PHARMACOLOGICAL PROPERTIES OF *PANAX GINSENG* ROOT

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A number of authors have reported the pharmacological properties of ginseng (1), but pharmacological studies were not complete as most have used only crude extracts and systematic data on the pharmacological active components of ginseng were not included. Substances listed in Tab. 1 have been found in ginseng by many organic chemists. What is the main pharmacological substance of ginseng and which substance has what kind of pharmacological activity in ginseng.? Surely, choline (2) is a precursor of acetylcholine, and has the same pharmacological actions as acetylcholine but is less active. Cholinergic action (3, 4) confirmed in ginseng is probably due to choline, but choline does not have other actions found in ginseng preparations. Ascorbic acid and vitamin B (5) are used as vitamins, but they don't show the pharmacological actions of ginseng reported. Moreover, choline and vitamins are not specific substances contained only in ginseng. Prof. S. Shibata and his coworkers have been determining the structure of saponins in ginseng (6) and provided us every fraction obtained in the step of saponin isolation and individual saponins. These saponins are specific compounds in ginseng, and were found to exert many pharmacological actions by blind and specific screenings. Surely, both ginsenoside Rb_1 (GRb_1) and ginsenoside Rg_1 (GRg_1) are among the most important pharmacological active components, but they have only part of the pharmacological properties of ginseng which have been noted. We would like to discuss our results and two topics on pharmacological studies of ginseng saponins afterwards. At the beginning of our pharmacological studies of ginseng, the first we had to do was to confirm the pharmacological properties which many researchers have reported. We adopted the blind screening consisted of 5 tests, and the specific screening to determine the pharmacological properties which have been noted in ginseng such as antifatigue activities and could not be presumed by blind screening. We reconfirmed many different pharmacological properties which have been already reported by many investigators and found a few pharmacological properties which have

Table 1. Substances contained in *Panax ginseng* root

Lipophilics;
Fatty acids
Panacene (β-elemene, panaxynol,
β-sitosterol, daucosterol
Saponins; ginsenoside Ro, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh
Amino acids, peptides
Basic compounds (choline)
Carbohydrates (glucose, fructose, sucrose, maltase, trisaccharides)
Ascorbic acid, Vitamin B complex
Germanium ion

never been reported yet in ginseng. We are separating pharmacological active substances. We also noticed that multiple pharmacodynamic activities of ginseng originated from various ingredients and there are many pharmacologically antagonistic actions in ginseng. Tab. 2 shows antagonistic actions reported by many pharmacologists with ginseng. In the oldest medical book in the world, Sheng-nung Pen-tsao Ching, we also found that ginseng is effective on what is normally assumed to be contradictory states. We can not say how these antagonistic activities play an important role in the medicinal effects of ginseng, but adaptogenic activity (7), proposed by Prof. Brekhman, may occur due to the existence of its antagonistic activities.

Table 2. Antagonistic activities obtained from *Panax ginseng* root

Shen-nung Pen-tsao Ching; a tonic to the five viscera, quieting the animal spirits, establishing the soul, allaying fear, expelling evil effluvia, brightening the eye, opening up the heart, benefiting the understanding.
Central nervous system stimulant and depressant activities
Histamine releasing and antihistamine-like activities
Cholinergic and anticholinergic activities
Blood pressure elevation and fall
Diuretic and antidiuretic activities
Hemolytic and antihemolytic activities
Aggravation and inhibition of stress ulcer
Adaptogenic activities

1. Pharmacological properties of saponins in ginseng and *Panacis japonica* Rhizoma (8, 9)

Tab. 3 shows the pharmacological properties of saponins in ginseng and *Panacis japonica* Rhizoma. There are three different types of saponins in them. GRb_1, GRb_2, GRc and chikusetsusaponin III are 20S-protopanaxadiol glycosides. They potentiated the sleeping time produced by the i.p. administration of 70 mg/kg of hexobarbital, inhibited the writhing syndrome induced by acetic acid, and protected restraint and water immersion stress ulcer. From these results they may have a sedative action. Moreover, some of them promoted intestinal motility. GRe and GRg_1 are 20S-protopanaxatriol glycosides. Restraint and water immersion stress ulcer of the mouse was aggravated by 50 mg/kg of GRg_1. GRe promoted intestinal motility. GR_0 and chikusetsusaponin IV and V are oleanolic acid glycosides. They have little influence on the sleeping time produced by hexobarbital, but inhibited the writhing syndrome induced by acetic acid. Moreover, they protected stress ulcer and promoted intestinal motility. From these results, they may have anti-inflammatory action without side effect such as ulceration.

2. Pharmacological properties of GRb1 and GRg1

Tab. 4 shows the pharmacological and biochemical properties of GRb_1 and GRg_1 CNS-depressant action of GRb_1 was confirmed with specific screenings. Anti-convulsant and analgesic actions were also significant. Body temperature lowering and antipyretic activity were observed. Antipsychotic action of GRb_1 was also confirmed with specific screenings. Conditioned avoidance response was remarkedly depressed in the pole-climbing and shuttle-avoidance tests. Performance of position discrimination behavior in the Y-maze test was depressed. Fighting behavior induced in pairs of mice by electric stimulation was also inhibited. CNS-stimulant action of GRg_1 was examined with specific screening as GRg_1 was supposed to have a mild CNS-stimulant action in blind screening. We could not find significant stimulant action of GRg_1 in any of the tests we conducted. Only a slight increase of motor activity was significantly observed with low doses administered chronically in the two tests. We thought GRg_1 might not have stimulant action, but it had stimulant action in Y-maze test. GRg_1 shortened response latency and running time. Accordingly GRg_1 possesses a somewhat different CNS-stimulant action from caffeine and methamphetamine. GRg_1 might affect animal behavior. We are studying the effect of GRg_1 on behavior using different types of apparatus. It is very difficult to give any definite conclusion on the results of behavioral experiments, but they may indicate that GRg_1 has a facilitatory effect on the acquisition of learning response. We would

Table 3. Pharmacological properties of saponins

Origin	Character	Aglycone	a	b	c	d
Panax ginseng	Crude Saponins	20S-protopanaxadiol 20S-protopanaxatriol	4	4	2	2
	Neutral saponins	20S-protopanaxadiol	3	3	0	3
	Ginsenoside Rb_1	20S-protopanaxadiol	3	4	4	3
	Ginsenoside Rb_2	20S-protopanaxadiol	4	4	0	3
	Ginsenoside Rc	20S-protopanaxadiol	3	3	3	2
	Ginsenoside Re	20S-protopanaxatriol	0	1	4	0
	Ginsenoside Rg_1	20S-protopanaxatriol	0	-3(1)	1	0
	Ginsenoside Ro	Oleanolic Acid	1	2	0	3
Panax pseudoginseng var. *japonicum*	Crude saponins	20S-protopanaxadiol Oleanolic Acid	2	2	0.5	1
	Chikusetsusaponin III	20S-protopanaxadiol	2	2	2	1
	Chikusetsusaponin IV	Oleanolic Acid	1	2	2	2
	Chikusetsusaponin V	Oleanolic Acid	1	2	0	3

a: Potentiation of hexobarbital (mice, i.p.)
b: Protection of water immersion stress ulcer (mice, i.p.)
c: Acceleration of traverse of carbon black (mice, p.o.)
d: Inhibition of writhing induced by acetic acid (mice, p.o.)

Score 0: No effect
0.5: 1/2 LD_{50} < Dose ($p < 0.10$)
1: 1/2 LD_{50} < Dose ($p < 0.05$)
2: 1/5 LD_{50} < Dose < 1/2 LD_{50} ($p < 0.05$)
3: 1/10 LD_{50} < Dose < 1/5 LD_{50} ($p < 0.05$)
4: Dose < 1/10 LD_{50} ($p < 0.05$)

like to show you one experiment of our results afterwards. The effect of GRg_1 on recovery from exhaustion was confirmed with specific screening. After various forced exercises or stresses were applied to the mouse to produce a fatigued state, a 4 hr oscillation movement was selected. After oscillation, GRg_1 was given i.p. and 6 indexes were used to measure fatigued states; rectal temperature, motor coordination, body and grip tones, and spontaneous and exploratory movements. GRg_1 produced significant facilitation of recovery from an exhausted state (10). Restraint and water immersion stress ulcer of the mouse was protected by GRb_1, but was aggravated by GRg_1. As inhibition of gastric secretion was not recognized in either, GRb_1 may have an anti-stress action. GRb_1 promoted intestinal motility but GRg_1 had no influence. A weak anti-inflammatory action of GRb_1 was confirmed with carrageenin edema test. I hit upon the potentiation of the nerve growth factor(NGF)—mediated fiber production by GRb_1 in culture of chicken embryonic dorsal root

Table 4. Pharmacological properties of ginsenoside Rb_1 and ginsenoside Rg_1

Ginsenoside Rb_1	Ginsenoside Rg_1
CNS-depressant action	A weak CNS-stimulant action
Anticonvalsant action	A slight increase of motor activity
Analgesic action	Potentiation of DB-performance (Y-maze test)
Antipyretic action	
Antipsychotic action	Behavioral effect
Inhibition of conditioned avoidance response (pole-climbing and shuttle box tests)	Acceleration of acquisition of CAR & DB (pole-climbing and Y-maze tests), reversal learning (Y-maze test) & one trial passive avoidance learning (step down method)
Protection of stress ulcer (anti-stress action)	
Increase of gastrointestinal motility	Antifatigue action
A weak anti-inflammatory action	Aggravation of stress ulcer
Potentiation of the NGF-mediated fiber Production in chicken embryonic DRG & Symp-G.	
Antihemolytic action	
Acceleration of glycolysis, cholesterol synthesis (serum & liver) & nuclear RNA synthesis	
Acceleration of serum protein synthesis	

ganglia (DRG) and lumbar sympathetic ganglia (Symp-G) (11). We are studying the mechanism of GRb_1 on the potentiation of the NGF effect. We would like to discuss this new topic of research afterwards. In the biochemical field, anti-hemolytic action of GRb_1 and GRg_1 was observed (12). GRb_1 and GRg_1 accelerated glycolysis, cholesterol synthesis and nuclear RNA synthesis. GRb_1 also accelerated serum protein synthesis (13, 14).

3. Effect of GRg1 on animal behavior

We studied the effect of GRg_1 on the acquisition of conditioned avoidance response in the chronic intraventricularly canulated rats. One week after the operation, the canulated rats were used in the pole-climbing test. Before the start of the pole-climbing test, absence of disturbing effect of GRg_1 on motor coordination, muscle tone, spontaneous and exploratory movements, and behavior on the Y-shaped plate were confirmed in doses used in the pole-climbing test. The non-trained canulated rat was placed in the apparatus, then 500 Hz sounds which occurred intermittently once a sec, were delivered as a conditioned stimulus for 20 sec; sounds only for the first 10 sec and sounds with electric shock for the other 10 sec. Shocks were delivered from the grid at the same time. When the rat climbed the pole, stimulations were immediately terminated. Rats were exposed to the conditioned stimulations until they had 10 continuous successes of conditioned avoidance response. The following day rats were exposed again to the same procedure at the same time of the day. GRg_1 was given 5 min before the beginning of the learning trial, just after the end of the learning trial, or 5 min before the testing trial. Fig. 1 shows the result when GRg_1 was administered 5 min before the learning trial. The black column shows the mean numbers of the first success of the rats. GRg_1 administered rats did not show the facilitation of the first success in the learning trial. The white column shows the mean numbers, from those of the first success to those which had 10 continuous successes response. We found a significant difference in the white column of the learning trial. GRg_1 administered rats also showed a tendency of facilitation of first success in the testing trial. This may indicate the acceleration of acquisition of

Table 5. Potentiation of the NGF-mediated fiber production by ginseng saponins in organ cultures of chicken embryonic dorsal root (DRG) and sympathetic (symp-G) ganglia

Saponins tested (30 μM)		Ratio of optimal concentration of NGF (ng/ml) to that of NGF with 30 μM of saponin whih induced maximal fiber outgrowth	
		DRG	Symp-G
20S-protopanaxadiol	R_1 = H, R_2 = H	2.5	2.7
Compound K	R_1 = H, R_2 = D-Glc	4.2	6.2
Dammarendiol-1-3-O-β-glucoside	R_1 = D-Glc, R_2 = H	2.8	8.0
Ginsenoside F_2	R_1 = D-Glc, R_2 = D-Glc	5.7	4.0
Chikusetsusaponin III	R_1 = D-Glc(β1 → 2) -D-Glc Xyl(β1 → 6), R_2 = H	5.0	
Ginsenoside Rd	R_1 = D-Glc(β1 → 2)D-Glc, R_2 = D-Glc	8.4	8.0
Ginsenoside Rb_1	R_1 = D-Glc(β1 → 2)D-Glc, R_2 = D-Glc(β1 → 6)D-Glc	7.0	8.0
Ginsenoside M_{6-2}	R_1 = D-Glc(β1 → 2)D-Glc, R_2 = D-Glc	6.1	4.0
Ginsenoside F_{6-bc}	R_1 = D-Glc(β1 → 2)D-Glc, R_2 = D-Glc	3.8	2.0

learning. We did not find a significant difference in numbers when GRg_1 was administered just after the learning trial or 5 min before the testing trial.

4. Potentiation of the NGF-mediated nerve fiber production by GRg_1 in culture of chicken embryonic DRG and Symp-G

NGF has been described as an agent promoting growth and differentiation in its target neurons (sensory and sympathetic neurons), and recently has been confirmed as an agent promoting the fiber production of cholinergic neurons in cell culture experiment. The recent results of studies on NGF have recognized an essential role of NGF for survival, regeneration and regulation of catecholaminergic neurons of brain and ganglion in adult animals. These findings suggest unrecognized facets of NGF's action and open new problems to be dealt with. I happened to use GRg_1 and GRb_1 instead of NGF or with NGF in culture DRG. Stimulation of fiber outgrowth from chicken embryonic DRG and Symp-G in 24 hr culture is one of the most frequently used criteria for estimating the biological activity of NGF. GRg_1 and GRb_1 did not promote fiber production, but the effect of NGF was markedly potentiated by GRb_1. Optimal fiber outgrowth was achieved with 0.83 nM of NGF, and these in the presence of 30 nM of GRb_1 was achieved with 0.12 nM of NGF. These results indicate about a 7 fold potentiation of the original effect of NGF by GRb_1. There is little difference in the potentiation of the NGF-effect by GRb_1 between both ganglia. Potentiation of the NGF-effect occurred in the presence of more than 3 μM of GRb_1, and reached a plateau in the presence of 300 μM of GRb_1. Potentiation of the NGF-effect by ginseng saponins was observed. GRb_1, GRd, GF_2 and GF_{6-a} potentiated the original effect of NGF markedly. They are all 20S-protopanaxadiol glycosides and have a few glucoses in their two branches. Tab. 5 shows the structure-activity relationship of potentiation of the NGF-effect in both ganglia by 20S-protopanaxadiol glycosides and a few glucoses in their branches. The marked potentiation of the NGF-effect was recognized by GRb_1 and GRd. Removal of glucose or hydroxylation of side chain of GRd reduced activity. Dammarene-1-3-O-β-glucoside potentiated the NGF-effect in Symp-G, but not in DRG. It is presently under discussion whether an increase in the cAMP level, RNA synthesis, protein synthesis and tubulin assembly occur in the process of fiber production by NGF. Then using GRb_1, we studied the effect of NGF on levels of cAMP, incorporation of ^{3}H-leucine and ^{3}H-uridine and the antagonism between neurite outgrowth by NGF and drugs which were supposed to have effect on tubulin assembly. NGF and GRb_1 did not alter the levels of cAMP. Though NGF raised the incorporation of both ^{3}H-leucine and ^{3}H-uridine from 10 hr incubation of both ganglia, GRb_1 neither alter the incorporation nor potentiated the original effect of NGF. We could also recognize that increase of RNA and protein synthesis by NGF had no direct correlation with neurite outgrowth by NGF. Colchicine and vinblastin were a few which antagonized the NGF-effect on fiber production. GRb_1 protected the NGF-effect from colchicine, but potentiated the inhibitory effect of vinblastin on the NGF-effect. There may be a possibility that GRb_1 has a membrane effect influencing the uptake of colchicine and vinblastin into the ganglionic neurons. It is very difficult to give any definite conclusion on the results of these experiments, but they may indicate that GRb_1 plays an important role in the process of fiber production. Can GRb_1 potentiate the effect of NGF *in vivo* which plays a physiologically important role as a regulator and regenerator of its target neurons? This is a problem for us to tackle from now on.

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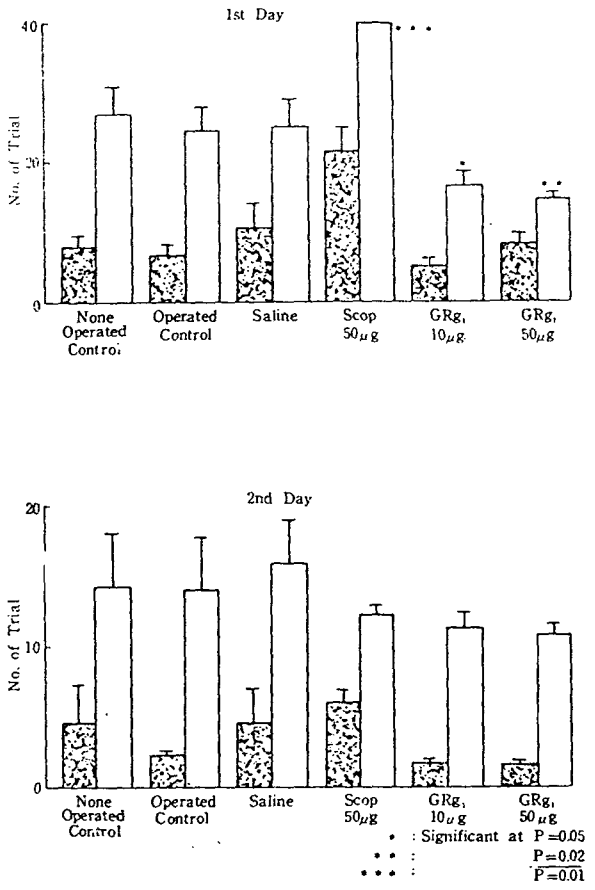

Fig. 1

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