STUDY ON THE NOOTROPIC MECHANISM OF GINSENOSIDES Rg₁ AND Rb₁

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Ginseng has long been used as a tonic in the Traditional Chinese Medicine. It can be used for treatment and prevention of many diseases, especially those associated with aging or low physiological function. In our previous study, we paid attention to the ability of ginseng and its ginsenosides Rg_1 and Rb_1 improve learning and memory in animals using several behavioral paradigms. Ginsenosides Rg_1 and Rb_1 were found to improve learning and memory in animals using several behavioral paradigms. Ginsenosides Rg_1 and Rb_1 were found to improve acquisition, consolidation and retrieval of memory impaired by hypoxia and a number of amnestic agents(1, 2).

In recent years, a study of nootropic mechanism of Rg_1 and Rb_1 on the biochemical and morphological level was carried out.

MATERIALS AND METHODS

Ginsenosides Rg_1 and Rb_1 are saponins isolated from notoginseng with a purity of 96% by the Guangzhou institute of Medicinal Industry. Laboratory animals Were purchased from the Animal Breeding Center, Chinese Academy of Medical Sciences.

Radioligand binding assays of d_1, d_2 and β – adrenoceptors, 5 – HT, DA and M – cholinergic receptors were performed essentially as previously described(3 – 5). Biosynthesis of protein and acetylcholine in brain was assayed by isotope labeling technique using ^3H – leucine and ^3H – choline. The rectus of abdominalis of flog was prepared as described previously for the determination of acetylcholine content of brain (6). The brain weight, thickness of cerebral cortex and synapses number in hippocampus were measured as the indicators of brain development. NA-DPH – Vit C and Fe^{-2} – cystein systems were used to induce lipid peroxidation in brain liver microsomes of rats and MDA formation was taken as measurement of lipid peroxidation. For the induction of superoxide anions, microsomes – NADPH – gossypol was employed and the rate of O_2 production was measured in terms of the reduction of cytochrome (7). Intracellular calcium measurement was performed using Fura – 2/AM fluoresent indicator and the experimental condtions and procedures were the same as previously reported (8). Determination of Ca^{2+} – Mg^{2+} ATPase and Na^+ – K^+ ATPase activity of brain were made according to the literatures (9). Lymphocyte proliferation was measured by radioactivity of ^3H TdR incorporation into the spleen cells of S.D.rats (10). IL – 2 activity was determined by the ability of the culture supernatants to support the growth of the IL – 2 – dependent cell line (CTLL – 20) (11). The level of IL – 2 mRNA was measured by total RNA slot hybridization as described by white et al (12).

RESULTS

1. Strengthening of Cholinergic System

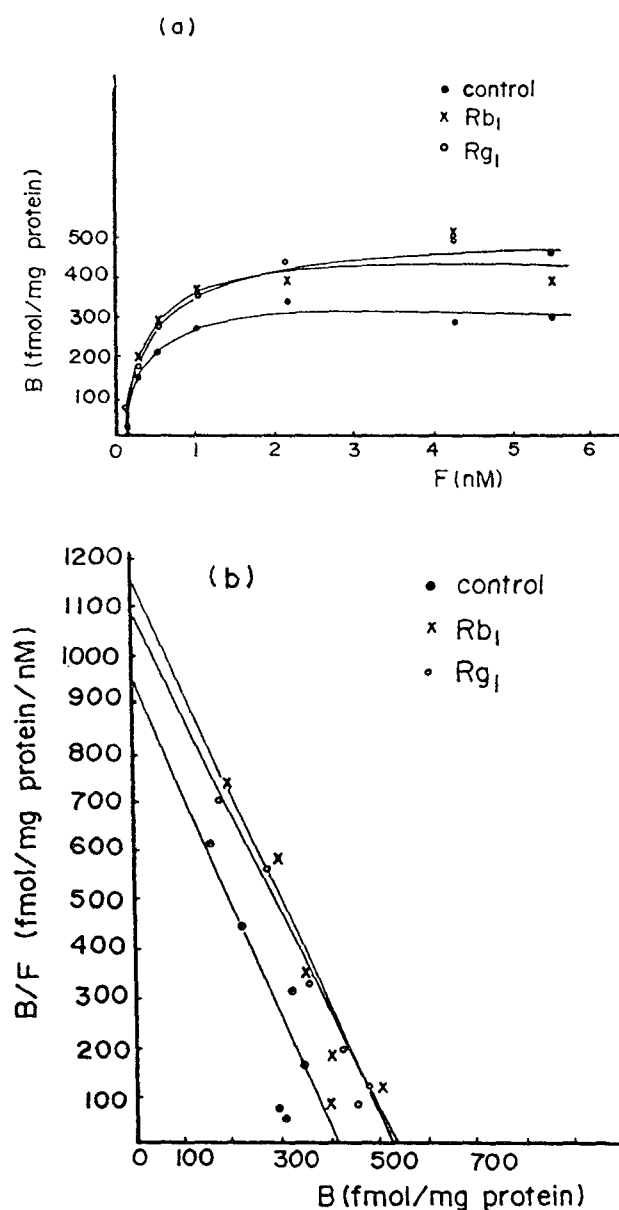Ginsenosides Rg_1 and Rb_1 Showed no specific binding to

Fig. 1. Saturation curve(a) and Scatchard plot (b) of ^3H – QNB – specific binding to M – cholinergic receptors of mouse brain obtained from control and Rb_1, Rg_1 – treated group

seven central neurotransmitter receptors including M – Cholinergic receptors. However, both Compounds increased significantly the density of M – cholinergic receptors after oral administration of Rg_1 and Rb_1 for 5 days (Fig. 1). In the mean time, Ach content in mouse brain was increased markedly. This increase of Ach content was accompanied by an increase in the high affinity uptake of ^3H – Choline in mouse synaptosomes (Tab. 1), indicating that synthesis of Ach was promoted by Rg_1 and Rb_1.
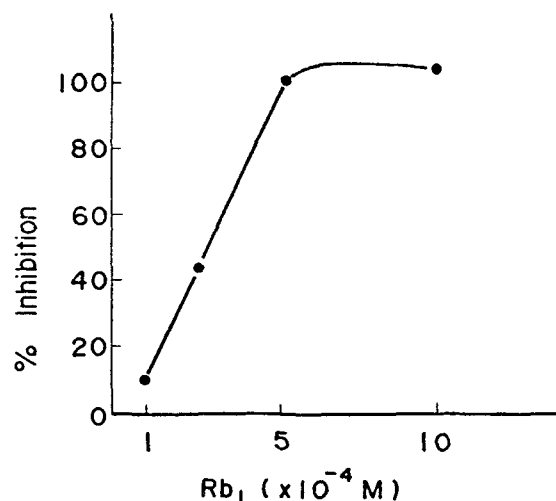
Table 1. Effect of ginsenoside Rg_1 and $Rb_1(5$ mg/kg) on ^3H – choline uptake of mouse brain synaptosomes

Group	^3H – choline uptake DPM	p
Control	5716± 1493	
Rg_1	8675± 2345	<0.05
Rb_1	8792± 2217	<0.02

N = 5

Fig. 2. Dose – effect curve of Rb_1 on gossypol – induced O_2^- generation in liver microsome of rats.
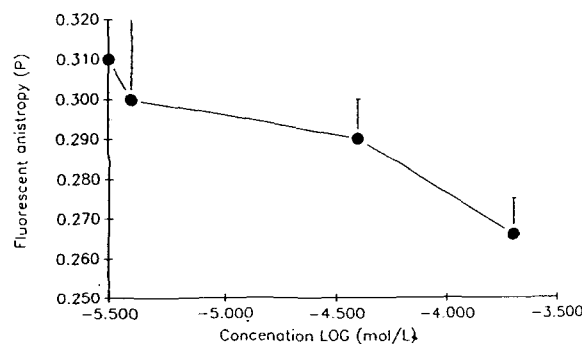
2. Scavenging of free radicals

Rg_1 and Rb_1 inhibited NADPH – Vit C and Fe^{2+} – cystein induced lipid peroxidation in rat brain microsomes, but only Rb_1 could scavenge oxygen radicals generated by liver microsome – NADPH – gossypol at 10 ^{4}mol/L (Fig. 2). On the other hand, Rb_1 decreased intracellular Ca^{2+} concentration and calmodulin activity, while Rg_1 showed no such effect. Rb_1 but not Rg_1 increased membrane fluidity impaired by Fe_2SO_4 cystein (Fig. 3). With the whole – cell patch clamp technique, Rb_1 had no obvious effect on calcium current and potassium current. In both cerebral cortical synaptosomes and hippocampus of rat, Rb_1 increased $Na^+ - K^+$ ATPase and $Ca^{2+} – Mg^{2+}$ ATPase at about 10^{-6}mol/L (Tab. 2, 3). Rg_1 had no effect on both ATPase at the same concentration.

Fig. 3. Effect of ginsenoside Rb_1 on fluorescent anisotropy in rat brain synaptosomes pretreated by $FeSO_4$ and cysteine.

Table 2. Effect of ginsonoside Rb_1 on $Ca^{2+} – Mg^{2+}$ ATPase activity in rat brain synatosomes

Dose (umol/L)	Sample No	Ca^{2+} Mg^{2+} ATPase activity (micromoles of product/mg/h) $(X± SE)$	%	P
0	5	0.55± 0.16	100	
2	7	0.76± 0.09	138.2	<0.05
5	7	0.54± 0.08	98.2	
50	7	0.22± 0.07	40.0	<0.01

Table 3. Effect of ginsonoside Rb_1 on $Na^+ - K^+$ ATPase activity in rat brain synatosomes

Dose (umol/L)	Sample No	Na^+ K^+ ATPase activity (micromoles of product/mg/h) $(X± SE)$	%	P
0	7	3.52± 0.37	100	
2	7	4.14± 0.39	117.6	<0.05
50	7	4.83± 0.36	137.2	<0.01
200	6	3.40± 0.62	97	

3. Promotion of brain development

The drinking water containing Rg_1 and Rb_1 was supplied to the weaning mice for successively 4 weeks. On the 15th day, the mice were trained to learn avoidance response to foot electric stimulation, then sacrified for measurement of brain develop-

ment. Results showed that Rg_1 and Rb_1 facilitated memory acquisition in step down and step through tests (Tab 4.). It can be seen from Tab 5, 6. and 7, the brain weight, thickness of cerebral cortex and synapses number of hippocample CA3 region in Rg_1 treated group at dosage of 27.4 and 53.9 mg/kg increased significantly. Rg_1 increased synapses number and tend to increase brain development.

Table 4. Effect of Ginsenoside Rb_1 and Rg_1 on the acquisition of memory in step-down and step-through tests in mice

	Group	Dose (mg/kg)	Latencies (sec.)	Number of errors	% of animals showing error response
	Control	········	120.0± 78.5	1.4± 1.5	40
	Rb_1	28.6	172.5± 23.8	0.2± 0.6*	10
Step-down	Rb_1	56.1	149.5± 64.3	0.4± 0.8	20
	Rg_1	27.4	180.0± 0.0	0.0± 0.0***	0
	Rg_1	53.9	176.0± 12.6	0.1± 0.3**	10
	Control	········	81.2± 86.1	2.9± 2.3	100
	Rb_1	28.6	156.4± 123.4	1.1± 1.4*	50*
Step-through	Rb_1	56.1	128.6± 105.0	1.2± 0.9*	80
	Rg_1	27.4	165.0± 115.4	1.5± 1.8	70
	Rg_1	53.9	187.0± 111.6*	1.7± 1.9	70

Note : The figures denote $\bar{x} \pm SD$; 10 mice per group
* $P \langle 0.01$ statistically significant defference as compared with the respective controls

Table 5. Effects of Ginsenoside Rb_1 and Rg_1 on the brain weight in mice

Group	Dose (mg/kg)	Brain Weight(g)
Control	······	5.6± 0.4
Rb_1	28.6	5.9± 0.4
Rb_1	56.1	6.4± 0.7
Rg_1	27.4	6.5± 0.4**
Rg_1	53.9	6.5± 0.5**

Note : The figures denote $\bar{x} \pm SD$; 5 mice per group
** $P \langle 0.01$ Significant difference from control group

Table 6. Effects of Ginsenoside Rg_1 and Rb_1 on the Thickness of Brain Cortex in Mice

Group	Dose (mg/kg)	Thickness of brain cortex (mm)
Control	·····	1.84± 0.07
Rb_1	28.6	1.86± 0.11
Rb_1	56.1	2.04± 0.19**
Rg_1	27.4	2.09± 0.09**
Rg_1	53.9	2.04± 0.16**

Note : The figures denote $\bar{x} \pm SD$; 10 mice per group
** $P \langle 0.01$ significant difference from control group

Table 7. Effects of Ginsenoside Rb_1 and Rg_1 on the number of synapses in the hippocampal CA_3 region in mice

Group	Dose (mg/kg)	Number/um^2
Control	·····	0.1718± 0.07128
Rb_1	28.6	0.2557± 0.1382**
Rb_1	56.1	0.2134± 0.07327**
Rg_1	27.4	0.2023± 0.08330**
Rg_1	53.9	0.2340± 0.07447**

Note : The figures denote $\bar{x} \pm SD$; 5 mice per group
** $P \langle 0.01$ Significant difference from control group

4. Stimulation of T-lymphocyte function

It has long been clear that aging leads to a substantial decline of T- cell function. In our study, Rg_1 increased the proliferation of lymphocytes and the optimal doses for Rg_1 were 20 mg/kg (in vivo) and 1×10^{-5}mol/L (in vitro). For determining IL-2 production, the culture supernatants were obtained from lymphocytes cultured in the presence of Con A (1.5 μg/ml) 24 hrs after initiating the culture. It was found that Rg_1 increased the IL-2 production of aged rats (Tab 8. and 9). The total RNA was extracted from lymphocytes that had been cultured with ConA (1.5 μg/ml) for 20 hrs. The cDNA probe to IL-2 was labeled by nick translation with ^{32}P - dATP and hybridiza-

Table 8. The effects of Rg_1 on lymphocyte proliferation and the production of IL-2 in vivo

Groups	[^{3}H]TdR incorporation (cpm)	IL - 2 Activity(10^7/ml) (units)
Control(5months rats)	32758± 3048.5	32.8± 3.42
Control(24months rats)	25045± 1973.4**	18.0± 3.16**
20 mg/kg(24months)	30508± 2157.5$^{\triangle\triangle}$	30.5± 3.69$^{\cdot\cdot}$
40 mg/kg(24months)	29125.8± 2287.6$^{\triangle\triangle}$	25.5± 3.45$^{\cdot\cdot}$

** $p<0.01$ VS control (5months) ; $^{\triangle}$ $p<0.05$; $^{\triangle\triangle}$ $p<0.01$ VS control(24months)

Table 9. The effects of Rg_1 on lyphocyte proliferation and the production of IL-2 in vitro

Groups	[^{3}H]TdR incorporation (cpm)	IL - 2 Activity(10^6/ml) (units)
Control(5months rats)	30683± 1865.4	22.8± 2.32
Control(24months rats)	20944± 2213.4*	14.9± 1.06**
Rg_1(1×10^{-7}M)	23354± 2290.8	16.0± 3.73
Rg_1(1×10^{-6}M)	26905± 1926.8$^{\triangle}$	18.7± 2.83$^{\triangle\triangle}$
Rg_1(1×10^{-5}M)	27510± 2357.4$^{\triangle\triangle}$	21.5± 2.59$^{\triangle\triangle}$
Rg_1(1×10^{-4}M)	26276± 2375.4$^{\triangle}$	19.9± 0.83$^{\triangle\triangle}$
Rg_1(1×10^{-3}M)	22289± 1994.5	16.3± 1.37

** $p<0.01$ VS control (5months) ; $^{\cdot\cdot}$ $p<0.05$; $^{\triangle\triangle}$ $p<0.01$ VS control(24months)
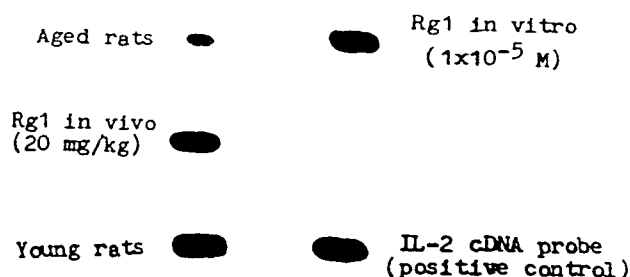

Fig.4. Slot blot analysis of IL-2 isolated from lymphocytes was hybridized to radioactively labled IL-2 cDNA probe

tion was detected by autoradiography (see Fig. 4). The slot blots in Fig. 3. showed that the size and slot intensity of hybridization signal was weaker in aged rats than in young rats. However, the level of mRNA in Rg_1 treated group was increased almost to the young rats mRNA level.

DISCUSSION AND CONCLUSION

1. Brain aging or memory impairment is frequently associated with cholinergic deficit in the central nervous system such as reduction of Ach content, M - cholinergic receptors' density and acetylcholine esterase activity (13). We found that ginsenosides Rg_1 and Rb_1 increased not only Ach biosynthesis and release, but also M - cholinergic receptors density. This indicated

that Rg_1 and Rb_1 may facilitate cholinergic neurotransmission and then benefit learning and memory. This finding has theoretical significance. According to the existing theory the regulation of density of M - cholinergic receptors is determined by the contents of acetylcholine in brain. For example, the increase of M - cholinergic receptors results from the decrease of acetylcholine in brain (14). However, our results that Rg_1 and Rb_1 induced up - regulation of M - cholinergic receptors and increased acetylcholine contents is necessary to establish for elucidation of ginseng's effect on the cholinergic system.

2. The structure and functions of cell membrane and lipids, protein and RNA are easily damaged by free radicals. Many pathological processes, such as radiation, ischemia, inflammatory disorders, aging are linked with generation of free radicals (14, 15). Recently, we provided an evidence of the antiaging effect of Rg_1 and Rb_1, that is, Rb_1 could inhibit MDA production, scavenge O_2 in vitro. Further study showed that Rg_1 and Rb_1 increased activities of glutatione peroxidase (GSH - PX) and catalase (CAT). So that the chain reactions of free radicals could be stopped through the following reactions :

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2, \quad 2H_2O_2 \xrightarrow{\text{GSH - PX}} H_2O + O_2$$
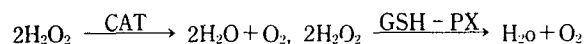
We also proved that Rb_1 reduced intracellular calcium level. This finding may be helpful to understand the antiaging effect of ginseng too. On the one hand, Rb_1 can decrease free radicals generation through decreasing intracellular calcium or eliminating calcium overload. On the other hand, it is now well accepted that cell death due to any cause is preceded by intracellular influx of calcium. This effect of Rb_1 modulating calcium metabolism may be a fruitful approach to the pharmacological treatment

of aged animals.

3. Cognitive function depends on the normal structure and development of brain. The basis of learning and memory is communication between synapses and formation of reflex. As noted by scientists that one of the characterization in aged and AD patients is neurons loses and degeneration of brain structure. We found for the first time that Rg_1 could accerelate brain development such as increase of brain weight thickness of cerebral cortex and synapses. Nishiyama et al (18) indicated that Rg_1 and Rb_1 promoted neuron survival of chick and rat cerebral cortex. Although the animals, methods and parameters they used were different from ours, the conclusion was the same that ginsenosides Rg_1 and Rb_1 had profund effect on the neural plasticity. Obviously, this adaptive capacity is of great significance in relation to a number of health - associated problems such as injury to CNS and developmental disorders, learning disabilities and senile dementia, etc.

4. Immune reactions involve the coordinate efforts of three cell types : B lymphocytes, T lymphocytes and antigen - presenting cells. Thymus - derived T cell, include at least two cell types : helper T cells, which initiate immune responses by providing signals required by T and B cell and by nonlymphoid effectors, and cytotoxic T cells, which can lyse antigen bearing target cells (19). It is well known that aging leads to a substantial declines in most measures of T cell function. G.Z. Yang demonstrated that ginsenosides (GS) enhanced the mitogenesis of T.B lymphocytes and promoted cytokines (IL - 2, IFN) and IL - 2 gene expression. We found that Rg_1 but not Rb_1 was the immune - modulating active principle which increased T lympholytes transformation and IL - 2 production as well as IL - 2 gene expression. This is new data underlying the antiaging effect of ginseng.

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