The Possible Involvement of GABAA Systems in the Antinarcotic Effect of Majonoside-R2, a Major Constituent of Vietnamese Ginseng, in Mice

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ABSTRACT—The effect of majonoside-R2 on morphine- and U-50,488H-induced antinociception was examined by the tail-pinch test in mice and compared with that of diazepam. Majonoside-R2 and diazepam inhibited the morphine- and U-50,488H-induced antinociception, and the actions were antagonized by the benzodiazepine receptor antagonist flumazenil and the GABA-gated Cl− channel blocker picrotoxin. Diazepam but not majonoside-R2 exhibited a protective activity against convulsion caused by the GABAA antagonists bicuculline and picrotoxin. These results indicate that GABAA systems are involved in the effect of majonoside-R2 on the opioid-induced antinociception and suggest that the mechanisms of action of majonoside-R2 may differ from those of diazepam.

Keywords: Majonoside-R2, Antinociception, GABAA-receptor

Majonoside-R2, a major constituent of Vietnamese ginseng, is an ocotillol-type saponin isolated and purified from Vietnamese ginseng saponin. This ocotillol-type saponin has not been isolated from Panax ginseng, American ginseng or Sanchi ginseng (1).

Our previous data demonstrated that majonoside-R2 reversed the psychological stress-induced decrease in pentobarbital sleep to the normal level in mice and that the effect of majonoside-R2 was abolished by flumazenil, a benzodiazepine receptor antagonist (2). Moreover, recent findings in this laboratory showed that orally administered Vietnamese ginseng extract and crude saponin attenuated the morphine- and U-50,488H-induced antinociception and suppressed the development of morphine tolerance (3). Taken together, it is possible that opioid and GABAA-receptor mechanisms are involved in the pharmacological action of majonoside-R2. In this study, to further test this possibility, we investigated the effects of majonoside-R2 on the morphine- and U-50,488H-induced antinociception in the tail-pinch test in mice and compared them with those of diazepam.

Male 5-week-old ddY mice (Japan SLC, Shizuoka) were used for the experiments.

Majonoside-R2 was purified from the saponin fraction of Vietnamese ginseng (yield: 5.29% of the dry material) as previously described (1). Test drugs, except diazepam, flumazenil and bicuculline, were dissolved in saline. Diazepam (Cercine®; Takeda Chemical Industries Ltd., Osaka) was dissolved in saline containing 40% propylene glycol. Bicuculline (Calbiochem, La Jolla, CA, USA) was dissolved in saline by adding a few drops of 1 N-HCl. All drug solutions were prepared just before starting the experiments and administered in a constant volume of 0.1 ml/10 g body weight.

The nociceptive response in the tail-pinch test was measured according to Haffner’s method as previously reported (4). To prevent tissue damage, a cut-off time of 6 sec was selected. The latency to show nociceptive responses was measured at 30 and 15 min after morphine HCl (Dainippon Pharmaceutical Co., Ltd., Osaka) and U-50,488H (trans-(±)-3,4-dichloro-N-methyl-N-[2-[1-pyrrolidinyl]-cyclohexyl] benzeneacetamide; Sigma Chem., Co., St. Louis, MO, USA) administration, respectively, when the effects of these opioids peaked (3).
The tail-pinch latency was expressed as the mean percentage maximum possible effect (5): \( \% \text{MPE} = \frac{\text{post-drug latency} - \text{pre-drug latency}}{\text{cut-off time} - \text{pre-drug latency}} \times 100. \)

Majonoside-R2 and diazepam were injected i.p. 30 min before morphine or U-50,488H administration. Morphine HCl and U-50,488H were injected subcutaneously (s.c.). Flumazenil (Anexate\textsuperscript{R}; Roche Co., Ltd., Basel, Switzerland) and picrotoxin (Sigma Chem.) were injected i.v. and i.p., respectively, just before opioid administration. In our preliminary study (6), we found that majonoside-R2 (3.1–6.2 mg/kg, i.p.) and diazepam (0.5–1.0 mg/kg) caused a dose-dependent suppression of morphine (5 mg/kg, s.c.)- and U-50,488H (15 mg/kg, s.c.)-induced antinociception in the tail-pinch test. Thus, in this study, we chose submaximal doses of majonoside-R2 (3.1 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.).

To evaluate the protective action on bicuculline- and picrotoxin-induced convulsion in mice, diazepam and majonoside-R2 were administered i.p. After 30 min, bicuculline (3 mg/kg) or picrotoxin (3 mg/kg) was administered s.c. and the latency to show the first generalized myoclonic seizures (characterized by quick, whole-body twitches and jerks), and mortality were recorded during

**Fig. 1.** Antagonism by flumazenil and picrotoxin of the suppressing effects of majonoside-R2 and diazepam on morphine-induced antinociception in the tail-pinch test in mice. After the basal nociceptive responses in the tail-pinch test were recorded, morphine (5 mg/kg, s.c.) was administered. The latency of the nociceptive response was measured at 30 min after morphine administration. Majonoside-R2 (MR2, 3.1 mg/kg) and diazepam (0.5 mg/kg) were administered i.p. 30 min before morphine. Flumazenil (A: 1 mg/kg, i.v.) and picrotoxin (B: 1 mg/kg, i.p.) were administered just before opioid administration. Each column represents the mean \( \% \text{MPE} \) (maximum possible effect) \( \pm \) S.E.M. (n = 10). **\( P < 0.01 \) vs vehicle groups. ***\( P < 0.01 \) vs majonoside-R2 or diazepam alone (Tukey's test).
an observation period of 30 (for bicuculline-induced convulsion) or 60 min (for picrotoxin-induced convolution).

The effects of drugs on the nociceptive response were analyzed with two-way analysis of variance (ANOVA) followed by Tukey's test. The protective effects on GABA_A antagonist-induced convulsion were analyzed with the Mann-Whitney test. Differences of P<0.05 were considered significant.

These 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.

As shown in Fig. 1, majonoside-R2 (3.1 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) exhibited inhibitory effects on the morphine-induced antinociception in the tail-pinchocken test. A significant interaction between majonoside-R2 (3.1 mg/kg, i.p.) and flumazenil (1 mg/kg, i.v.) treatment was observed in the morphine-induced antinociception [F_majonoside-R2 x flumazenil (1,34)=9.436, P<0.01]. An interaction between diazepam (0.5 mg/kg, i.p.) and flumazenil (1 mg/kg, i.v.) treatment in the morphine-induced antinociception was also statistically significant [F_diazepam x flumazenil (1,34)=8.444, P<0.01]. Flumazenil

![Diagram](image-url)

**Fig. 2.** Antagonism by flumazenil and picrotoxin of the suppressing effects of majonoside-R2 and diazepam on U-50,488H-induced antinociception in the tail-pinchocken test in mice. After the basal nociceptive responses in the tail-pinchocken test were recorded, U-50,488H (15 mg/kg, s.c.) was administered. The latency of the nociceptive response was measured at 15 min after U-50,488H. Majonoside-R2 (MR2, 3.1 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) were administered i.p. 30 min before U-50,488H. Flumazenil (A: 1 mg/kg, i.v.) and picrotoxin (B: 1 mg/kg, i.p.) were administered just before opioid administration. Each column represents the mean %MPE (maximum possible effect)±S.E.M. (n=10). **P<0.01 vs vehicle groups. #P<0.05 and a#P<0.01 vs majonoside-R2 or diazepam alone (Tukey's test).
significantly antagonized not only the effect of diazepam but also that of majonoside-R2 on the morphine-induced antinociception in the tail-pinch test (Fig. 1A). Moreover, significant interactions between majonoside-R2 (3.1 mg/kg, i.p.) and picrotoxin (1 mg/kg, i.p.) treatment and between diazepam (0.5 mg/kg, i.p.) and picrotoxin (1 mg/kg, i.p.) treatment in the morphine antinociception were observed [Fig. 1B: Fmajonoside-R2 x picrotoxin (1,36) = 10.071, P <0.01; Fdiazepam x picrotoxin (1,36) = 15.045, P <0.01]. Picrotoxin, as well as flumazenil, significantly attenuated not only the suppressing effect of majonoside-R2, but also that of diazepam, on morphine-induced antinociception.

Moreover, the selective κ-agonist U-50,488H-induced antinociception in the tail-pinch test was antagonized by majonoside-R2 (3.1 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.). A two-way ANOVA revealed significant interactions between diazepam and flumazenil (1 mg/kg, i.v.) treatment [Fdiazepam x flumazenil (1,34)= 20.548, P<0.01], and between majonoside-R2 and flumazenil (1 mg/kg, i.v.) treatment [Fmajonoside-R2 x flumazenil (1,35)= 9.447, P<0.01] in U-50,488H-induced antinociception (Fig. 2A). Significant interactions between diazepam (0.5 mg/kg, i.p.) and picrotoxin (1 mg/kg, i.p.) treatment [Fdiazepam x picrotoxin (1,36)= 6.170, P<0.05] and between majonoside-R2 (3.1 mg/kg, i.p.) and picrotoxin (1 mg/kg, i.p.) treatment interaction [Fmajonoside-R2 x picrotoxin (1,36)= 12.808, P<0.01] were also observed (Fig. 2B). Both flumazenil and picrotoxin significantly blocked the effects of diazepam and majonoside-R2 on U-50,488H-induced antinociception, without changing the antinociceptive action of morphine or U-50,488H by themselves.

To further clarify whether majonoside-R2 has the similar pharmacological profiles to diazepam, we tested the effects of majonoside-R2 on the GABA<sub>A</sub> antagonist-induced convulsion, and compared them with those of diazepam. As summarized in Table 1, diazepam (0.5 mg/kg, i.p.) but not majonoside-R2 (1.5, 3.1 and 6.2 mg/kg, i.p.), significantly delayed the onset of convulsions caused by bicuculline (3 mg/kg, s.c.) and picrotoxin (3 mg/kg, s.c.).

| Table 1. Effects of majonoside-R2 and diazepam on bicuculline- and picrotoxin-induced convulsion |
|-----------------------------------------------|----------|-----------------|-----------------|
| Groups                                      | Dose (mg/kg) | Latency (min) | No. of animals showing convulsion | Mortality (%) |
| Exp. I. Bicuculline (3 mg/kg, s.c.)-induced convulsion |
| Vehicle                                     | 3.8±0.6    | 8/8             | 100                           |
| Majonoside-R2 (1.5 mg/kg, i.p.)             | 3.4±0.3    | 9/9             | 100                           |
| Majonoside-R2 (3.1 mg/kg, i.p.)             | 3.3±0.4    | 8/8             | 100                           |
| Majonoside-R2 (6.2 mg/kg, i.p.)             | 2.6±0.25   | 8/8             | 100                           |
| Diazepam (0.5 mg/kg, i.p.)                  | 15.2±3.3*  | 8/8             | 25                            |
| Exp. II. Picrotoxin (3 mg/kg, s.c.)-induced convulsion |
| Vehicle (15.6±0.9 mg/kg, i.p.)               | 12/12     | 16.7            |
| Majonoside-R2 (1.5 mg/kg, i.p.)             | 16.4±1.5   | 12/12           | 8.3                           |
| Majonoside-R2 (3.1 mg/kg, i.p.)             | 14.8±1.2   | 12/12           | 8.3                           |
| Majonoside-R2 (6.2 mg/kg, i.p.)             | 13.5±0.9   | 12/12           | 16.7                          |
| Diazepam (0.5 mg/kg, i.p.)                  | 48.8±4.8*  | 4/12            | 0                             |

Majonoside-R2 and diazepam were administered i.p. 30 min before s.c. administration of bicuculline or picrotoxin. The latency to show the first generalized myoclonic seizures and mortality were recorded. Animals without seizures during the test session were assigned the maximum latency of 30 and 60 min in the bicuculline- and picrotoxin-induced convulsion experiments, respectively. The latency data are expressed as the mean ± S.E.M. *P<0.05 vs vehicle groups (Mann-Whitney test).

It has been reported that diazepam attenuates the antinociceptive effect of opioid agonists and that the action is partially reversed by GABA<sub>A</sub> antagonists (7-9). Consistent with these findings, the present results showed that the antinociception caused by μ- and κ-opioid agonists in the tail-pinch test were significantly suppressed by diazepam, and that the effect of diazepam was significantly antagonized by the benzodiazepine receptor antagonist flumazenil and the GABA-gated chloride channel blocker picrotoxin. Thus, these findings support the idea that enhancement of GABAergic transmission negatively modulates the opioid receptor-mediated antinociception.

Previously, we reported that flumazenil significantly blocked the reversing effects of diazepam and majonoside-R2 on the psychological stress-induced decrease in pentobarbital sleep in mice (2). In this study, we also found that both flumazenil and picrotoxin completely blocked the antagonistic effects of majonoside-R2 on morphine- and U-50,488H-induced antinociception in the tail-pinch test. Taken together, it is possible that enhancement of the function of GABA<sub>A</sub> receptor complex is at least partly involved in the pharmacological action of majonoside-R2. Although the exact mechanism of the antagonistic action of majonoside-R2 on the opioid-induced antinociception remains unclear, majonoside-R2 does not seem to share the same mechanism of action as diazepam, since in contrast to diazepam majonoside-R2 failed to suppress convulsion caused by GABA<sub>A</sub> antagonists bicuculline and picrotoxin.

It has been demonstrated that the GABA<sub>A</sub> receptor function in the brain can be negatively or positively modulated by various neuroactive steroids (10), and that adrenalectomy attenuates the swim stress-induced increase in neurosteroid levels not only in the plasma but also in the cortex (11). These findings suggest that neuroactive steroids are in part derived from the peripheral system. Ginsenosides have been demonstrated to produce an estrogen-like effect and bind to glucocorticoid receptors (12). Moreover, Vietnamese ginseng extract report-
edly produces steroid hormone-like effects that are mediated by the hypothalamus-pituitary-adrenal axis (13). Thus, it would be quite interesting to speculate that majonoside-R2 and/or its metabolites may have properties like a GABA agonistic neurosteroid because of the steroid-like structure of majonoside-R2. Clarification of the possible interactions between majonoside-R2 and the GABAnergic receptor complex and between majonoside-R2 and opioid receptors would require further investigation such as receptor binding experiments. Nevertheless, taking into account the data that, in contrast to diazepam, majonoside-R2 failed to block the convulsion caused by GABA<sub>A</sub>-receptor antagonists, there is a possibility that the suppressive effect of majonoside-R2 on opioid-induced antinociception is due to its indirect action on the GABAergic systems negatively modulating the function of opioid systems.

In conclusion, the present results suggest that majonoside-R2 exhibits the attenuating action on the opioid-induced antinociception by modulating the function of GABA<sub>A</sub> receptor systems.

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REFERENCES

6 Huong NTT, Matsumoto K, Yamasaki K, Duc NM, Nham NT and Watanabe H: Majonoside-R2, a major constituent of Vietnamese ginseng, attenuates opioid-induced antinociception. Pharmacol Biochem Behav (in press)