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Majonoside-R2, a Major Constituent of Vietnamese Ginseng, Attenuates Opioid-induced Antinociception

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HUONG, N. T. T., K. MATSUMOTO, K. YAMASAKI, N. M. DUC, N. T. NHAM AND H. WATANABE. *Majonoside-R2, a major constituent of Vietnamese ginseng, attenuates opioid-induced antinociception.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 285–291, 1997.—The effects of majonoside-R2 on antinociceptive responses caused by the μ-opioid receptor agonist morphine and the selective κ-opioid receptor agonist U-50,488H were examined by the tail-pinch test in mice. Intraperitoneal (IP) or intracerebroventricular (ICV) injection of majonoside-R2 (3.1–6.2 mg/kg, IP or 5–10 μg/mouse, ICV) and diazepam (0.1–0.5 mg/kg, IP or 0.5–1.0 μg/mouse, ICV), as well as an opioid receptor antagonist naloxone (2 mg/kg, IP or 5 μg/mouse, ICV), dose-dependently attenuated the antinociception caused by subcutaneously administered morphine and U-50,488H. Moreover, when co-administered ICV or intrathecally (IT) with morphine (4 μg/mouse) or U-50,488H (60 μg/mouse), majonoside-R2 (5–20 μg/mouse) also exhibited antagonism against the antinociceptive action of these opioid receptor agonists in the tail-pinch test. The inhibitory effects of majonoside-R2 (10 μg/mouse, ICV) and diazepam (1 μg/mouse, ICV) were reversed by flumazenil (2.5 μg/mouse, ICV), a selective benzodiazepine receptor antagonist, and picrotoxin (0.25 μg/mouse, ICV), a GABA-gated chloride channel blocker. These results suggest that majonoside-R2 attenuates the opioid-induced antinociception by acting at the spinal and supraspinal levels, and that the $GABA_A$ receptor complex at the supraspinal level is involved in the effect of ICV administered majonoside-R2. © 1997 Elsevier Science Inc.

Antinociception Morphine U-50,488H Vietnamese ginseng Majonoside-R2 $GABA_A$ antagonists

VIETNAMESE ginseng (VG), a wild Panax species, has attracted much attention as a new medicinal resource in Vietnam since its discovery. VG is known to contain not only ginseng saponins but also the ocotillol-type saponins. The latter saponins account for over 50% of total saponins and have not been isolated from *Panax ginseng*, American ginseng or Sanchi ginseng (2,13,14). Although *Panax ginseng* has received much attention, the pharmacological profiles of VG have not been well evaluated yet.

Majonoside-R2, an ocotillol-type saponin, has been reported to be one of the major constituents of Vietnamese ginseng (2). Recently, we demonstrated that Vietnamese ginseng saponin and majonoside-R2 attenuated the psychological stress- and foot shock stress-induced antinociception in the tail-pinch test in mice (5). We also found 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 (6). These findings suggest that Vietnamese ginseng has protective effects on the pathophysiological changes caused by stressful stimuli and indicate the possible involvement of opioid and $GABA_A$-receptor mechanisms in the effect of majonoside-R2. In the present study, to further clarify the involvement of opioid and $GABA_A$ receptor mecha-

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nisms in the action of majonoside-R2, we investigated the effects of majonoside-R2 on the morphine- and U-50,488H-induced antinociception in the tail-pinch test in mice.

MATERIALS AND METHODS

Animals

Male 5-week-old ddY mice (Japan SLC, Shizuoka, Japan) were used for the experiments. The animals were housed in groups of 20–25 per cage for at least 1 week before starting the experiments, with free access to food and water. Housing conditions were thermostatically maintained at 24 ± 1°C and a relative humidity of 55 ± 5% with a 12-h light:dark cycle (lights on: 0730–1930). All studies were done in compliance with the Guide for Animal Experiments, Toyama Medical and Pharmaceutical University. Each animal was used only once.

Drug Administration

Systemic administration. All drugs referred to as the salt form were administered in a constant volume of 0.1 ml/10 g body weight. Majonoside-R2 was purified from the saponin fraction of Vietnamese ginseng (yield: 5.29% of dry material) as previously described (2), and the purity of majonoside-R2 was over 85%. Test drugs, except diazepam, were dissolved in saline just before starting the experiments. Diazepam (Cercine®, Takeda Chemical Industries Ltd., Osaka, Japan) was dissolved in saline containing 40% propylene glycol. Morphine HCl (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) and U-50,488H [trans-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]-cyclohexyl) benzeneacetamide; Sigma Chem., Co., St. Louis, MO] were injected subcutaneously (s.c.) at time 0. Majonoside-R2 and diazepam were injected intraperitoneally (IP) 30 min before morphine or U-50,488H administration. Naloxone HCl (Sigma Chem., Co., St. Louis, MO) was injected IP 10 and 30 min before morphine and U-50,488H administration, respectively. The time courses and routes of administration of test drugs were chosen based on the preliminary experiments in which they produced a maximal inhibition of the antinociception caused by morphine and U-50,488H.

Intracerebroventricular (ICV) and Intrathecal (IT) Administration. ICV and IT injection of test drugs were performed in a constant total volume of 5 μl according to the methods of Haley and McCormick (4) and of Hylden and Wilcox (8), respectively. When testing antagonism at the spinal or supraspinal level, test drugs were injected ICV or IT at the same time. In some experiments, majonoside-R2, Picrotoxin (Sigma Chem. Co., St. Louis, MO) and flumazemil (Anexate®, Roche Co. Ltd., Basel) were co-administered by a single ICV injection just before s.c. administration of opioids.

Measurement of Antinociception by the Tail-Pinch Test

The nociceptive response in the tail-pinch test was measured according to Haffner's method as previously reported (20). Briefly, hemostatic forceps (3 mm width, 500 g constant pressure) were applied to the root of the tail, and the latency of the biting response to the forceps was measured. To prevent tissue damage, a cut-off time of 6 s was selected.

The nociceptive response was measured every 30 min over a 120-min observation period and every 15 min over a 60-min observation period in morphine- and U-50,488H-treated animals, respectively.

Statistical Analysis

In the tail-pinch test, data are expressed as the mean percent maximum possible effect (%MPE ± SEM) according to Dewey et al. (1)

$$\%\text{MPE} = (\text{post-drug latency} - \text{pre-drug latency})/(\text{cut-off time} - \text{pre-drug latency}) \times 100.$$

The %MPE was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test or by two-way ANOVA followed by Tukey's test for multiple comparison among groups. When testing the effect of naloxone, the %MPE was analyzed by the unpaired Student's t-test. Differences of $P < 0.05$ were considered statistically significant.

RESULTS

Effects of Majonoside-R2 and Diazepam on Morphine-Induced Antinociception

As shown in Fig. 1, morphine (5 mg/kg, s.c.) exhibited marked suppression of nociceptive responses in the tail-pinch test and the effect was maximal at 30 min after administration. Naloxone (2 mg/kg, IP and 5 μg/mouse, ICV) antagonized the antinociceptive action of morphine. IP and ICV administration of majonoside-R2 (3.1-6.2 mg/kg, IP and 5-10 μg/mouse, ICV) significantly attenuated the morphine-induced antinociception in a dose-dependent manner [IP administration: $F(2, 38) = 15.818$, $P < 0.001$, and ICV administration: $F(2, 23) = 5.259$, $P < 0.05$ at 30 min after morphine administration] (Fig. 1A, 1C). Moreover, diazepam (0.1–0.5 mg/kg, IP or 0.5–1.0 mg/mouse, ICV) also dose-dependently and significantly suppressed the effect of morphine in the tail-pinch test [IP administration: $F(2, 37) = 18.114$, $P < 0.001$, and ICV administration: $F(2, 23) = 11.519$, $P < 0.001$ at 30 min after morphine administration] (Fig. 1B, 1D).

Effects of Majonoside-R2 and Diazepam on U-50,488H-Induced Antinociception

U-50,488H (15 mg/kg, s.c.), as well as morphine, produced an antinociceptive action in the tail-pinch test which was maximal at 15 min after administration (Fig. 2). The antinociceptive action of U-50,488H was antagonized by systemic (2 mg/kg, IP) or ICV administration (5 μg/mouse) of naloxone. Majonoside-R2 (1.5-6.2 mg/kg, IP and 5-10 μg/mouse, ICV) dose-dependently suppressed the U-50,488H-induced antinociception [IP administration: $F(3, 42) = 18.194$, $P < 0.001$; ICV administration: $F(2,29) = 3.518$, $P < 0.05$ at 15 min after administration of U-50,488H] (Fig. 2A, 2C). Moreover, IP and ICV injections of diazepam also significantly blocked the effect of U-50,488H [IP administration: $F(3, 36) = 7.844$, $P < 0.001$, and ICV administration: $F(2, 26) = 4.220$, $P < 0.05$ at 15 min after administration of U-50,488H] (Fig. 2B, 2D).

Effects of Majonoside-R2 on Opioid-Induced Antinociception at Spinal and Supraspinal Levels

ICV and IT injection of morphine (4 μg/mouse) produced antinociceptive actions in the tail-pinch test. The effects of ICV and IT morphine were maximal at 30 and 15 min after morphine injection, respectively (data not shown). Moreover, ICV and IT injection of U-50,488H (60 μg/mouse) also caused antinociception in the tail-pinch test and the effects of ICV and IT U-50,488H were maximal at 15 min after injection (data not shown). ICV and IT injection of majonoside-R2 (5-

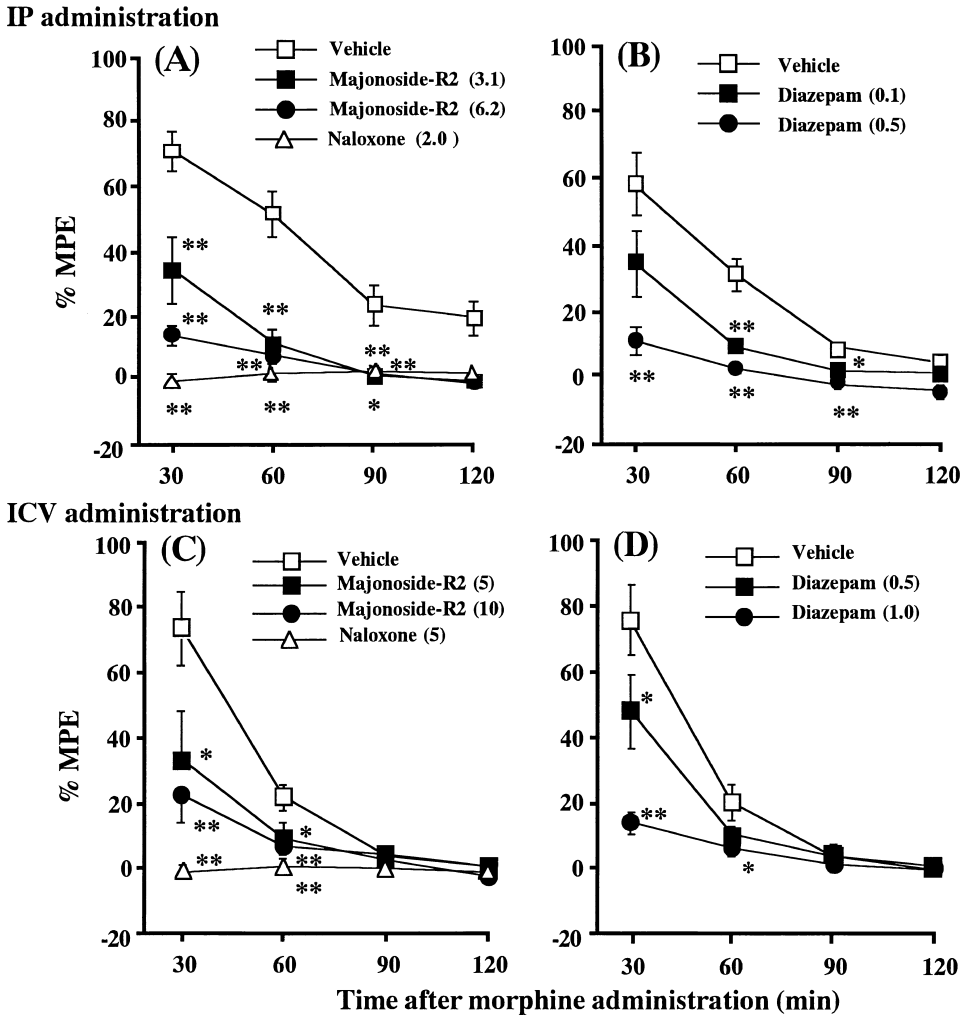

FIG. 1. Effects of majonoside-R2, naloxone and diazepam on the morphine-induced antinociception in the tail-pinch test in mice. After the basal nociceptive responses were recorded, morphine was administered (5 mg/kg, s.c.). The latency of nociceptive response was measured every 30 min over a 120-min observation period. For IP administration, majonoside-R2 (A), naloxone (A) and diazepam (B) were administered 30, 10 and 30 min before morphine, respectively. For ICV administration, majonoside- R2 (C), naloxone (C) and diazepam (D) were administered just before morphine administration. The number in each parenthesis is the dose (A and B: mg/kg; C and D: μg/mouse). Each point represents the mean %MPE ± SEM. (n = 10–12). *$P < 0.05$, **$P < 0.01$ vs. vehicle groups (Dunnett's test or Student's t-test).

20 μg) significantly attenuated the antinociception caused by ICV and IT administration of morphine (4 μg/mouse), respectively [$F(3, 37) = 5.851$, $P < 0.01$ and $F(2, 27) = 12.557$, $P < 0.001$ for ICV and IT administration, respectively; Fig. 3A and 3B]. Moreover, the same treatment with majonoside-R2 (5-20 μg/mouse, ICV and 5–10 μg/mouse, IT) also significantly suppressed the antinociception caused by ICV and IT administration of U-50,488H (60 μg/mouse) [$F(3, 36) = 5.718$, $P < 0.01$ and $F(2, 26) = 18.931$, $P < 0.001$ for ICV and IT administration, respectively; Fig. 3C and 3D].

Flumazenil and picrotoxin reverse the suppressing effects of majonoside-R2 and diazepam on the morphine-induced antinociception in the tail-pinch test

ICV administration of flumazenil had no effect on the tail-pinch latency, while picrotoxin produced a significant increase in the tail-pinch latency at 0.25 μg/mouse (ICV) (%MPE: −2.69 ± 1.25 and 32.27 ± 11.5 in saline-treated control and picrotoxin-treated mice, respectively; $P < 0.01$). Neither flumazenil nor picrotoxin had an effect on the antinociceptive action of morphine in the tail-pinch test. In terms of morphine-induced antinociception, a significant interaction between majonoside-R2 (10 μg/mouse) and flumazenil (2.5 μg/mouse) was observed following ICV administration of these drugs [$F_{\text{majonoside-R2} \times \text{flumazenil}}(1,32) = 6.885$, $P < 0.05$; Fig. 4A]. The post hoc test revealed that the suppressing effect of majonoside-R2 on the morphine-induced antinociception was significantly reversed by ICV flumazenil. Furthermore, a significant interaction between diazepam (1 μg/mouse, ICV) and flumazenil (2.5 μg/mouse, ICV) in morphine-induced antinociception was also observed [$F_{\text{diazepam} \times \text{flumazenil}}(1,31) = 6.880$, $P < 0.05$, Fig. 4A]. ICV administration of flumazenil(2.5 μg/mouse) completely

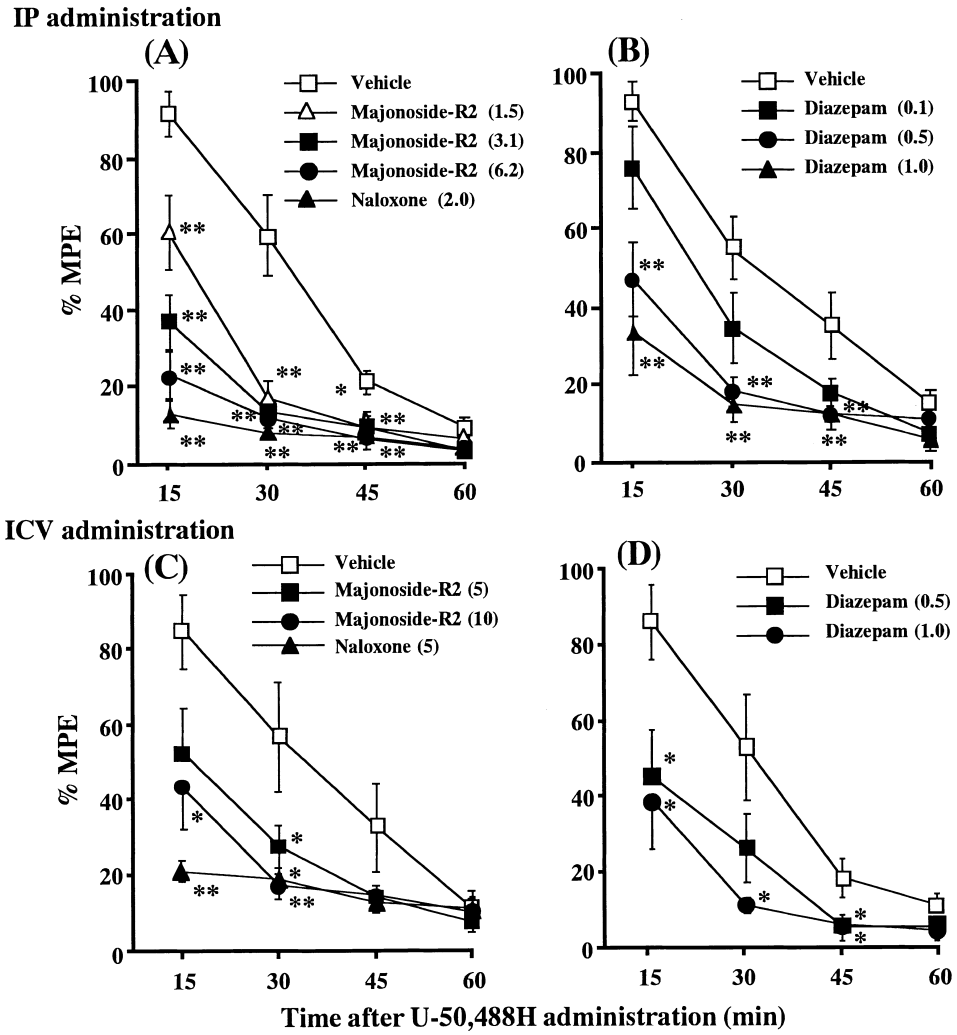

FIG. 2. Effects of majonoside-R2, naloxone and diazepam on the U-50,488H-induced antinociception in the tail-pinch test in mice. After the basal nociceptive responses were recorded, U-50,488H was administered (15 mg/kg, s.c.). The latency of the nociceptive response was measured every 15 min over a 60-min observation period. For IP administration, majonoside-R2 (A), naloxone (A) and diazepam (B) were administered 30 min before U-50,488H administration. For ICV administration, majonoside R2 (C), naloxone (C) and diazepam (D) were administered just before U-50,488H administration. The number in each parenthesis is the dose (A and B: mg/kg; C and D: μg/mouse). Each point represents the mean %MPE ± SEM. (n = 10–12). *$P < 0.05$, **$P < 0.01$ vs. vehicle groups (Dunnett's test or Student's t-test).

antagonized the effect of diazepam (1 μg/mouse, ICV) on morphine-induced antinociception. Likewise, significant majonoside-R2-picrotoxin and diazepam-picrotoxin interactions in the morphine-induced antinociception were observed following ICV administration of these drugs [$F_{\text{majonoside-R2} \times \text{picrotoxin}}(1,29) = 6.368$, $P < 0.05$ and $F_{\text{diazepam} \times \text{picrotoxin}}(1,29) = 24.698$, $P < 0.001$, Fig. 4B].

Neither diazepam nor picrotoxin exhibited motor dysfunction in mice at the doses tested in this experiment.

Flumazenil and picrotoxin reverse the suppressing effects of majonoside-R2 and diazepam on the U-50,488H-induced antinociception in the tail-pinch test

As shown in Fig. 5, when flumazenil (2.5 μg/mouse) or picrotoxin (0.25 μg/mouse) was co-administered ICV with majonoside-R2 (10 μg/mouse), both drugs significantly blocked the reversing effect of majonoside-R2 on the antinociceptive action of U-50,488H [$F_{\text{majonoside-R2} \times \text{flumazenil}}(1,32) = 14.739$, $P < 0.01$ and $F_{\text{majonoside-R2} \times \text{picrotoxin}}(1,31) = 8.700$, $P < 0.01$]. Moreover, the suppressing effect of diazepam (1 μg/mouse, ICV) on the U-50,488H antinociception was also significantly antagonized by flumazenil (2.5 μg/mouse, ICV) or picrotoxin (0.25 μg/mouse, ICV) [$F_{\text{diazepam} \times \text{flumazenil}}(1,34) = 23.904$, $P < 0.001$ and $F_{\text{diazepam} \times \text{picrotoxin}}(1,33) = 8.002$, $P < 0.01$]. Neither flumazenil nor picrotoxin itself had a significant effect on the U-50,488H-induced antinociception in the tail-pinch test.

DISCUSSION

The present data demonstrated that majonoside-R2 dose-dependently attenuated the antinociception caused by the

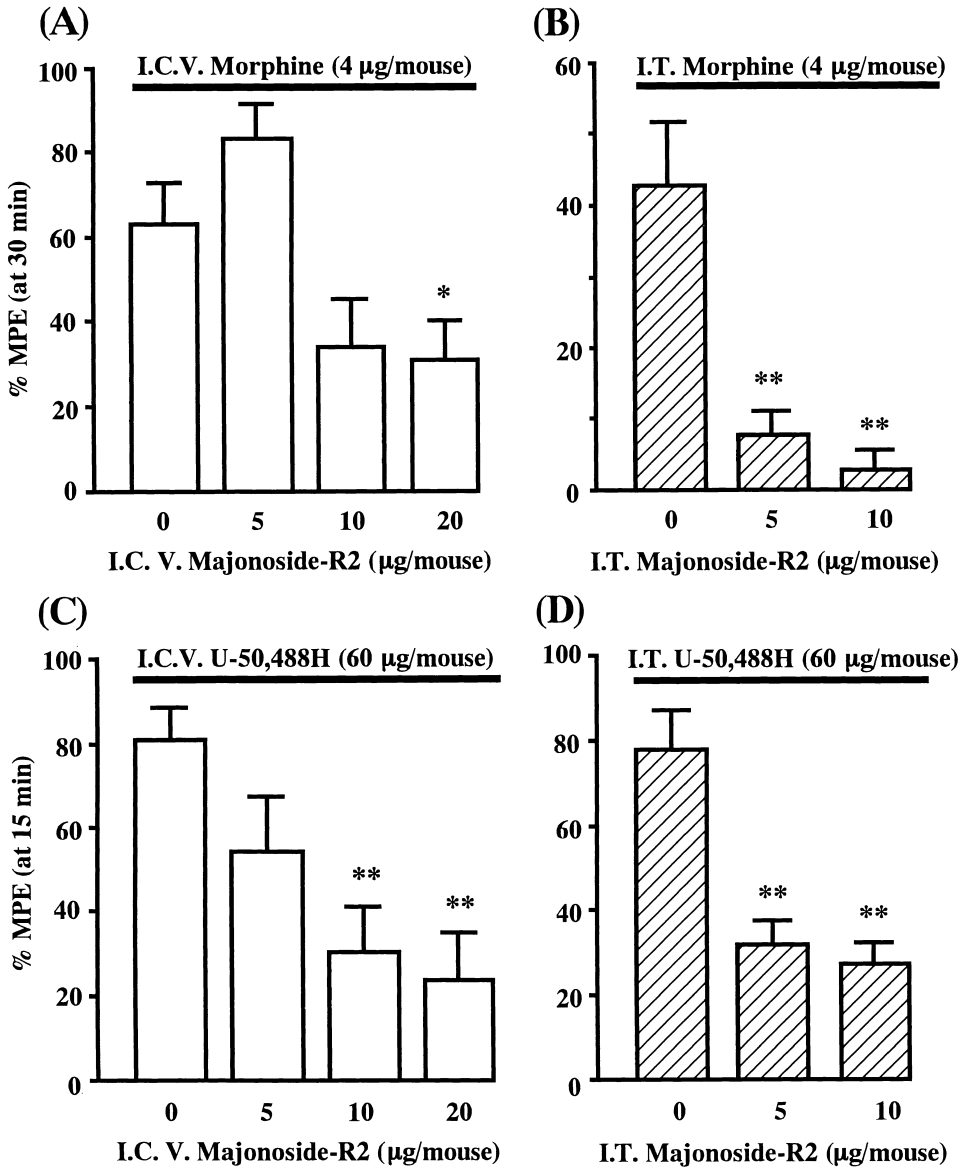

FIG. 3. Effects of centrally administered majonoside-R2 on the antinociception caused by centrally administered morphine and U-50,488H in the tail-pinch test. After the basal nociceptive responses in the tail-pinch test were recorded, morphine (4 μg) or U-50,488H (60 μg) was administered ICV or IT. The latency of the nociceptive responses was measured at 30 min and 15 min after morphine and U-50,488H, respectively. Majonoside-R2 was co-administered ICV (A, C: 5-20 μg/mouse) or IT (B, D: 5-10 μg/mouse) with either morphine (ICV or IT, 4 μg/mouse) or U-50,488H (ICV or IT 60 μg/mouse). $*P < 0.05$, $**P < 0.01$ compared with respective vehicle control (Dunnett's test).

μ-opioid agonist morphine and the κ-opioid agonist U-50,488H in the tail-pinch test by acting at the spinal and supraspinal levels, and that $GABA_A$ receptor mechanisms are partly involved in the effect of supraspinally administered majonoside-R2.

Majonoside-R2 itself had no effect on the nociceptive response in the tail-pinch test (data not shown). However, when administered IP or ICV, majonoside-R2 significantly attenuated the antinociceptive action of systemically administered morphine and U-50,488H. Moreover, ICV and IT administration of majonoside-R2 also exhibited an antagonist effect on the antinociception caused by ICV and IT administration of the opioids, respectively. Although no information is available on the bioavailability or metabolism of majonoside-R2 following systemic administration, the present findings indicate that majonoside-R2 itself may modulate the effects of opioids at the spinal and supraspinal levels if it can pass through the blood brain barrier.

At least three mechanisms seem to explain the action of majonoside-R2 in this study. First, majonoside-R2 indirectly inhibits the antinociceptive action of morphine and U-50,488H in the tail-pinch test. We previously demonstrated that majonoside-R2 reversed the psychological stress-induced decrease in

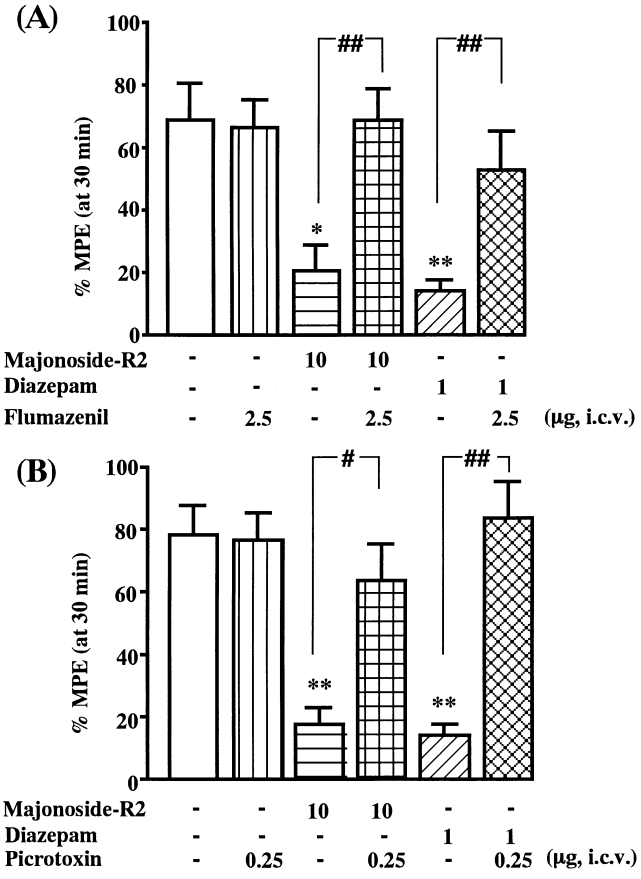

FIG. 4. Antagonism by flumazenil and picrotoxin of the suppressing effects of majonoside-R2 and diazepam on the morphine-induced antinociception in the tail-pinch test. After the basal nociceptive responses in the tail-pinch test were recorded, morphine (5 mg/kg, s.c.) was administered. The latency of the nociceptive responses was measured at 30 min after morphine administration. Majonoside-R2 (10 μg/mouse) and diazepam (1 μg/mouse) were administered ICV with or without flumazenil (A; 2.5 μg/mouse) and picrotoxin (B; 0.25 μg/mouse) just before morphine. Each column represents the mean %MPE ± SEM. ($n = 10$). *$P < 0.05$, **$P < 0.01$ vs. vehicle groups. #$P < 0.05$, ##$P < 0.01$ vs. majonoside-R2 or diazepam alone (Tukey's test).

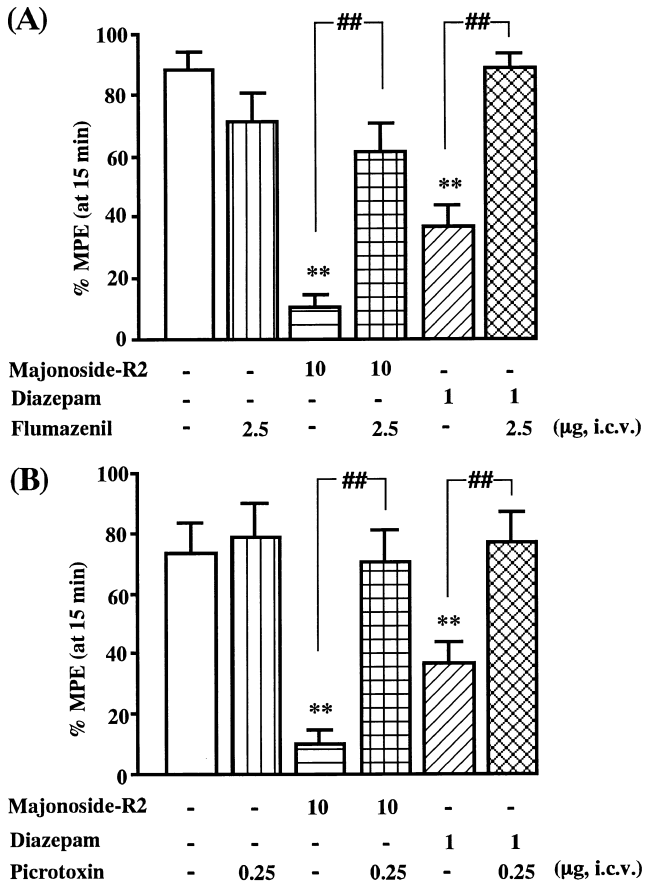

FIG. 5. Antagonism by flumazenil and picrotoxin of the suppressing effects of majonoside-R2 and diazepam on the U-50,488H-induced antinociception in the tail-pinch test. After the basal nociceptive responses in the tail-pinch test were recorded, U-50,488H (15 mg/kg, s.c.) was administered. The latency of nociceptive responses was measured at 15 min after U-50,488H administration. Majonoside-R2 (10 μg/mouse) and diazepam (1 μg/mouse) were administered ICV with or without flumazenil (A; 2.5 μg/mouse) and picrotoxin (B; 0.25 μg/mouse) just before U-50,488H. Each column represents the mean %MPE ± SEM. ($n = 10$). **$P < 0.01$ vs. vehicle groups. ##$P < 0.01$ vs. majonoside-R2 or diazepam alone (Tukey's test).

pentobarbital sleep in mice, and that the effect of majonoside-R2 was attenuated by flumazenil, a selective benzodiazepine receptor antagonist, suggesting the involvement of benzodiazepine receptor mechanisms in the action of majonoside-R2 (6). Recent evidence indicates that the opioid-induced antinociception can be modulated by the descending GABA ergic systems (3,11,16,17,19). Diazepam reportedly attenuates the morphine antinociception and such an apparent antagonistic action of diazepam is reversed by bicuculline and picrotoxin, specific GABA antagonists, indicating the involvement of the $GABA_A$ receptor complex (12,15,18,22). Although the role of the GABAergic systems in κ-opioid receptor-mediated nociceptive response has not been fully elucidated, in this study we found that ICV injection of diazepam significantly suppressed both morphine- and U-50,488H-induced antinociception in the tail-pinch test, and that the effect of diazepam was antagonized by ICV administered flumazenil and picrotoxin. Thus, these findings not only support the idea that enhancement of GABA-ergic transmission negatively modulates the antinociception caused by μm- and κ-opioid receptor agonists in mice, but also suggest that the $GABA_A$-benzodiazepine receptor complex is also involved in the inhibitory action of majonoside-R2 on the morphine- and U-50,488H-induced antinociception. This idea can be strongly supported by the findings that when administered ICV, both flumazenil and picrotoxin also completely blocked the antagonistic effects of ICV administered majonoside-R2 on the morphine- and U-50,488H-induced antinociception in the tail-pinch test. Thus, it is possible that majonoside-R2 exerts its pharmacological activity by enhancing the function of $GABA_A$-benzodiazepine receptor complex in the brain. To clarify whether majonoside-R2 modulation of opioid-induced antinociception is due to its direct interaction with $GABA_A$-benzodiazepine receptor complex in the brain requires further investigation such as in vitro binding experiments.

Secondly, the inhibitory action of majonoside-R2 on the

morphine- and U-50,488H-induced antinociception may be due to its direct and non-selective blockade of the μ- and κ-opioid receptors. In this study, majonoside-R2, as well as naloxone, dose-dependently suppressed the morphine-induced antinociception in the tail-pinch test. We previously found that VG saponin and majonoside-R2 blocked the opioid-mediated stress-induced antinociception (5). Moreover, repeated administration of Vietnamese ginseng saponins and majonoside-R2 suppressed the development of morphine tolerance in the tail-pinch test (7). Taken together, majonoside-R2 may be able to directly antagonize the effect of morphine and U-50,488H at the μ- and κ-opioid receptor sites in a similar manner to naloxone.

The present results do not exclude the possibility that majonoside-R2 modulates the opioid-induced antinociception in the tail-pinch test by affecting other neuronal pathways. It has been reported that the descending monoaminergic systems are involved in the antinociceptive action of opioids. For example, the descending noradrenergic system plays a predominant role in the antinociceptive effect of morphine in the tail-pinch test, while the descending serotonergic system appears to be predominantly implicated in the action of morphine in the tail-flick and hot-plate tests (9, 10). Moreover, the κ-opioid receptor-mediated antinociception also appears to be modulated by monoaminergic agents (21). Thus, a speculative explanation is that the antagonistic effect of majonoside-R2 on the opioid-induced antinociception in the tail-pinch test may be due to modulation of these descending monoaminergic pathways by majonoside-R2.

In summary, we have tested the effect of systemic, ICV and IT administration of majonoside-R2 on the μ- and κ-opioid receptor agonist-induced antinociception in the tail-pinch test, and found that majonoside-R2 attenuates the antinociceptive action of these opioids by acting at the spinal and supraspinal levels. The finding that flumazenil and picrotoxin abolished the action of majonoside-R2 indicates that the $GABA_A$-benzodiazepine receptor complex at the supraspinal level is at least partly involved in the majonoside-R2 modulation of the antinociceptive action of opioids.

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