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Suppressive Effects of Vietnamese Ginseng Saponin and Its Major Component Majonoside-R2 on Psychological Stress-Induced Enhancement of Lipid Peroxidation in the Mouse Brain

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YOBIMOTO, K., K. MATSUMOTO, N. T. T. HUONG, R. KASAI, K. YAMASAKI AND H. WATANABE. *Suppressive effects of Vietnamese ginseng saponin and its major component majonoside-R2 on psychological stress-induced enhancement of lipid peroxidation in the mouse brain.* PHARMACOL BIOCHEM BEHAV **66**(3) 661–665, 2000.—We investigated the in vivo effects of Vietnamese ginseng saponin (VG saponin) and its major component majonoside-R2 (MR2) on psychological stress-induced enhancement of lipid peroxidation in the mouse brain. Psychological stress exposure using a communication box system for 4 h significantly increased the content of thiobarbituric acid reactive substance (TBARS), an index of lipid peroxidation activity, in the brain. Pretreatment with VG saponin (15–25 mg/kg, PO) and MR2 (1–10 mg/kg, IP) significantly attenuated the psychological stress-induced increase in TBARS content in the brain. The aglycone of MR2 (MR2-aglycone: 1.2 mg/kg, IP), at the equivalent dose of MR2 (i.e., 3 mg/kg, IP), also produced the suppressive effect on the increase in the TBARS content. The in vivo suppressive effect of MR2 was dose dependently attenuated by flumazenil (3 and 10 mg/kg, IP), a benzodiazepine receptor antagonist, and pregnenolone sulfate (10 mg/kg, IP), a neurosteroidal negative allosteric modulator of $GABA_A$ receptors. These findings suggest that VG saponin and its major component MR2 have preventive effects on the psychological stress-induced brain cell membrane damage, and that the effect of MR2 is partly due to enhancement of $GABA_A$-ergic systems in the brain. © 2000 Elsevier Science Inc.

Psychological stress Lipid peroxidation Vietnamese ginseng Majonoside-R2 In vivo antioxidant activity

IT has been proposed that lipid peroxidation caused by oxidative stress produces marked damage to the structure and function of cell membranes not only in peripheral tissues, but also in the central nervous system (6). The brain is particularly sensitive to free radical insults because it contains high concentrations of easily peroxidizable polyunsaturated fatty acid (1–3), and is not particularly enriched with protective antioxidant enzymes or other antioxidant compounds (13).

Stressors such as immobilization, electric foot shock, cold swim, etc., with a physical factor have been demonstrated to produce oxidative damage to lipid in the brain in rodents (16), although there are conflicting reports (14,22). The inhibition of such free radical-mediated pathophysiological changes has become a central focus of research efforts designed to prevent or ameliorate free radical-induced degenerative tissue injury in the brain. Our previous study (17) has demonstrated that

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psychological stress exposure using a communication box paradigm (19,20) markedly enhances lipid peroxidation activity in the mouse brain via an increase of neuronal nitric oxide synthase (nNOS)-mediated NO production in the brain, and that drugs with a benzodiazepine or 5-HT_{1A} receptor agonist profile have a protective effect on oxidative brain membrane damage caused by psychological stress.

Vietnamese ginseng (VG, *Panax vietnamensis* Ha et Grushv. Araliaceae) is a wild Panax species that has been used as a herbal medicine in Central Vietnam. The saponin fraction of VG contains not only Panax ginseng (PG) saponins such as ginsenoside Rb_1, -Rg_1, -Rd, -Re, etc., but also ocotillol-type saponins. The latter type saponins, especially majonoside-R2 (MR2), has not been found in other ginsengs such as *Panax ginseng* (5,18). In previous studies, we reported that VG extract and total VG saponin attenuate psychological stress-induced changes in the nociceptive response, the duration of pentobarbital sleep and gastric lesion, and that enhancement of $GABA_A$-ergic systems is involved in the effects of MR2 on the psychologically stressed mice or socially isolated mice [see Huong et al. (8) for review]. Recently it was found that VG saponin, but not MR2, exerted the in vitro inhibitory effect on the free radical generating system-induced lipid peroxidation in the mouse brain and liver homogenates (7). However, it remains unclear if the systemic administration of these substances is capable of suppressing the psychological stress-induced oxidative damage to brain membrane. In this study, we investigated the effect of VG saponin and MR2 on psychological stress-induced increase in thiobarbituric reactive substance in the brain, an index of lipid peroxidation, to clarify their in vivo effects on oxidative damage to brain membrane in psychologically stressed mice.

METHOD

Animals

Male ICR mice (5–7 weeks old, Japan SLC, Shizuoka, Japan) were used for the experiments. The animals were housed in groups of 12–20 per cage (35 × 30 × 16 cm) for at least 1 week before the start of the experiments. Housing condition was thermostatically maintained at 24 ± 1°C, with a constant humidity (65%) and a 12 L:12 D cycle (lights on 0700–1900 h). Food and water were given ad lib. The present studies were conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

Apparatus

Mice were exposed to psychological stress according to the previous method (8,10,11,17) using a communication box paradigm (19,20). Briefly, the communication box consists of two types of compartments; compartments A and B (10 × 10 cm each). These compartments (25 compartments in total) are arranged like a "checkerboard," and are separated by transparent Plexiglas walls. All compartments have stainless steel grid floors, but the floors of the B compartments are covered with Plexiglas plates. Animals were individually placed in each compartment and intermittent electric shocks (2-mA, 10-s duration, 110-s intershock interval) were delivered through the grid floor by a shock generator (Muromachi-Kikai Co., Ltd., Tokyo, Japan). Thus, the animals in the A compartments (sender) received foot shock through the grids floor, while the animals in the B compartments (responder) were only exposed to psychological stress by watching and hearing the struggle, jumping, and vocalization of the sender mice in the adjacent compartments. The sender mice were used once in each experiment. The unstressed control mice were placed individually in the compartments of the control box (10 × 10 cm) without electric grid floor and without exposure to the senders for the same period as the stressed mice. Based on the data obtained in our previous study, the animals were exposed to psychological stress for 4 h and decapitated 30 min after termination of the stress.

Measurement of Lipid Peroxidation Activity

Lipid peroxidation in the brain was measured as previously described (7,17) by modifying the method of Ohkawa et al. (21). The whole brain (excluding cerebellum) was homogenized in 10 vol. of ice-cold phosphate buffer (5 mM, pH 7.4) using a Potter-Elvehjem homogenizer with a Teflon pestle. The brain homogenates (1 ml) were supplemented with 1 ml of 10% trichloroacetic acid and then centrifuged at 8000 × g for 10 min at 4°C. The supernatant was incubated with 1 ml of 0.8% (w/v) 2-thiobarbituric acid at 100°C for 15 min. After a cooling period, TBARS concentration was spectrophotometrically determined at 532 nm (Beckman DU640 Spectrophotometer) using malondialdehyde (MDA) as a standard. The protein contents of tissue homogenates and serum were measured by the Biuret method (15).

Drug Treatment

Vietnamese ginseng saponin (VG saponin), MR2 and an aglycone of MR2 (MR2-aglycone) were obtained according to our previous reports (4,5). Other drugs were obtained from the following sources: flumazenil (Yamanouchi Pharm, Tokyo) and pregnenolone sulfate (Sigma Chem. Co., St. Louis, MO). Flumazenil was suspended in saline containing 0.1% Tween 80. MR2-aglycone and pregnenolone sulfate were suspended in saline containing 0.5% sodium carboxymethyl cellulose. Other test drugs were dissolved in saline. Drug solutions were prepared just before starting the experiments and administered PO or IP at a constant volume of 0.1 ml/10 g body weight. VG saponin was given PO, and MR2 and MR2-aglycone were given IP just before stress. Flumazenil (3 and 10 mg/kg) or pregnenolone sulfate (10 mg/kg) was injected IP 15 min before the stress exposure.

Statistical Analysis

Data were analyzed by one- or two-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for multiple comparisons among different groups. Differences with $p < 0.05$ were considered significant.

RESULTS

As shown in Fig. 1, psychological stress exposure for a 4-h period significantly increased the TBARS content, an index of lipid peroxidation, in the brain. The per oral administration of VG saponin (15–25 mg/kg) had no effect on the brain TBARS content in unstressed control mice, but significantly antagonized the psychological stress-induced increase in the brain TBARS content in a dose-dependent fashion. MR2, a major component of VG saponin, also significantly and dose dependently attenuated the effect of psychological stress on the TBARS content in the brain. Moreover, MR2–aglycone, at the dose (1.2 mg/kg, IP) that is equivalent to the dose of 3 mg/kg MR2, suppressed the psychological stress-induced increase in the brain TBARS content.

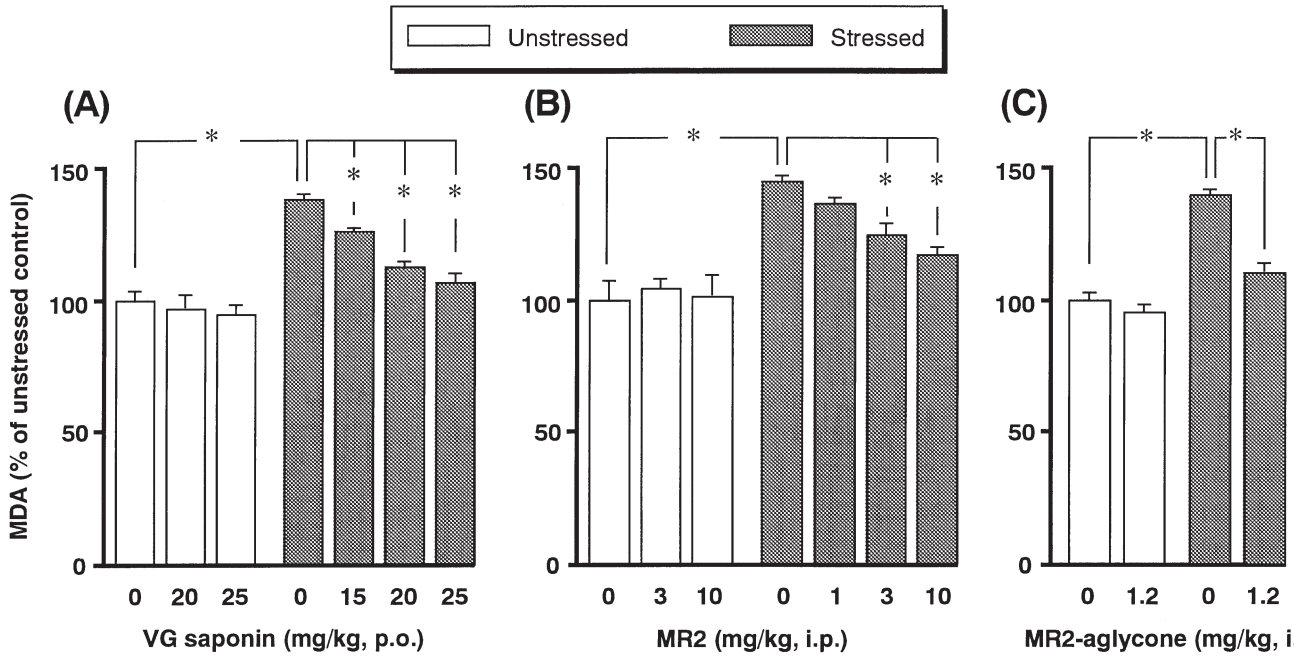

FIG. 1. The in vivo effects of Vietnamese ginseng saponin (A: VG saponin), majonoside-R2 (B: MR2), and majonoside-R2 aglycone (C: MR2-aglycone) on psychological stress-induced enhancement of lipid peroxidation in the mouse brain. Mice were exposed to psychological stress for 4 h as described in the text. Thirty minutes after stress, the animals were decapitated and thiobarbituric acid reactive substances (TBARS) in the brain homogenate was determined by using malondialdehyde (MDA) as a standard. The brain TBARS contents of unstressed vehicle control animals in A, B, and C of this figure were: 76.8 ± 2.6, 70.2 ± 5.0, and 75.6 ± 1.9 pmol MDA/mg protein (mean ± SEM, $n = 6$), respectively. *$p < 0.05$ (the Student–Newman–Keuls test).

Flumazenil (3 and 10 mg/kg, IP), a selective benzodiazepine receptor antagonist, and pregnenolone sulfate (10 mg/kg, IP), a neurosteroidal negative allosteric modulator of the $GABA_A$ receptor, significantly attenuated the effect of MR2 on the brain TBARS content in psychologically stressed mice (Fig. 2).

DISCUSSION

The present results demonstrate that the systemic administrations of Vietnamese ginseng saponin and MR2, a major saponin component of Vietnamese ginseng, exert the protective effect on brain membrane lipid damage caused by psychological stress in mice, and that the effect of MR2 is partly mediated by facilitation of GABAergic systems in the brain.

We previously reported that Vietnamese ginseng saponin inhibited lipid peroxidation reaction elicited by free radical-generating systems, iron ferrous plus ascorbic acid and iron ferrous plus hydrogen peroxide, in brain and liver homogenates, while MR2 had no effect on the reaction, indicating that Vietnamese ginseng saponin but not MR2 has radical-scavenging activity in vitro (7). Thus, it is of quite interest to note that both Vietnamese ginseng saponin and MR2 are capable of producing antioxidative effects in vivo. Recent findings (17) in this laboratory indicate that psychological stress exposure for a 4-h period enhances brain lipid peroxidation activity without affecting the activity in the liver or serum. This enhancement appeared to be triggered by an increase of nNOS-mediated NO production in the brain (17). Taken together, these results suggest that in vivo antioxidant effect of Vietnamese ginseng saponin is at least partly attributable to its radial scavenging activity. Moreover, the present results raise the possibility that systemically administered MR2 may be converted to metabolites with a radical scavenging activity or an inhibitory effect on the activity of nNOS in the brain and thereby exert the in vivo antioxidant activity. However, this possibility seems to be slight because the inhibitory effect of MR2 on psychological stress-induced enhancement of brain lipid peroxidation was significantly attenuated by systemic administrations of flumazenil and pregnenolone sulfate, drugs capable of interacting with the $GABA_A$ receptor complex.

MR2 is an ocotillol-type glycoside saponin. In our preliminary studies, it was found that the intracerebroventricular injection of MR2–aglycone produced the same pharmacological effects as MR2 in the mice exposed to long-term social isolation stress (Huong et al., unpublished data). In the present study, systemically administered MR2–aglycone also inhibited the psychological stress-induced enhancement of brain lipid peroxidation at the dose equivalent to the effective dose of MR2. Thus, it is very likely that aglycone of MR2 plays an important role in the in vivo antioxidant effect of MR2 in the brain.

Flumazenil, a selective benzodiazepine receptor antagonist, and pregnenolone sulfate, a negative allosteric neuromodulator of the $GABA_A$ receptor, significantly reversed the preventive effect of MR2 on the psychological stress-induced membrane damage in the brain. We previously reported that flumazenil and the $GABA_A$ receptor antagonist picrotoxin blocked the antagonistic effect of MR2 on opioid-induced antinociception (9). Moreover, recently it was found that pregnenolone sulfate antagonized the reversing effect of MR2 on social isolation stress-induced decrease in pentobarbital sleep

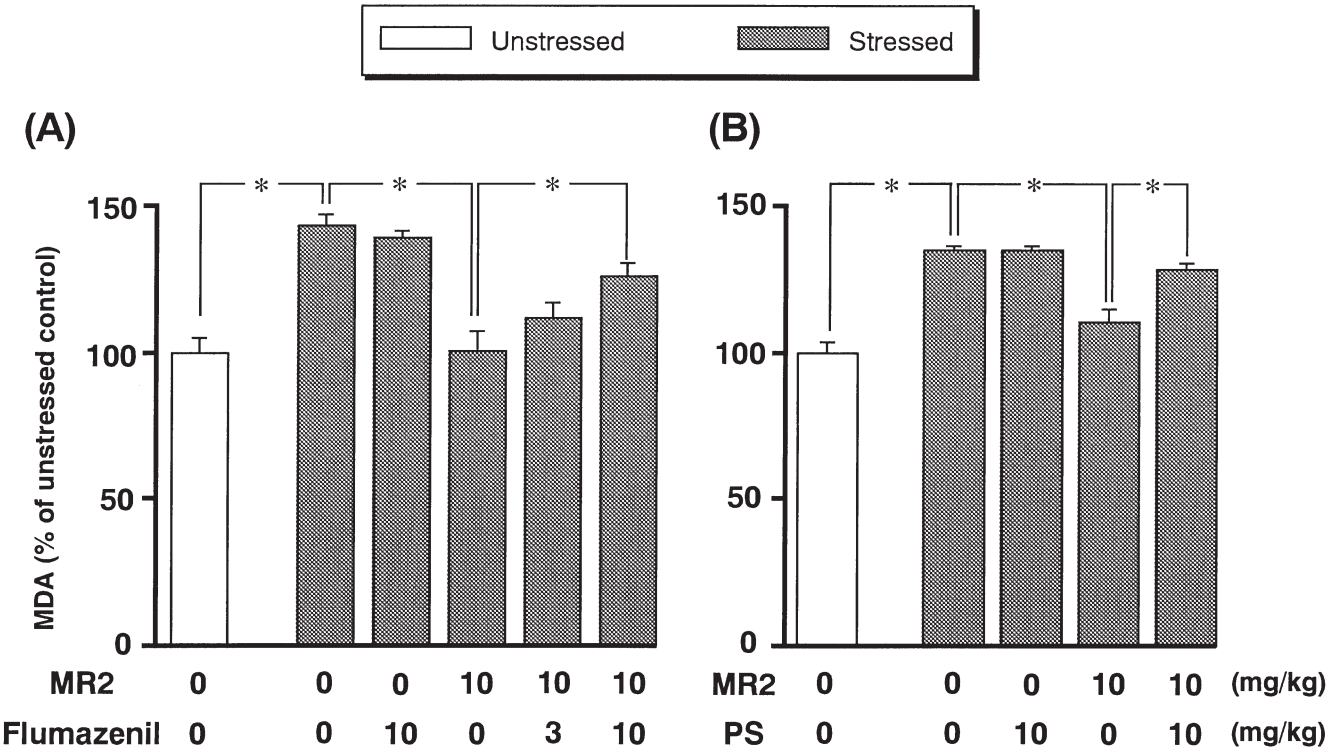

FIG. 2. Effects of flumazenil (A) and pregnenolone sulfate (B) on majonoside-R2–induced suppression of thiobarbituric acid reactive substances (TBARS) production in the psychologically stressed mouse brain. Flumazenil (3 and 10 mg/kg) or pregnenolone sulfate (PS: 10 mg/kg) was injected IP 15 min before the stress exposure. Majonoside-R2 (MR2) was given IP just before stress. Thirty minutes after stress, the animals were decapitated and TBARS in the brain homogenate was determined by using malondialdehyde (MDA) as a standard. The mean of the brain TBARS content in each unstressed vehicle control group is expressed as 100%. Each data column represents the mean ± SEM of five to six mice. $*p < 0.05$ (the Student–Newman–Keuls test).

in mice, suggesting the involvement of neuroactive steroids in the effect of MR2 (12). Thus, the antagonistic interaction between MR2 and $GABA_A$ receptor-related compounds observed in this study is consistent with those previous findings.

In our previous study (17), systemic administrations of diazepam, an anxiolytic benzodiazepine receptor agonist, and FG7142, an anxiogenic benzodiazepine receptor inverse agonist, exerted an opposite effects on lipid peroxidation activity in the brains of psychologically stressed animals, i.e., suppression and exacerbation of the activity, respectively, in a manner sensitive to flumazenil (17). These findings suggested a modulatory role for the $GABA_A$/benzodiazepine receptor/chloride ionophore complex in the psychological stress-induced enhancement of brain lipid peroxidation activity (17). Taken together, the present results that both flumazenil and pregnenolone sulfate significantly reversed the preventive effect of MR2 on the psychological stress-induced brain membrane damage suggest that the enhancement of $GABA_A$-ergic systems in the brain is at least partly involved in the in vivo antioxidative effect of MR2. Nevertheless, further investigations using more selective $GABA_A$ receptor antagonists will be required to elucidate the role of GABAergic systems in the in vivo antioxidant activity of MR2 in the psychologically stressed animals.

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