonistic confronations were conducted between 13:00 and 16:00 hours.

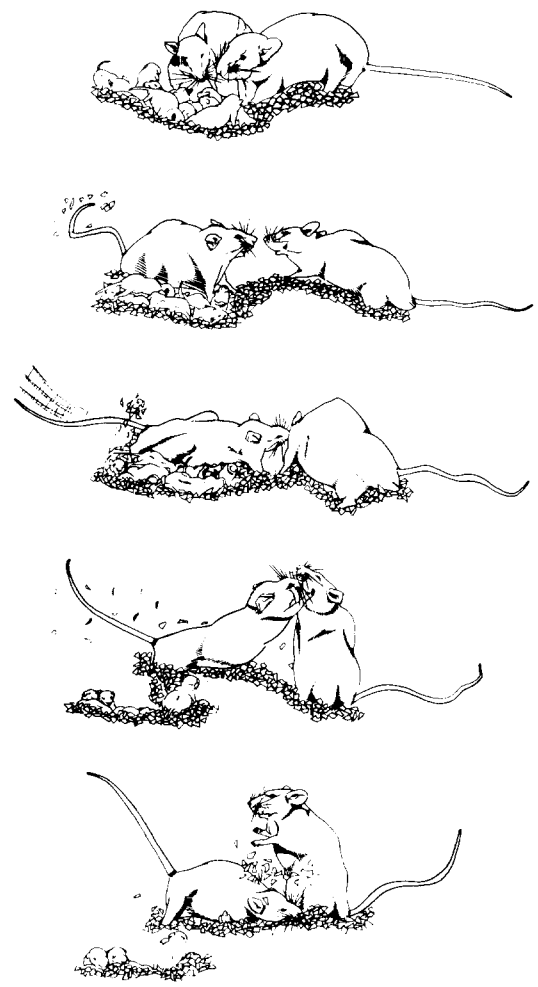

Fig.2. *Agonistic acts and postures shown by a parturient female mouse(left). Maternal aggression in mice is characterized by immediate attack bites and threat such as mincing and sideways posture is actually rare. Young, sexually naive male mice were used as intruders.*

Drugs: The drugs were given intraperitoneally in an isotonic saline vehicle in a volume of 0.1 m*l* per 10g body weight. Both GS-b1 and GS-g1 employed in this study contain a high percentage of each sapogenins(more than 98%) and their purities were confirmed with TLC and NMR methods. CGS was extracted by alcohol from the powder of Red ginseng, and includes all saponins.

Statistical analysis: The statistical evaluation was performed by means of analysis of variance or analysis of covariance, and multiple comparisons of means were performed using the Duncan's multiple-range test.

Experiment 1.

Procedure: Each resident male mouse was housed together with an age-matched female. The intruder male mice were housed five animals per cage. At approximately 5 weeks after initial cohabitation, the behavioral testing began: the resident male mouse was confronted in his home cage with a group-housed male intruder. Immediately before the introduction of the intruder, the female mouse and, if present, pups were removed. After completion of the test for agonistic behavior, the removed mice were returned to their home cage. Three separate series of experiments were conducted, using CGS, GS-b1, and GS-g1. Drug tests were conducted twice a week and each test was separated by 3-4 days from the next. Residents and intruders were drugged on alternate test days, so that only one mouse in a test was drugged, and no animal was drugged more than once per week: the drugged residents were tested in their home cage with nondrugged intruders, and the drugged intruders were tested in the home cage of nondrugged resident mice. In each experiment, the different doses of drug were given in a randomized order to resident or intruder mice, so that each animal was subjected to all doses during the 4 weeks of the experimental period. All drugs were administered 30 min before testing.

Experiment 2.

Procedure: Each resident female mouse was housed together with an age-matched male, and on the 5th day of initial cohabitation her partner male was removed. Then, all the female mice had been housed alone during pregnancy and lactation. The intruder male mice were housed 10 animals per cage. Behavioral testing for evaluating acute effects of a drug was performed on postpartum days 5 and 7; drug was administered only on the 7th day, and the frequency of each behavioral item was then compared with the corresponding pre-levels which had been determined on the 5th postpartum day. All drugs were administered 30 min before behavioral testing. For evaluating chronic effects of a drug, drug treatment(once per day between 9:00 and 10:00 a.m.) was started immediately after the removal of the partner

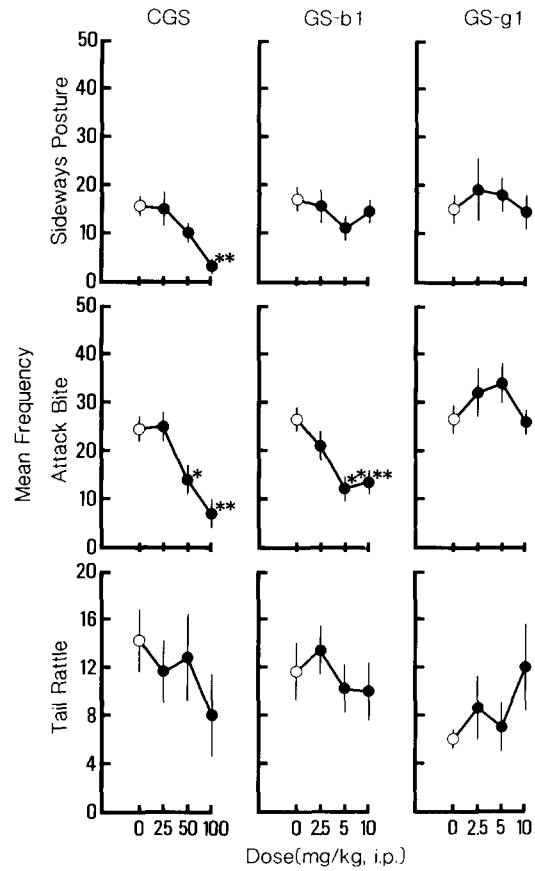

Fig.3. *Effects of various ginseng saponins on resident's sideways posture(top), attack bite(middle), and tail rattle(bottom) when the resident mice were the drug recipients. Open circles indicate vehicle control values; vertical lines indicate ±S.E.M. Significant differences from the corresponding vehicle control levels are indicated by* $*p<0.05$ and $**p<0.01$.

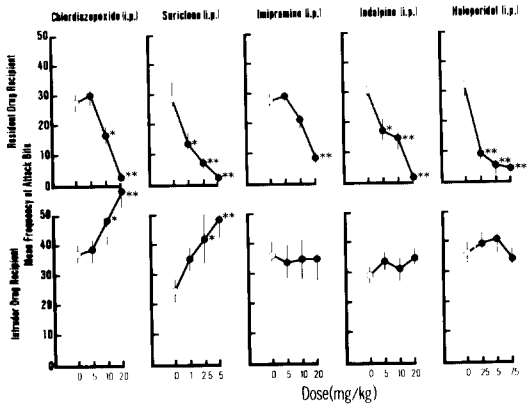

Fig.5. *Effects of psychotropic drugs on the frequency of attack bites in male mice. Top panels indicate the resident's attack bites when the resident mice were the drug recipients, and bottom panels show the resident's attack bites when the intruder mice were the drug recipients. Open circles indicate vehicle control values; vertical lines indicate ±S.E.M. Significant differences from the corresponding vehicle control levels are indicated by * $p<0.05$ and ** $p<0.01$.*

drugs which have an anxiolytic action caused intruder mice to be more frequently attacked by nondrugged resident mice. It is especially noteworthy that a nonbenzodiazepine anxiolytic like suriclone increased the frequency of the resident's attack bites when the intruder was drugged. The question that arises is why only drugs which possess anxiolytic actions show indirect effects on the male resident's aggression. It is likely that fear or anxiety in intruder mice, produced in such confrontations, are suppressed by anxiolytics. On the other hand, antidepressants(imipramine and indalpine) and antipsychotics(haloperidol) have only direct suppressive effects on the resident's attack behavior. In the present study, we found no significant changes in agonistic behavior when intruder mice were the drug recipients. Therefore, it appears that the behavioral effects of ginseng saponins are comparable to those of antidepressants, which have only a direct suppressive effect on male resident's attack behavior at doses which cause on motor dysfunction.

The results of experiment 2, that either acute or chronic administration of CGS and GS-b1 significantly suppressed maternal aggression, is interesting in the light of our recent study(Yoshimura and Ogawa, 1987) that antidepressant imipramine(5 and 10mg/kg) showed a significant decrease in the frequency of attack bite by females through either acute of chronic administration of the drug, without any impairment of motor activity. Chlordiazepoxide(5 and 10 mg/kg) significantly increased maternal aggression and haloperidol(0.2 and 0.4 mg/kg) suppressed both maternal aggression and locomotion. These pieces of evidence suggest the possibility that suppressive effect of GS-b1 on maternal aggression is similar in nature to the effect of antidepressants(see Table 1).

The present findings that CGS and GS-b1 significantly suppressed agonistic behavior in male and female mice, while GS-g1 was ineffective, clearly indicate that the root of *Panax ginseng* contains psychoactive ingredient. In evaluating the effect of a drug on aggressive behavior, it is important to note whether or not the dose employed alters motor performance. To assess the animal's motor activity, we determined changes in locomotion during agonistic confrontations, and found that none of the treatments employed significantly suppressed locomotion, except 100 mg/kg CGS which suppressed resident motor behavior. Thus, the suppressive effects of CGS and GS-b1 are specific to agonistic behavior, and it appears that 100 mg/kg CGS may be a relatively high dose in comparison with GS-b1 and GS-g1.

In evaluating the pharmacological action of a drug, pharmacokinetic factors such as distribution, excretion and metabolism are important. Because of the methodological difficulty of detecting tissue concentration of ginseng saponins, however, there has been no study in which the distribution of ginseng saponin in the brain was clearly demonstrated. At present, therefore, it is impossible to determine whether or not the observed behavioral effect is due to

male(5th day of initial cohabitation), and terminated on the 3rd postpartum day. Behavioral testing was performed on the 5th postpartum day without any injection of drug.

Results

Experiment 1: The effect of ginseng saponins on resident's aggressive episodes(offensive sideways posture, attack bite, and tail rattle) when resident was drugged is shown in Fig.3. Administration of CGS to resident mice significantly suppressed the frequency of attack bite and sideways posture. GS-b1 also showed a significant suppressive effect on resident's attack bite, but sideways posture and tail rattle were not reliably affected by the drug. By contrast, GS-g1 at all doses employed failed to alter the frequency of sideways posture, attack bite, and tail rattle. When the resident's behavior was altered by injection of ginseng saponins, defensive behaviors such as upright posture and escape were also altered. There was no significant change in intruder escape behavior when confronted with GS-g1-treated residents. CGS significantly suppressed resident locomotion at the highest dose(100mg/kg). GS-b1 did not affect the frequency of locomotion, whereas GS-g1 significantly increased the frequency of locomotion by residents.

When intruder mice were treated with one of three ginseng saponins, aggressive episodes by untreated residents were not altered. The frequency of upright posture, escape, or locomotion was also not altered by these drugs.

Experiment 2: The acute effect of various ginseng saponins on the frequency of attack bite in female residents is shown in Fig.4. Analysis of covariance revealed that there was a significant differences among the 7 groups. The multiple comparisons indicated that CGS and GS-b1 significantly suppressed the frequency of attack bite. Administration of neither vehicle nor GS-g1 affected the maternal aggression. Although CGS at the high dose(100mg/kg) significantly suppressed the frequency of locomotion, administration of vehicle, GS-b1 and GS-g1, did not affect the frequency of locomotion at the doses employed.

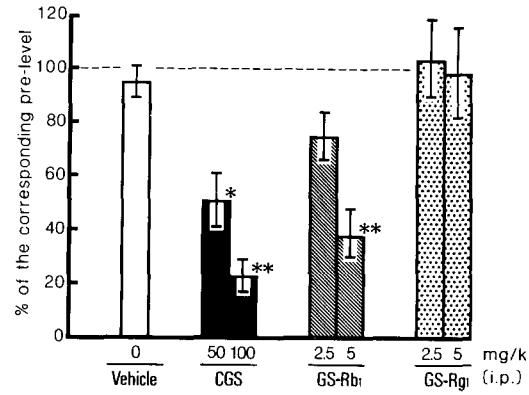

Fig.4. *Acute effects of various ginseng saponins on the frequency of attack bites in female mice. Each column shows the post-level and is refered as the percentage of the corresponding pre-level(for details see in the text) Vertical lines indicate ±S.E.M. Significant differences form the corresponding prelevels are indicated by* $*p<0.05$ *and* $**p<0.01$.

Chronic treatment with either CGS(25 and 50mg/kg) or GS-b1(1.25 and 2.5mg/kg) significantly suppressed the frequency of attack bite by females in a dosedependent manner. Females which has been treated chronically with GS-g1(2.5mg/kg) showed a tendency to increase the frequency of attack bite. Analysis of variance did not show any significant difference in the frequency of locomotion among the 7 threatment groups.

Discussion

In experiment 1, we clearly demonstrated that CGS and GS-b1 significantly suppressed intermale aggression. The intensity of agonistic behavior in male mice is known to be altered by the confronted opponent's nature, and the effect of a drug on agonistic interactions depends on whether aggressive or defensive animals are the drug recipients(Yoshimura, 1987; Yoshimura and Ogawa, 1984). As shown in Fig.5,

Table 1. *Effect of Psychotropic Drugs on Agonistic Behavior in Male and Female Mice*

Drugs	Male Aggression						Female Aggression			
	Direct Effect			Indirect Effect			Acute Effect		Chronic Effect	
Anxiolytics										
Chlordiazepoxide	5 ±	10 ↓↓	20 ↓↓↓	5 ±	10 ↑↑	20 ↑↑↑	5 ±	10 ↑↑	5 ±	10 ±
Suriclone	1 ↓	2.5 ↓↓↓	5 ↓↓↓	1 ±	2.5 ↑↑↑	5 ↑↑↑	1.5 ±	3 ↓↓	1.5 ±	3 ↓↓↓
Antidepressants										
Imipramine	5 ±	10 ±	20 ↓↓↓	5 ±	10 ±	20 ±	5 ↓↓	10 ↓↓↓	5 ±	10 ↓↓
Indalpine	5 ↓↓	10 ↓↓↓	20 ↓↓↓	5 ±	10 ±	20 ±	5 ±	10 ↓↓↓	5 ?	10 ?
Antipsychotics										
Haloperidol	0.25 ↓↓↓	0.5 ↓↓↓	0.75 ↓↓↓	0.25 ±	0.5 ±	0.75 ±	0.2 ↓↓↓	0.4 ↓↓↓	0.1 ±	0.2 ±
Candidates										
Ginseng Saponin	25 ±	50 ↓↓	100 ↓↓↓	25 ±	50 ±	100 ±	50 ↓↓	100 ↓↓↓	25 ±	50 ↓↓↓
Ginsenoside-Rb1	2.5 ±	5 ↓↓	10 ↓↓	2.5 ±	5 ±	10 ±	2.5 ↓	5 ↓↓	1.25 ±	2.5 ↓↓↓
Ginsenoside-Rg1	2.5 ±	5 ±	10 ±	2.5 ±	5 ±	10 ±	2.5 ±	5 ±	1.25 ±	2.5 ±

±: not significant, ↓ or ↑ 10~24% decrease or increase, ↓↓ or ↑↑ 25~49% decrease or increase, ↓↓↓ or ↑↑↑ 50~100% decrease or increase, Each number indicates the dose level(mg/kg, *i.p.*) For details see in the text.

some direct action of GS-b1 on brain function.

In conclusion, CGS and GS-b1 administered intraperitoneally have potent antiaggressive effects, and it is clear that GS-b1 is one of psychoactive ingredients of ginseng root. The present findings have important implications for the clinical usefulness of ginseng saponins in psychiatric medicine.

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