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Anti-stress Activities of Ginsenoside Rb_1 is Related with GABAnergic Neuron

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Abstract – The main aim of this study was to investigate stress related activities of ginsenosides and their action mechanism. Control group and ginsenoside supplemented groups were exposed to stress while no-stress group was not done. Animals of each group (n =8~10) were orally administerd 100 mg red ginseng extract (R-G), or 10 mg ginsenosides/ kg body weight once a day. Animals were given materials for 5 days without stress, and then were given supplements for 5 days with restraint and electroshock stress. Mice were given materials for 5 days for experiments on anti-fatigue effect. After loading final stress, stress-related behavioral changes of experimental animals were examined and plasma corticosterone levels were measured. R-G and ginsenoside Rb_1 supplementation partially blocked the stress effects on locomotion and elevated plus-maze test in rats and mice. They also partially blocked stress induced behavioral changes such as freezing, smelling, face-washing, rearing behavior in rats. R-G and Rb_1 decrease adrenal gland size and plasma corticosterone level, which were increased by stress in rats. R-G increased enduring time on the Rota rod, cold water and horizontal wire, but Rb_1 didn't. Effects of Rb_1 on plusmaze test were inhibited by administration of flumazenil. These results suggest that Rb_1 is the main anti-stress principle in ginseng and it's effect is modulated by GABAnergic nervous system.

Key words □ anti-stress, ginsenoside, behavioral activity, corticosterone, GABA

INTRODUCTION

Ginseng, the root and rhizome of *Panax ginseng* C A Meyer, has been used as a tonic remedy in orient traditional medicine for over 2000 years. It is one of the most popular medicinal plants throughout the world because of its beneficial effects (Tachikawa and Kudo, 2004). Red ginseng (R-G) is made by steaming and drying the fresh ginseng, suggesting chemical transformation by heat (Park *et al.*, 2002). Roots of this plant contain a variety of saponins which are the characteristic major constituents of ginseng and believed to be responsible for most of the effects (Attele *et al.*, 1999). This herb has been used as a general tonic in traditional oriental medicine to increase vitality, health and longevity, especially in older persons (Tang and Eisenbrand, 1992). Many researchers believe that ginseng has many beneficial effects, including alleviating learning and memory impairment, reversing pathological and physiological changes induced by stress and aging, etc. It was reported that ginseng or ginsenosides show anti-stress activities in stressful circumstances such as footshock (Takahashi *et al.*, 1992), cold(Choi *et al.*, 2003; Kaneko *et al.*, 1996) and heat (Yuan *et al.*, 1988). Thus anti-stress activities of ginseng or ginsenosides may account for their clinical efficacy in stress related disorders such as hypertension, diabetes mellitus, peptic ulcer, depression and anxiety disorder (Cheng *et al.*, 2005; Attele *et al.*, 1999; Bhattacharya *et al.*, 1990). Various kinds of stress, especially chronic stress, cause many diseases and accelerate aging, so anti-stress drugs are in high demand. Ginseng has long been considered to act as an adaptogen, but the mechanisms underlying its effect are still unclear.

Selye has defined stress to refer to the combination of interactions to various stimuli and responses that our body attempts in coping to maintain biochemical constancy (homeostasis) (Chrousos and Gold, 1992; Selye, 1974). It is the nonspecific response the body makes to all specific demands placed on it. Stress is any change we have to adjust to (Seley, 1978). Whether the change is positive or negative, it will cause stress. However, stress may build up over time if we experience numerous changes in our lives all at once (Seley, 1993). In

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humans, overstress has been suggested to contribute to heart disease (Kamarck and Jennings, 1991), high blood pressure (Snider and Kuchel 1983), and emotional disorders, such as depression (Kessler, 1997). Some studies stated that over 60% of diseases are related to stress especially life-threatening diseases like cardiovascular diseases, hypertension, cancer and gastrointestinal tract disorders (McCain and Smith, 1994; Brier *et al.*, 1887; Hurst *et al.*, 1976). Furthermore, stress has been postulated to be involved in the etiopathogenesis of various disease states, including hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions (Staratakis and Chrousos, 1995; Hurst *et al.*, 1976), and behavioral disorders like anxiety (Kessler, 1997; Blazer *et al.*, 1987), due to the involvement of the central nervous system (CNS), endocrine system, and metabolic system (Staratakis and Chrousos, 1995; Brier *et al.*, 1887). The resultant disturbances may vary due to the type, intensity and duration of a particular stressor and the strain of the experimental subjects (Kioukia-Fougia *et al.*, 2002; Dimsdale *et al.*, 2000). Stress can also result in feelings of distrust, rejection, anger and depression, which in turn can lead to health problems such as headaches, stomach ulcer, liver disease, rashes, insomnia, heart disease, stroke, diabetes, immune disorders, and sexual disorders (9, 10, Dimsdale *et al.*, 2000; Glaser *et al.*, 1990; Blazer *et al.*, 1987; Brier et al., 1887).

Recently, acute, chronic, and repeated stress models were used to observe the effects of ginsenosides on stress and more than 40 ginsenosides have been isolated from several species of ginseng. Among all the active ingredients isolated from ginseng, we selected Rg_1, Rb_1, Rc, C, K, for study. Ginseng extracts block the uptake of GAB_A into rat brain synaptosomes (Tsang *et al.*, 1985). Moreover, some of its saponins are reported to alter the binding of GABAA (Kimara *et al.*, 1994). In addition, ginseng is demonstrated to have diazepam like activity (Bhattacharya and Mitra 1991). Therefore, anti-stress activities of ginseng or ginsenosides may be also modulated by GABAnergic nervous system. We especially intended to apply methods to induce psychologically or physically overstress into experimental animal and tested it to evaluate anti-stress effect or stress-resistant activities. We selected simple methods that could psychologically or physically expose overstress to animals and could detect signs of overstress. Animals were given an electroshock stimulation and a restraint stress. We examined the behavioral changes, the changes of organ weights, and plasma corticosterone levels induced by overstress. There have been many reports suggesting that levels of blood corticosterone can be used as a parameter of stress level because stress alters corticosterone secretion and adrenal function (Tan-Lee *et al.*, 2004; Kim *et al.*, 2003; 18-23).

In this study, anti-stress effects of various ginsenosides were compared to the effects of ginseng total extract. We also invested whether anti-stress activity is modulated by GABAnergic nervous system.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley(SD) rats (8-10 weeks of age) and the male ICR mice (20-25g) used in this study were obtained from Hanlim experimental animal Co. (Hwasung, Korea) We used ginsenosides (Rg_1, Rb_1, Rc, compound K, C, MA, MB) which were produced by Bitigin Corp. (Daejeon, Korea), red ginseng extract (R-G) produced by Korean Ginseng Corp. (Seoul Korea). We chose red ginseng extract (R-G) as a positive control. All animals were housed in a temperature (22±2°C) and humidity(55±5%) controlled animal room on a 12 hr/12 hr light/dark (6 A.M.-6 P.M.) schedule. They had free access to food and water throughout the experiments. The animals were divided into four groups after stabilizing them for 1 week in our animal room. Animals belonging to the normal group were not exposed to any stress. The rats of control group were orally administered with saline 1 mL/100 g body weight and were exposed to stress, and the mice of control group were given saline 0.1 mL/10 g body weight and exposed to stress. The animals of red ginseng and ginsenosides supplemented group were orally administered once a day 100 mg of red ginseng extract or 5 and 10 mg of Rb_1/kg body weight and exposed to stress. Animals were given only supplementary materials for 5 days after stabilizing them, and then were supplemented materials for 5 days with stress.

Induction of stress

Animals were given supplementary materials before exposing them to stress. The mice were usually subjected to restraint stress by keeping them in a well ventilated conical plastic tubes (3 cm in diameter and 7 cm in length) for 30 minutes each day. During the restraining period, the mice did not have any access to food and water. At the end of each restraint stress, the mice were exposed to electroshocks with intensity of 0.5 mA (1 sec. duration; 20 sec inter-shock interval) for 5 minutes (Tan-Lee *et al.*, 2004). The rats were usually subjected to restraint stress by keeping them in a well ventilated conical polypropylene tubes (6.2 cm in diameter and 16.5 cm in length) for 30 minutes each

day. During the restraining period, the rats did not have any access to food and water. At the end of each restraint stress, the rats were exposed to electroshocks with intensity of 2 mA (1 sec duration; 20 sec inter-shock interval) for 5 minutes (Tan-Lee *et al.*, 2004).

Behavioral apparatus

The equipment was located in the animal room allowing the observer to view and observe the animals through a computer outside the room. After inducing terminal stress (in the manner described above), behavioral changes of animals were monitored automatically using a computerized EthoVision system (Noldus IT b.v., Netherlands). In the locomotor activity and elevated plus-maze tests, the behavioral parameters were analyzed by an automatic video-tracking system.

Locomotor activity

The apparatus consisted of 9 black plastic boxes (47 × 47 cm), and the field was bordered by 42-cm-high side walls. The total moved distance and total movement time were monitored for 20 minutes after terminal stress (Tan-Lee *et al.*, 2004; Noldus *et al.*, 2001).

Elevated plus-maze test

The Elevated plus-maze box and arms were made of plastic. The apparatus consisted of two open arms (50 × 10 cm in rats; 30 × 6 cm in mice), alternating at right angles, with two arms enclosed by high walls of 30 cm in rats and 20 cm in mice. The four arms delimited a central area of 10 × 10 cm. The whole apparatus was placed 50 cm above the floor. Animals were placed in the central square after measuring stress related activity and allowed to explore the maze freely for 5 minutes. The parameters measured were the times spent in open and closed areas (Tan-Lee *et al.*, 2004; Noldus *et al.*, 2001).

Stress related activity tests

After terminal stress, animals were placed alone in individual plastic cage (40 × 20 × 18 cm in rats; 26 × 20 × 13 cm in mice). The behavioral activities were measured soon after stress. Smelling, feeding, burrowing, freezing, tailing, face washing and grooming time were recorded for 5 minutes (Tan-Lee *et al.*, 2004; Takeuchi *et al.*, 2003). Rearing frequency was measured using EthoVision system for 20 minutes after the terminal stress (Tan-Lee *et al.*, 2004; Noldus *et al.*, 2001).

Blood sampling and measurement of plasma corticosterone

After monitoring locomotor activity, blood samples (rat 4 mL; mouse 1.5 mL in heparinized tubes) were taken through heart puncture between 10:00 A.M. - 2:00 P.M., and then adrenal gland in rat and spleen in mouse were dissected and weighed.

The serum corticosterone level was measured by a modified method (Lee *et al.*, 2004; Kim *et al.*, 2003) using HPLC system composed with SI-2 3001 pump, SI-2 3002 UV-Visible detector, SI-2 3004 column oven, separation (Shiseido, Tokyo, Japan), and column Capacell Pak C18 MG 120 (5 μm, 3 × 250 mm). Corticosterone and dexamethasone (Sigma, St. Louis, MO, U.S.A.) were used as the internal standard. We injected the 40 μL of treated sample solution into HPLC column. We used acetonitrile : methanol : 0.03% sulfuric acid solution (32 : 4 : 64) as the mobile phase with a flow rate of 500 μL/min. We determined corticosterone level as absorbance in wavelength 240 nm using dsCHROM computing program (Shiseido, Tokyo, Japan).

The resistance to physical stress

After giving supplementary materials for 7 days, the resistance to physical stress was evaluated using horizontal wire test, Rota rod test and swimming test. A horizontal wire test was carried out, according to the method described by Hui et al, with minor modifications (Lee *et al.*, 2004; Hui *et al.*, 2003). The mice were lifted by the tail and were allowed to grasp a horizontal wire (5 mm diameter, 150 cm long) with their forepaws and tail elevated 80 cm above the table. The time spent balancing on the horizontal wire was recorded manually by using a stop watch. The animals were placed on the rota rod with speeds of 60 rpm. The mice were allowed to run in rotating rod until they're exhausted to the point of dropping from rod (Lee *et al.*, 2004). The total time running on the rotating rod were recorded. The mice were forced to swim in cold water maintained at 8±2°C (Lee *et al.*, 2004; Choi *et al.*, 2003). The apparatus is a circular water tank (diameter: 150 cm; height: 50 cm) made of stainless steel. The pool was filled with water to a depth of 15 cm. The mice (5/session) were allowed to swim in the pool until they're exhausted to the point of drowning. The time spent swimming in the water was recorded manually using a stop watch.

Pentylentetrazole (PTZ) induced seizures

Seizures were induced with PTZ in a mouse model, as previ-

ously described (Yuhas *et al.*, 1995). Groups of 10 mice were injected subcutaneously (ip) with PTZ (35, 70 or 100 mg/kg) and observed for 30 min. Whether tonic seizures (hind legs rigidly extended to the rear) was occurred or not was observed.

Statistical analysis

Data are expressed as the mean±S.E.M.. ANOVA was used to compare the scores among the groups for one variable. This was followed by post hoc comparisons using the Newman-Keuls test.

RESULTS

Effects of ginsenosides on locomotor activity.

Figure 1 show that stress affects locomotor activity in both rats and mice. The total moved time and distance were significantly different between animals exposed to stress and those not exposed to. The stress condition resulted in the significant decrease of total moved time and distance in both animals whereas red ginseng (R-G) supplementation partially blocked this stress-induced suppression of locomotion. Ginsenosides didn't affect on locomotor activity.

Effects of ginsenosides on the exploratory activity in the elevated plus-maze

The time spent in the open or closed arm for 10 minutes significantly differed between the animals exposed to stress and the unexposed animals as shown in Figure 2. The animals exposed to stress spent less time in the open arms than the unexposed animals. Furthermore, the animals exposed to stress spent more time in the closed arm than the unexposed animals. R-G and ginsenosides Rb_1 supplementation significantly reversed this stress-induced response in the elevated plus-maze test.

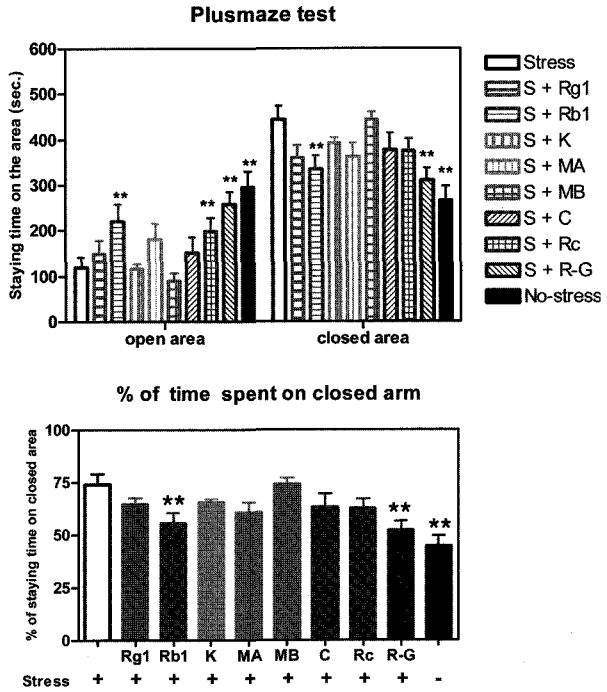

Fig. 2. Effects of ginsenosides on the exploratory activity in the elevated plusmaze in mice (n=10). Each bar represents mean± S.E. of the time spent on the open and closed arms. No-stress, R-G + stress (Red ginseng 100 mg/kg p.o. + stress) or ginsenosides (Rg_1, Rb_1, MA, MB, K, Rc, C) + stress (ginsenosides 10 mg/kg p.o. + stress) group, versus stress group, (** $p<0.01$; * $p<0.05$).

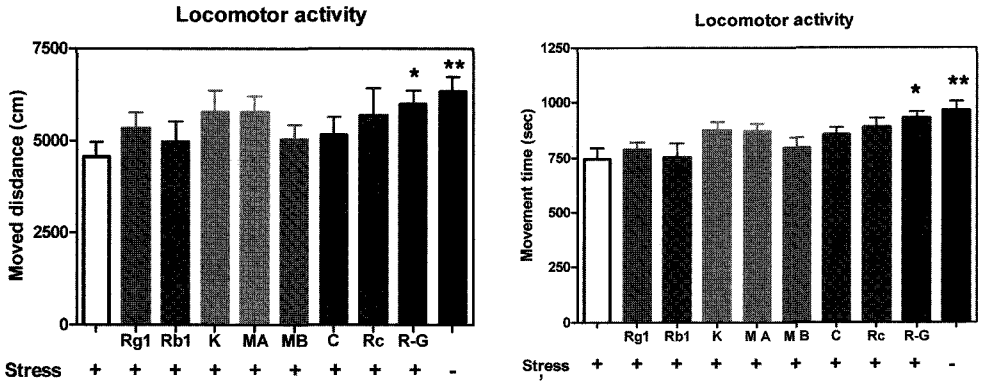

Fig. 1. Effects of ginsenosides on Locomotor activity in mice (n=9). Each bar represents the mean±S.E. of the moved distance and movement duration for 20 min after loading stress. The no-stress group(-) were not exposed to any stress and the control group (+) were exposed to stress. The other group were supplemented R-G or ginsenosides (Rg1, Rb1, MA, MB, K, Rc, C) and exposed to stress. No-stress (-), R-G + stress (Red ginseng 100 mg/kg p.o. + stress) or ginsenosides (Rg1, Rb1, MA, MB, K, Rc, C) + stress (ginsenosides 10mg/kg p.o. + stress) group, versus stress group (Saline p.o. + stress), (** $p<0.01$; * $p<0.05$).

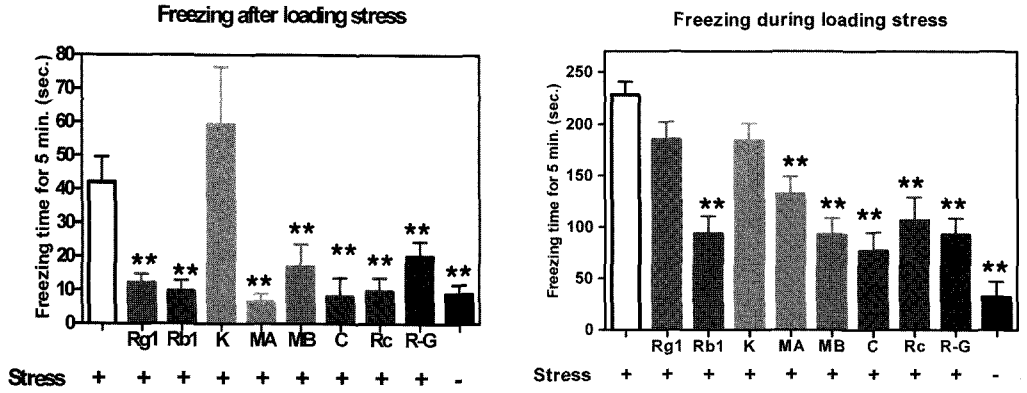

Fig. 3. Effects of ginsenosides on freezing behavior in mice (n=10). Each bar represents mean±S.E. of the freezing time for 5 min after(left) or during(right) loading stress. No stress, R-G + stress (Red ginseng 100 mg/kg p.o. + stress) or ginsenosides (Rg_1, Rb_1, MA, MB, K, Rc, C) + stress (ginsenosides 10 mg/kg p.o. + stress) group, versus stress group, (** $p<0.01$; * $p<0.05$).

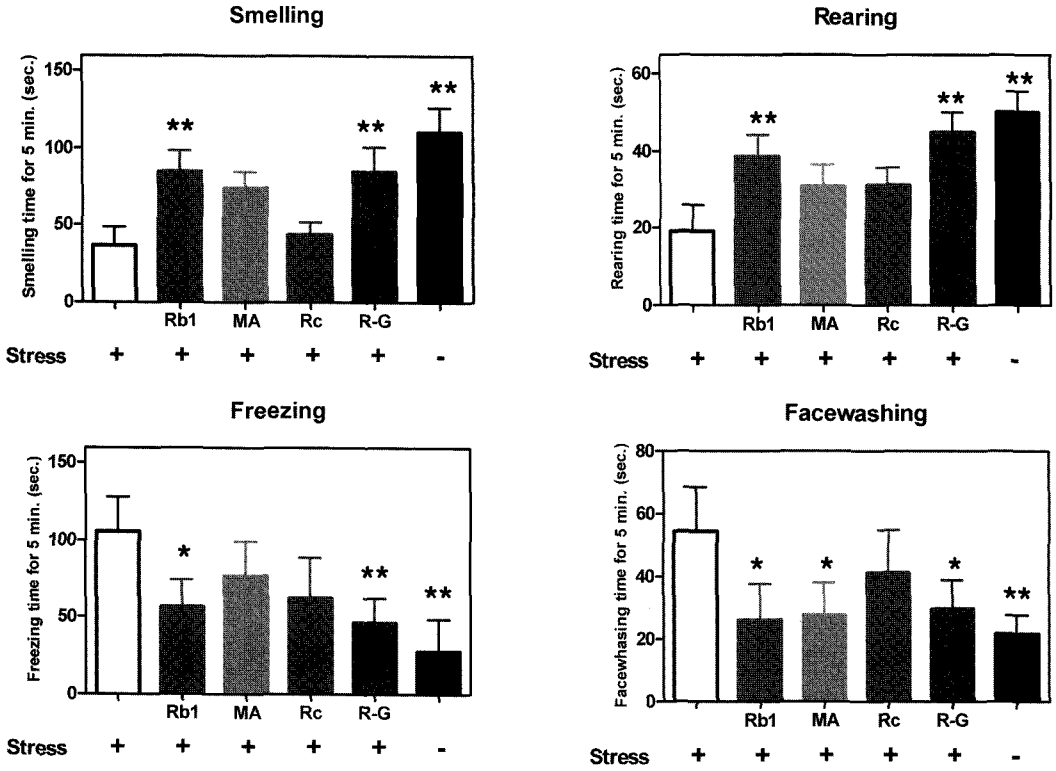

Fig. 4. Effects of ginsenosides on stress-related behavioral activities in rats (n=9). Each bar represents mean±S.E. of the total activity time for 5 min and rearing frequency for 20 min after loading stress. No stress, R-G + stress (Red ginseng 100 mg/kg p.o. + stress) or ginsenosides (Rb_1, MA, Rc,) + stress (ginsenosides 10 mg/kg p.o. + stress) group, versus stress group, (** $p<0.01$; * $p<0.05$).

Effects of ginsenosides on specific stress related behaviors.

As shown in Figure 3, the stress exposure resulted in a significant increase of the time spent in freezing behavior of mice. R-G and ginsenosides supplementation blocked these stress-induced freezing behavior except compound K. Figure 4 shows that stress significantly increase the time spent in freezing and face-washing behaviors of rats for 5 minutes and decrease the time spent in smelling behavior for 5 minutes and rearing frequency of rats for 20 minutes. R-G and ginsenosides Rb_1 supplementation partially blocked these stress-induced behavioral changes.

Effects of ginsenosides on plasma corticosterone levels and organ weight

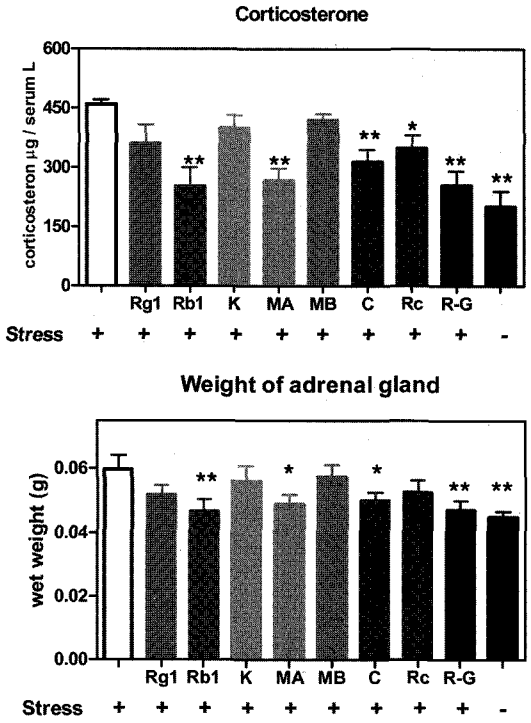

Fig. 5. Effects of ginsenosides on plasma corticosterone levels and wet weight of adrenal glands in rats (n=9). Each bar represents mean±S.E. of wet weights of adrenal glands and plasma corticosterone levels after loading stress. No stress, R-G + stress (Red ginseng 100 mg/kg p.o. + stress) or ginsenosides (Rg_1, Rb_1, MA, MB, K, Rc, C) + stress (ginsenosides 10mg/kg p.o. + stress) group, versus stress group, (** $p<0.01$; * $p<0.05$).

As shown in Figure 5, the stress condition resulted in a significant increase of wet weight of adrenal glands in rats. The stressed animals have higher corticosterone levels than the stress free animals. R-G and ginsenoside Rb_1 supplementation significantly attenuated the stress induced plasma corticosterone levels and the hypertrophied adrenal glands.

Effects of ginsenosides on resistance to physical stress

Anti-stress effect (physical stress) of ginsenosides was evaluated using the Horizontal wire test, cold swimming test and Rota rod test. Motor coordination and endurance ability were evaluated. As shown in figure 6, R-G prolonged significantly swimming time, balancing time on the wire and running time on the Rota rod but Rb_1 didn't. These results mean that R-G enhances physical capacity or blocks stress responses but Rb_1 didn't.

Effects of ginsenosides on GABAnergic activities

Figure 7 shows that effects of Rb_1 in plus maze test were interfered by $GABA_A$ receptor antagonist, flumazenil. Ginsenoside Rb_1 partially blocked convulsion induced by GABA antagonist pentylenetetrazole.

DISCUSSION

Stress was found to affect locomotor activity in rats. The ani-

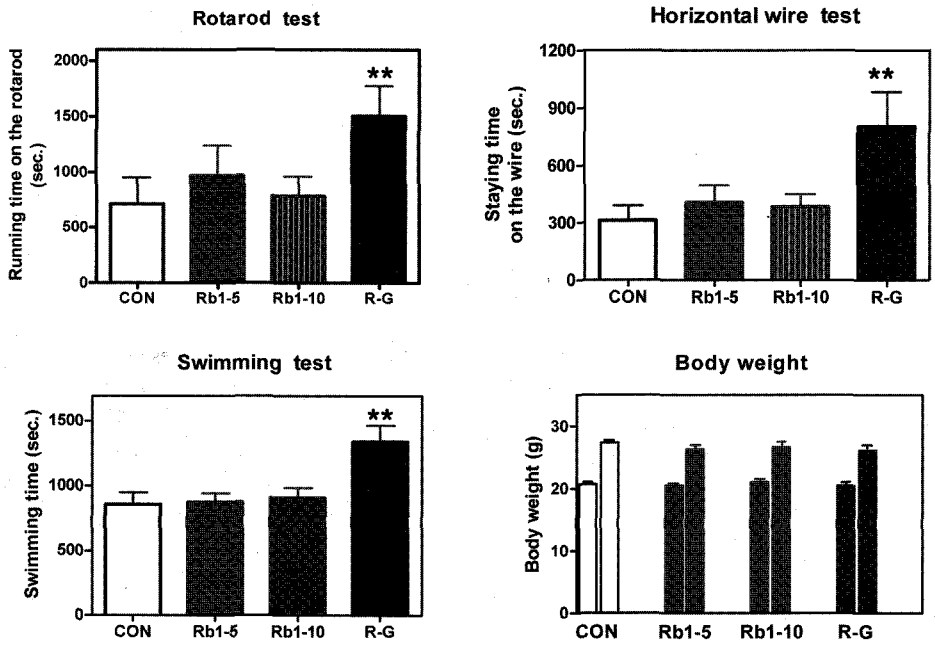

Fig. 6. Effects of ginsenosides on resistant activities to physical stress in mice (n=10). Each bar represents mean±S.E. of endurance time on Rota rod, the horizontal wire or swimming pool. Mice were given R-G or ginsenosides for 5 days. R-G (Red ginseng 100 mg/kg p.o.), ginsenosides Rb_1-5 (Rb_1 5mg/kg p.o.) and Rb_1-10 (Rb_1 10 mg/kg p.o.) group, versus control group, (** $p<0.01$; * $p<0.05$).

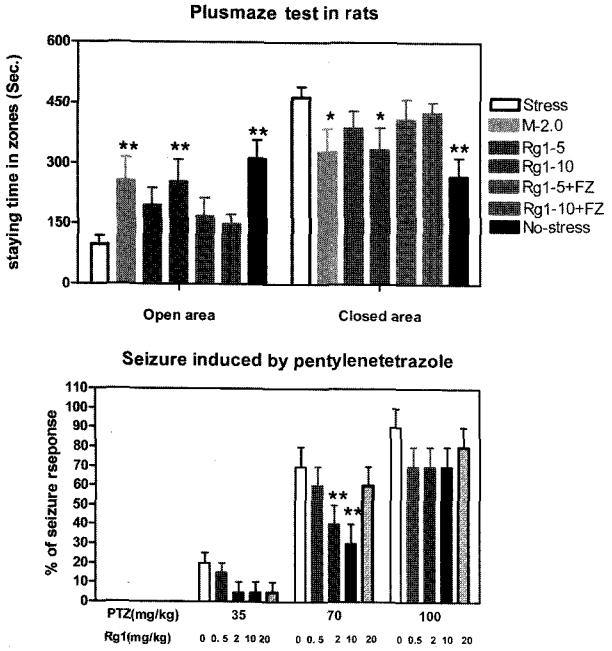

Fig. 7. Effects of ginsenosides on GABAnergic activities in rats and mice (n=10). Each bar represents mean±S.E. of the time spent on the open and closed arms in rats (upper). Each bar represents % of seizure response induced by pentylenetetrazole in mice (lower). No-stress, M-2.0 (Muscimol 2 mg/kg i.p.), ginsenosides Rb1-5 (Rb1 5mg/kg p.o.), Rb_1-10 (Rb_1 10 mg/kg p.o.), Rb_1-5+FZ (Rb_1 5mg/kg p.o. + Flumazenil 3 mg/kg i.p.), or Rb_1-10+FZ (Rb_1 10mg/kg p.o. + Flumazenil 3 mg/kg i.p.) group versus stress group, (** $p<0.01$; * $p<0.05$).

mals exposed to stressors showed a decline in locomotor activity. In the previous studies, this method was applied to induce stress response in rats and mice, and the stress responses were similar to the results of the other stress conditions (Lee *et al.*, 2004; Tan-Lee *et al.*, 2004; Kim *et al.*, 2003a; Takeuchi *et al.*, 2003). The decline in locomotor activity was alleviated by the supplementation of R-G. The animals exposed to stress spent less time in the open arms than the unexposed animals. Furthermore, the animals exposed to stress spent more time in the closed arm than the unexposed animals. R-G and ginsenosides Rb_1 supplementation significantly reversed stress-induced response in the elevated plus-maze test. Therefore, the stressed state reaction to this paradigm, like that experienced following stress, would be associated with a clear reduction in open-arm activity, which is similar with the other studies (Takeuchi *et al.*, 2003; Albonetti and Farabollini, 1994). R-G and ginsenosides Rb_1 supplementation partially blocked stress-induced behavioral changes such as increase in freezing and face washing and decrease in smelling and rearing. This results are similar to Takeuchi's and Tan's (Tan-Lee *et al.*, 2004; Takeuchi *et al.*, 2003). It is well known that an environmental stress leads to grooming and face washing, and corticotropin-releasing hormone (CRH) may be involved in this reaction (Gispen and Isaacson, 1981). It was reported that electrical stimulus elicits behavioral effects including freezing, increase in blood pressure and heart rate (Brandao *et al.*, 1995).

Stress has been thought to be a non-specific response to specific stressors accompanied by the activation of adrenal glucocorticoid and catecholamine release (Djordjevic *et al.*, 2002; Finlay *et al.*, 1995; Ida *et al.*, 1985; Munck *et al.*, 1984). The hypothalamic-pituitary-adrenal (HPA) axis is regarded as the fundamental and traditionally documented system mediating stress response (Kim *et al.*, 2003a; Kim *et al.*, 2003; Kioukia-Fougia *et al.*, 2002). The main regulation of stress related activity of the HPA axis occurs at the level of parvicellular subdivision of the hypothalamic paraventricular nuclei, and the majority of these neurons secrete corticotropin-releasing hormone and vasopressin, which synergistically stimulate ACTH secretion by the pituitary corticotropic cells ((Kim *et al.*, 2003a; Kim *et al.*, 2003; Djordjevic *et al.*, 2002; Koob and Bloom, 1985). ACTH enters the systemic circulation, and stimulates synthesis and release of corticosterone which results from enlargement of the adrenal glands (Djordjevic *et al.*, 2002). The adrenal hypertrophy takes place in response to the secretion of ACTH from the pituitary for increased corticosterone secretion from cortical cells to combat stress (Djordjevic *et al.*, 2002; Walker *et al.*, 1986). The stress condition resulted in the enlargement of adrenal glands and increase the rate of corticosterone secretion (Tan-Lee *et al.*, 2004; Djordjevic *et al.*, 2002; Morimoto *et al.*, 1984). Consistent with a number of evidence, exposure to various kinds of stressors induced a clear increase in corticosterone secretion. Stress condition resulted in a significant increase in the wet weight of adrenal glands, indicating the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis which is highly responsive to stress (Djordjevic *et al.*, 2002; Finlay *et al.*, 1995; Walker *et al.*, 1986; Ida *et al.*, 1985; Munck *et al.*, 1984; Morimoto *et al.*, 1984). Stressed animals have higher plasma corticosterone levels than the non-stressed group.

Findings from the present study showed that the changes in locomotor activity, stress behavior, exploratory activity on the plus maze, plasma corticosterone level, and organ weights induced by the manipulation of different kinds of stress condition which we established in this study and the previous studies were similar to the results of the other studies (Tan-Lee *et al.*, 2004; Takeuchi *et al.*, 2003; Djordjevic *et al.*, 2002; Morimoto *et al.*, 1984). R-G and ginsenoside Rb_1 supplementation par-

tially blocked above most stress responses. Many researchers reported that ginseng extract and its constituents such as ginsenosides have anti-stress activity on animals subjected to stressful stimuli such as foot-shock, cold, heat and immobilization (Lee *et al.*, 2004; Tan-Lee *et al.*, 2004; Takeuchi *et al.*, 2003; Kim *et al.*, 2003a; Takahashi *et al.*, 1992). Our results were similar to them except response to physical stress. R-G prolonged significantly swimming time, balancing time on the wire and running time on the Rota rod but Rb_1 didn't. These results mean that R-G enhances physical capacity or stamina but Rb_1 didn't. They suggest that the anti-stress effects of Rb_1 were not caused by enhancing physical capacity or resistance to physical stimulation but acting on the central nervous system or endocrine system. Active constituents found in most ginseng species include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids (Attele *et al.*, 1999; Lee, 1992). Most pharmacological actions of ginseng are attributed to ginsenosides (Attele *et al.*, 1999; Huang, 1999). Ginsenosides possess the four *trans*-ring rigid steroid skeleton, with a modified side chain at C-20. Among ginsenosides, Rb_1 is panaxdiol with 4 sugars (Attele *et al.*, 1999). Ginsenoside Rb_1 showed the ability to reverse all the changes induced by psychological stress. Therefore, Rb_1 is believed to be the main anti-stress principle of ginseng in case of psychological stress.

Ginseng extracts block the uptake of GAB_A into rat brain synaptosomes (Tsang *et al.*, 1985). Moreover, ginseng saponins are reported to alter the binding of GABAA (Kimara *et al.*, 1994). In addition, ginseng is demonstrated to have diazepam like activity (Bhattacharya *et al.*, 1991). Like ginsenosides, steroidal compounds regulate GABAergic neurotransmission in the brain. Several endogenous steroids such as progesterone, androsterone, neurosteroids, and their metabolites stimulate GABAA-mediated chloride ion flux (Attele *et al.*, 1999; Wehling, 1997). Effects of Rb_1 were interfered by $GABA_A$ receptor antagonist, flumazenil in plus maze test and Rb_1 partially blocked convulsion induced by GABA antagonist pentylenetetrazole. This result means that anti-stress effect of Rb_1 is modulated by GABAnergic nervous system.

These results suggest that Rb_1 protects partially the living organism from stress attack in some cases and it has the potential to be used as a drug in order to alleviate psychological stress response.

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