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Crude Sponin Extracted From Vietnamese Ginseng and Its Major Constituent Majonoside-R2 Attenuate the Psychological Stress- and Foot-Shock Stress-Induced Antinociception in Mice

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HUONG, N. T. T., K. MATSUMOTO, K. YAMASAKI, N. M. DUC, N. T. NHAM AND H. WATANABE. *Crude saponin extracted from Vietnamese ginseng and its major constituent majonoside-R2 attenuate psychological stress- and foot shock stress-induced antinociception in mice.* PHARMACOL BIOCHEM BEHAV **52**(2) 427–432, 1995. – Effects of Vietnamese ginseng (VG) crude saponin and majonoside-R2, a major saponin constituent, on the psychological stress- and foot shock stress-induced antinociception in the tail pinch test were examined in mice. VG crude saponin (6.2, 12.5, and 25 mg/kg, PO) attenuated psychological stress- but not foot shock stress-induced antinociceptive response, whereas majonoside-R2 (3, 6.2, and 12.5 mg/kg, PO and IP), as well as naloxone (2 mg/kg, IP), suppressed both psychological stress- and foot shock stress-induced antinociception. Pretreatment with the crude saponin (12.5 mg/kg, PO) or majonoside-R2 (6.2 mg/kg, PO) for 5 days followed by the treatment in combination with stress for next 5 days did not affect the development of adaptation to foot shock stress, but they significantly suppressed the antinociceptive action of the stress measured on the first, second, and third day during the stress exposure period. Majonoside-R2 (6.2 mg/kg, PO) but not the crude saponin (12.5 mg/kg, PO) significantly blocked the development of adaptation to psychological stress. These results suggest that VG crude saponin has the suppressing effect on psychological stress- and foot shock stress-induced antinociception and that majonoside-R2 is important for the action of the saponin.

Vietnamese ginseng crude saponin Majonoside-R2 Psychological stress-induced antinociception Footshock stress-induced antinociception

VIETNAMESE ginseng (VG), a wild Panax species, has been used as a herbal medicine in central Vietnam for treating many serious diseases and for enhancing physical strength. This ginseng contains not only Ginseng saponins such as ginsenoside Rb_1, -Rg_1, -Rd, -Re, etc., but also ocotillol-type saponins, especially majonoside-R2 (Fig. 1), which accounts for 5.29% in VG but does not exist in Panax ginseng (PG) (12–15). Because of such characteristic composition, studies on this plant have been done to discover new pharmacological actions for medicinal use.

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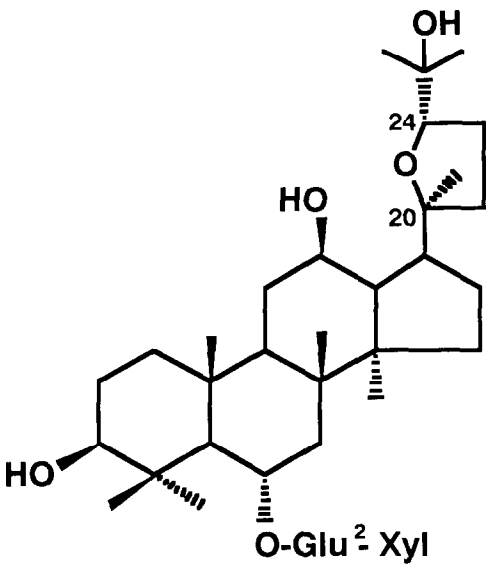

FIG. 1. Chemical structure of majonoside-R2.

Physical stress manipulations such as electric shock are known to cause antinociception in experimental animals (3,5, 8). Emotional factors like anxiety and fear also play an important role in the mechanism of the stress-induced antinociception (6,23). PG extract has been demonstrated to suppress the development of adaptation to psychological stress in mice (24). In the present study, to clarify antistress effect of VG we investigated whether VG saponins and its major saponin majonoside-R2 exerted effects on psychological stress- and foot shock stress-induced antinociception in mice.

METHOD

Animals

Male 5-week-old ddY mice (25–30 g, Japan SLC, Inc., Hamamatsu, Japan) were used in the experiments. The animals were housed in groups of 20–25 per cage (29 × 36 × 18 cm) for at least 1 week before the experiment, with free access to food and water. Housing conditions were thermostatically maintained at 24 ± 1°C, with a 12 L : 12 D cycle (lights on: 0730–1930 h).

Apparatus

The apparatus (Fig. 2) for stress exposure consists of four compartments (A1, A2, B1, B2; 12.5 × 12.5 × 30 cm each) with transparent Plexiglas walls and stainless steel grid floor (1.5 mm diameter rods 0.9 cm apart from each other). A scrambled electric shock (1 mA, 0.2 Hz, 1 s duration) is delivered through the floor grids by a shock generator (Muromachi-Kikai Co., Ltd., Tokyo, Japan), but the grids of two compartments (B1, B2) are covered with Plexiglas plates. Mice were individually placed in each compartment. Thus mice in B1 and B2 were not given electric shock but only exposed to psychological stress by watching and hearing the struggle, jumping, and vocalization of shocked animals in the adjacent compartments (A1, A2).

Preparation of Vietnamese Ginseng Crude Saponin and Majonoside-R2

Powdered root and rhizome of Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv. Araliaceae; 5-year-old plant age) were extracted four times with 96%, 48%, 24%, and 0% v/v ethanol, respectively (percolation method). The extracts were combined and evaporated under reduced pressure and then lyophilized to yield VG crude extract. Following extraction with ethyl ether to remove lipids from the extract, water-

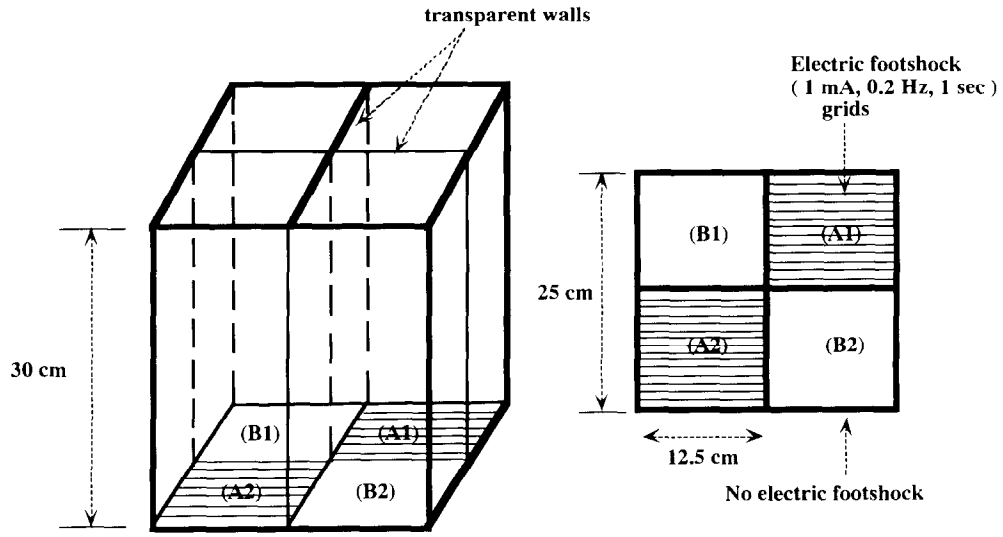

FIG. 2. A schematic drawing of the communication box to expose mice to either psychological stress or foot shock stress. Mice were placed individually into four compartments (A1, A2, B1, B2). The animals in A1 and A2 received electric foot shock through stainless steel grid floor, whereas those in B1 and B2 received no electric foot shock but they were exposed to psychological stress by watching the behavior or hearing the vocalization of the foot shocked mice.

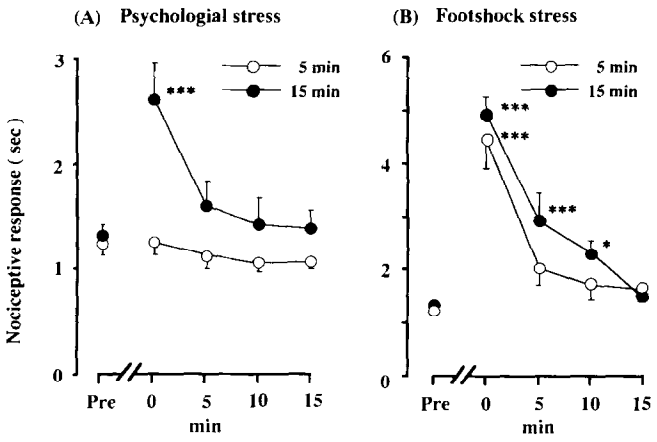

FIG. 3. Psychological stress- and foot shock stress-induced antinociception. Mice were exposed to either psychological stress (A) or foot shock stress (B) for 5 (○) or 15 min (●). The latency of nociceptive responses caused by the tail-pinch method was measured every 5 min for 15 min immediately after the termination of stress exposure. Each point is the mean ± SEM of 12–15 mice. $*p < 0.05$ and $***p < 0.001$ compared with prestress values (Dunnett's test).

saturated *n*-butanol was added. The *n*-butanol extract was evaporated to dryness to yield the crude saponin fraction. Majonoside-R2 was purified from the fraction as described previously (12–14). Yields of VG crude saponin and majonoside-R2 were 13.2 and 5.29% of the raw material, respectively.

Drug Administration

VG crude saponin and majonoside-R2 were dissolved in distilled water. Either these agents or distilled water was administered PO 1 h before stress exposure. In some experiments, naloxone-HCl (Sigma Chem., Co., St. Louis, MO) and majonoside-R2 dissolved in saline were administered IP 10 and 30 min before stress, respectively. All drugs were administered in a constant volume of 0.1 ml/10 g body weight.

Effect on the Development of Adaptation to Psychological and Foot Shock Stress

For examining the effect of tested agents on the development of adaptation to the stressful manipulation, mice were administered daily with 12.5 mg/kg VG crude saponin (PO), 6.2 mg/kg majonoside-R2 (PO) or distilled water for 5 days before and during 5 days of the experiment period. During the experiment period, the animals were exposed daily to either psychological stress or foot shock stress for 15 min. Administration of the test agents during this period was carried out 1 h before the stress exposure

Measurement of Antinociception

Nociceptive responses in the tail pinch test was measured by the modified Haffner's method (20). Briefly, mice were pretested by pinching their tails with crammer (500 g pressure), and only the mice that showed nociceptive responses such as biting the crammer within 2 s were used for the experiments. Immediately after termination of stress exposure, the nociceptive responses were measured every 5 min for 15 min, using a 6-s cutoff time (the maximum latency accepted to avoid the tissue damage owing to longer application of the crammer). The antinociceptive effect was calculated as the area under the curve (AUC) obtained by plotting the response time on the ordinate and the time intervals on the abscissa. A significant decrease of AUC value compared with that recorded on the initial stress exposure, indicates the development of stress adaptation.

Statistical Analysis

Data were expressed as the mean ± SEM and analyzed with one-way analysis of variance followed by Dunnett's test. Difference was considered statistically significant at $p < 0.05$.

RESULTS

Psychological Stress- and Foot Shock Stress-Induced Antinociception in the Tail Pinch Test

As shown in Fig. 3A, exposure to psychological stress for 15 min but not 5 min significantly increased the latency of nociceptive responses of mice in the tail pinch test. The antinociceptive effect of the psychological stress reached the maximum immediately after the termination of stress application. Exposure to foot shock stress for 5 or 15 min also produced a significant antinociceptive effect. The effect was more potent and persisted longer than that of psychological stress (Fig. 3B).

Effects of VG Crude Saponin and Majonoside-R2 on Psychological Stress- and Foot Shock Stress-Induced Antinociception

VG crude saponin, at 6.2–25 mg/kg (PO), significantly suppressed the antinociceptive effect of the psychological stress (Fig. 4A). The saponins at the same doses had no effect on foot shock stress-induced antinociception (Fig. 4B). As shown in Fig. 5A and B, oral administration and intraperitoneal injection of majonoside-R2, as well as IP injection of

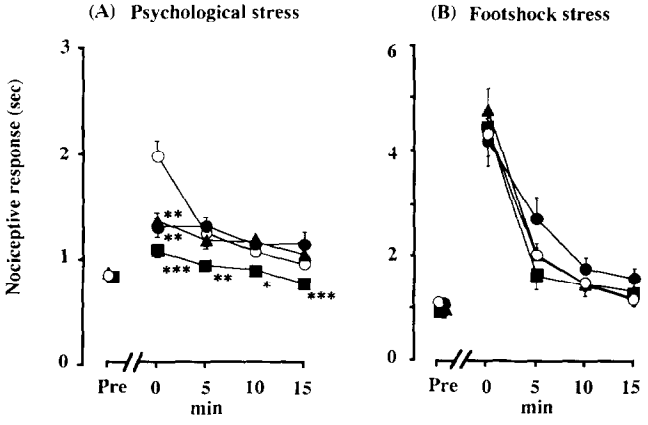

FIG. 4. Effect of Vietnamese ginseng (VG) crude saponin on the antinociception induced by psychological stress or foot shock stress. Mice were exposed to psychological stress (A) or foot shock stress (B) for 15 min. Either vehicle (○) or VG crude saponin [6.2 (■), 12.5 (●), and 25 mg/kg (▲)] was administered PO 1 h prior to stress exposure. The latency of nociceptive responses caused by the tail pinch was measured every 5 min for 15 min immediately after the termination of stress exposure. Each point is the mean ± SEM of 15 mice. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared with the vehicle-treated group (Dunnett's test).

naloxone (2 mg/kg), significantly attenuated the antinociception induced by psychological stress or foot shock stress exposure at half the doses that VG saponin required to produce the effect.

Effects of VG Crude Saponin and Majonoside-R2 on the Development of Adaptation to Psychological Stress and Foot Shock Stress

The psychological stress- and foot shock stress-induced antinociceptive responses were gradually decreased by repeated exposure to the same stressors, indicating the development of adaptation to these stressors. Daily treatment with the crude saponin (12.5 mg/kg, PO) or majonoside-R2 (6.2 mg/kg, PO) had no effect on the development of adaptation to foot shock stress (Fig. 6B), although both agents significantly suppressed the foot shock stress-induced antinociception measured on the first, second, and third day during the stress exposure period as compared with the vehicle control. The crude saponin treatment did not affect the development of adaptation to psychological stress, while in majonoside-R2-treated mice no significant development of stress adaptation was observed (Fig. 6A).

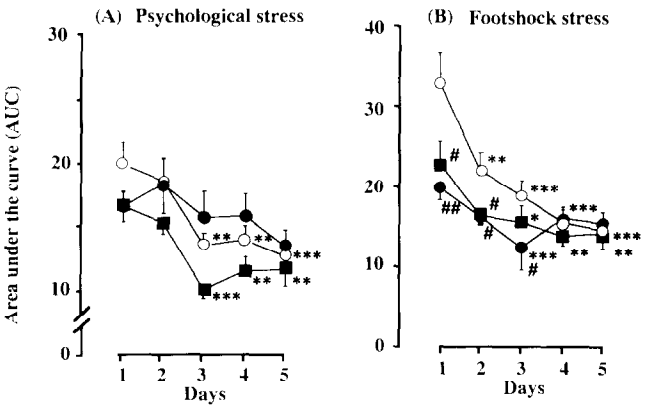

FIG. 6. Effects of repeated administration of VG crude saponin and majonoside-R2 on the development of adaptation to psychological stress or foot shock stress. Daily changes in the antinociceptive effect of psychological stress (A) and foot shock stress (B) were expressed as the area under the curve by plotting the latency of nociceptive response (s) on the ordinate and the nociceptive stimulation intervals (min) on the abscissa. Mice were pretreated daily with vehicle (○), VG crude saponin (■; 12.5 mg/kg, PO), or majonoside-R2 (●, 6.2 mg/kg, PO) for 5 days, and from the sixth day, tested agents were given daily 1 h before the stress exposure for 5 days. Each point is the mean ± SEM of 12 mice. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared with the respective value recorded on the day 1. $\#p < 0.05$ and $\#\#p < 0.01$ compared with the vehicle-treated group (Dunnett's test).

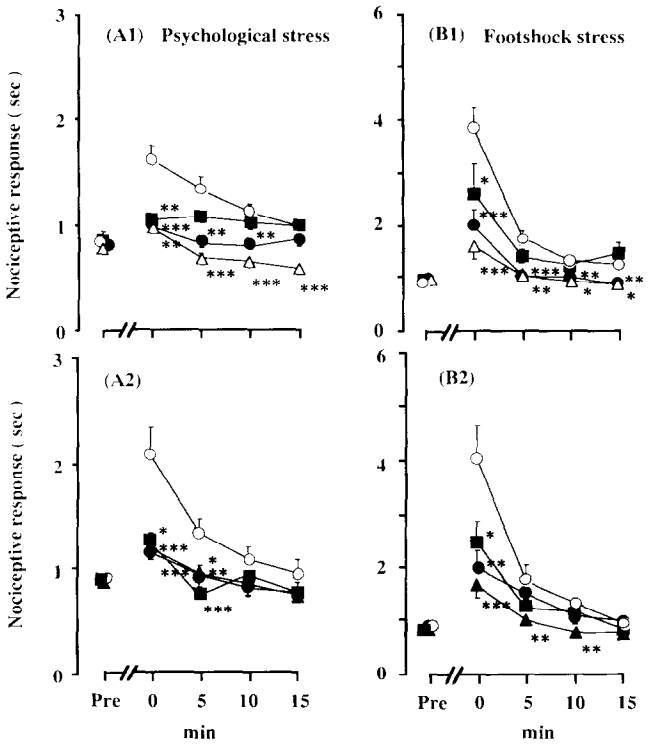

FIG. 5. Effects of majonoside-R2 and naloxone on the antinociception induced by psychological stress or foot shock stress. Mice were exposed to psychological-stress (A1, A2) or foot shock-stress (B1, B2) for 15 min. In A1 and B1, vehicle (○), naloxone [2 (△) mg/kg, IP], and majonoside-R2 [3 (■), 6.2 (●), and 12.5 (▲) mg/kg, PO] were given 10, 10 min, and 1 h before stress exposure, respectively. In A2 and B2, vehicle (○) and majonoside-R2 [3 (■), 6.2 (●), and 12.5 (▲) mg/kg] were administered IP 30 min before stress exposure. The latency of nociceptive responses caused by the tail pinch was measured every 5 min for 15 min immediately after the termination of stress exposure. Each point is the mean ± SEM of 12–15 mice. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared with the vehicle-treated group (Dunnett's test).

DISCUSSION

Noxious, non-noxious, and environmental stimuli are known to attenuate nociceptive sensitivity in experimental animals as well as in humans (1,8,30). In the present study, the exposure of mice to psychological stress for 15 min significantly increased the latency of nociceptive responses in the tail pinch test, and the antinociceptive effect was short lasting. These findings agree with the data reported by Takahashi et al. (22) that psychological stress exposure produced a short-lasting antinociceptive effect in the tail pinch test in mice. To induce antinociception, a 15-min period of psychological stress exposure was required in this study, while Takahashi et al. reported that psychological stress exposure (using a shock box that consists of nine compartments and electric foot shock 2 mA with intershock interval of 4 s and shock duration of 1 s) for 5 min significantly induced antinociception. This may be due to difference in the stress paradigms such as strength of foot shock.

It is of interest to note that the effects of VG on stress-induced antinociception are quite different from those of the standardized PG extract G115 (trademark for the standardized ginseng extract containing 4% ginsenosides). Takahashi et al. (24) demonstrated that acute administration of G115 had no effect on the foot shock stress- or psychological stress-induced antinociception, whereas repeated administration of this extract suppressed the development of adaptation to psychological stress but did not so to foot shock stress. In the present study, the acute administration of VG crude saponin (6.2–25 mg/kg, PO) significantly suppressed the antinociceptive response caused by the psychological stress without affect-

ing the response caused by foot shock stress. Moreover, repeated administration of the VG saponin had no effect on the development of adaptation to either psychological stress or foot shock stress exposure, although it suppressed the foot shock stress-induced antinociception in the initial stage. We have previously demonstrated that new saponin compounds (Vina-ginsenosides) and ocotillol-type saponins, especially majonoside-R2, which account for over 50% of VG saponins, do not exist in PG saponin fraction (12–14). Thus, the different action profiles of VG saponins from PG saponins may be due to the difference in the chemical composition between these two saponin extracts.

Acute administration of majonoside-R2 (PO and IP) but not VG crude saponin (PO) attenuated the antinociceptive response caused by foot shock stress, whereas repeated administration of these agents significantly suppressed the response. The reason for this discrepancy between majonoside-R2 and VG crude saponin remains to be clarified, but it is possible that other components of VG crude saponin may apparently counteract the effect of majonoside-R2. Nevertheless, these results suggest sufficiently that majonoside-R2 is important for the action of VG crude saponin.

An opioid mechanism is suggested to be involved in psychological stress- and foot shock stress-induced antinociception, because naloxone, an opioid antagonist, attenuates antinociception caused by the stress in the tail-pinch test (22). On the other hand, the data reported by other groups (2,17,21,28) suggest that serotonergic mechanisms are not only involved in the opioid receptor-mediated antinociception but also in the development of stress adaptation following repeated stress exposure. Cancela et al. reported that naloxone pretreatment fully antagonized the appearance of adaptive changes of 5-HT_1 receptors after chronic stress (2). In this study, majonoside-R2, as well as naloxone, attenuated both antinociceptive response caused by both psychological stress and foot shock stress. It also suppressed the development of adaptation to the psychological stress. Thus, although the underlying mechanism of action of majonoside-R2 remains unclear, it is of interest to speculate that at least two neuronal mechanisms, an opioid system and a serotonergic system, may play important roles in the effect of this compound on the stress-induced antinociception and the development of stress adaptation in mice.

In conclusion, Vietnamese ginseng crude saponin attenuated psychological stress- and foot shock stress-induced antinociceptive response in the tail-pinch test. Its major saponin constituent majonoside-R2 is important for the effect of Vietnamese ginseng saponins. Nevertheless, to clarify the mechanisms of action of majonoside-R2 on the stress-induced antinociception requires further investigation.

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