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Inhibitory Effect of Ginseng Total Saponins on the Development of Tolerance to U-50,488H-Induced Antinociception is Dependent on Serotonergic Mechanisms

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Abstract□We have previously reported that the antagonism of U-50,488H-induced antinociception in mice pretreated with ginseng total saponins (GTS) was abolished by pretreatment with a serotonin precursor, 5-hydroxytryptophan (5-HTP), but not by a noradrenaline precursor, L-dihydroxyphenylalanine (L-DOPA) in the tail flick test. In the present experiments, the effect of the same GTS on the development of tolerance to U-50,488H-induced antinociception was determined. GTS inhibited the development of tolerance to U-50,488H-induced antinociception. The inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception was reversed by 5-HTP, but not by L-DOPA. These findings suggest that the inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception is dependent on serotonergic mechanisms.

Key words□Ginseng total saponin, U-50,488H, tolerance, serotonin.

Introduction

U-50,488H displays antinociceptive actions in a variety of assays (thermal, pressure and irritant) in mice and rats.[1] Since different opioids exhibit different specificities toward different receptor types, tolerance development of U-50,488H might be qualitatively different from that of morphine. These observations suggest that the antinociceptive effects of U-50,488H and morphine are mediated via different opioid receptors. Morphine- and U-50,488H-induced antinociceptions appear to be mediated by the so-called μ- and κ-opioid receptors, respectively.[2,3] Of special interest is the work of VonVoigtlander *et al.*[4] in mice, which showed that the depletion of serotonin with *p*-chlorophenylalanine (pCPA) slightly reduced morphin-induced antinociception but resulted in a marked antagonism of U-50,488H analgesic potency in the tail-flick and hot plate assays. And, the antagonism by pCPA was abolished by pretreatment with the serotonin precursor, 5-HTP, suggesting that the serotonergic system could be involved in the opioid receptor subtypes.[4] The association of serotonin with pain pathways has been widely studied and reviewed[5], as has the more controversial relationship of the serotonergic system and morphine analgesia.[2]

We reported that ginseng total saponins (GTS) prevented morphine-induced antinociception, and the antagonistic effect of GTS on morphine-induced antinociception was presumed to be associated with the reduction of the brain biogenic monoamines.[6] Similar findings were obtained with reserpine.[7,8] There has been a report that the daily administration of ginseng extract for 5 days decreased the serotonin levels in the brainstem as well as cerebral cortex in rats.[9] In addition, single administration of GTS antagonized U-50,488H-induced antinociception and the antagonism of U-50,488H-induced antinociception by GTS was abolished by pretreatment with 5-HTP, suggesting that this antagonism was dependent on serotonergic mechanisms.[10]

Tolerance development of U-50,488H-induced antinociception might be qualitatively different from that of morphine since U-50,488H showed no cross-tolerance to morphine.[1,11] The possible mechanism

is still controversial. For these reasons, it is of interest to test whether GTS inhibits the development of tolerance to U-50,488H-induced antinociception and also whether the inhibition of the development of tolerance to U-50,488H-induced antinociception by GTS is dependent on serotonergic mechanisms.

Materials and Methods

Male mice of the ICR strain weighing 18~20 g were used for these experiments. They were kept at an ambient temperature of 22± 1℃ and given normal laboratory diet and tap water *ad libitum*.

Antinociceptive effect was measured by the tail flick (TF) test, a modified D'Amour and Smith method.[12] U-50,488H (Sigma, USA) 30 mg/kg was injected subcutaneously (SC) once a day for 10 days. GTS [saponins mixture containing at least 10 glycosides known as ginsenosides from *Panax ginseng* C.A. Meyer, extracted and purified by the method of Namba *et al.*[13] and supplied by Korea Ginseng and Tobacco Research Institute] 100 mg/kg or 200 mg/kg was injected intraperitoneally (IP) 4 hours prior to the administration of U-50,488H. The 100 mg/kg of L-DOPA (Sigma) or 5-HTP (Sigma) was pretreated IP 30 min prior to the administration of U-50,488H. The antinociceptive effect of U-50,488H was calculated as the area under the curve (AUC) by plotting the changes in latency time (sec) on the ordinate and the interval (min) on the abscissa. To assess tolerance development, the antinociceptive effect of U-50,488H measured on day 11, 24 hours after the final administration of U-50,488H, was expressed as a percentage of the antinociceptive effect obtained with single administration of U-50,488H. The results were expressed as the mean± S.E. Differences between the individual mean values in various groups were analyzed by Students' t-test.

Results and Discussion

U-50,488H-induced antinociception measured on day 11 was reduced to about 20% compared with

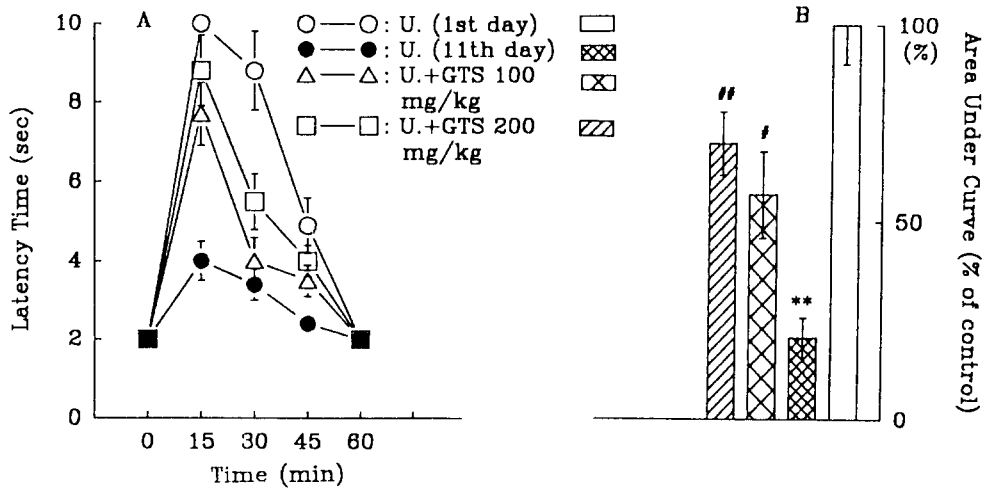

Fig. 1. Inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception in the TF test. U-50,488H (U) 30 mg/kg was injected SC once a day for 10 days. Saline or GTS (100 or 200 mg/kg) was injected IP 4 hours prior to U-50,488H administration. On 11th day, antinociceptive effect was measured by the TF method before and every 15 min for 60 min after U-50,488H administration (panel A), and data in panel A transformed into as the area under the time latency curve (AUC) after substraction of basal values was expressed as a percentage of the effect obtained in control animals (panel B).
**$p<0.01$: compared with that of U-50,488H control.
#$p<0.05$, ##$p<0.01$: compared with that of consecutive U-50,488H treatment. Values are significantly different from the control values as determined by Students' t-test.

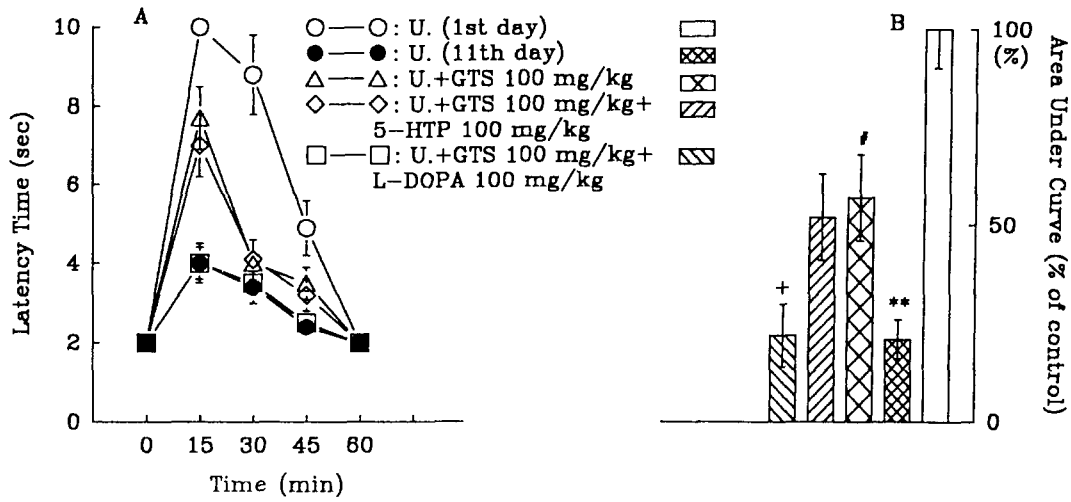

Fig. 2. Inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception and its reversal by 5-HTP or L-DOPA. U-50,488H 30 mg/kg was injected SC once a day for 10 days and the antinociceptive effect of U-50,488H was determined by the TF method on 11th day. Saline or GTS (100 mg/kg) was injected IP 4 hours prior to U-50,488H administration. The 100 mg/kg of 5-HTP or L-DOPA was injected IP 30 min prior to every U-50,488H administration. For other detail, refer Fig. 1.
**$p<0.01$: compared with that of U-50,488H control.
#$p<0.05$: compared with that of consecutive U-50,488H treatment.
†$p<0.05$: compared with that of consecutive U-50,488H treatment + GTS.

that of single administration in the TF test. However, the 100 and 200 mg/kg of GTS inhibited the development of tolerance to U-50,488H-induced antinociception showing 60% and 70% of U-50,488H-induced antinociception, respectively (Fig. 1). The inhibitory effect of tolerance to U-50,488H-induced antinociception by 100 mg/kg of GTS was reversed down to 20% of U-50,488H-induced antinociception by 5-HTP, but not L-DOPA (Fig. 2).

We reported that the standardized ginseng extract G115 [trademark for the standardized ginseng extract containing 4% ginsenosides (Pharmaton Ltd., Lugano-Bioggio/Switzerland)] inhibited the development of morphine-induced tolerance without antagonizing morphine-induced antinociception in mice.[14] However, GTS separated from ginseng extract prevented morphine-induced antinociception and also inhibited the development of morphine-induced antinociception.[6,15] Therefore, it is proposed that GTS is able to antagonize morphine-induced antinociception.

Antagonisms of morphine by reserpine and pCPA have been widely studied in mice and rats, indicating an important contribution of serotonergic mechanisms to morphine-induced antinociception.[16,17] Recently, it was reported that U-50,488H-induced antinociception was highly dependent upon serotonin in the mouse TF test, whereas morphine analgesia was minimally reliant on serotonin. Under the same conditions, it suggests that κ-opioid analgesia, in contrast to μ-opioid analgesia, is manifested principally through serotonergic pathway.[4] In addition, serotonin antagonists, cyproheptadine, ketanserine and pirenperone caused dose-related antagonism of U-50,488H in the TF test.[4] None of serotonin antagonists significantly affected morphine analgesia. Based upon the antagonism of U-50,488H by selective serotonin depletors and various antagonists, it is proposed that κ-opioid analgesia is dependent on serotonergic mechanisms.

Ginseng extract decreased serotonin levels in the brain stem and cerebral cortex.[9] In the previous report, GTS prevented U-50,488H-induced antinociception in the TF test and the antagonism was abolished by pretreatment with 5-HTP, but not by L-DOPA. This is also in agreement with the result

that the loss of k-opioid analgesia by reserpine was abolished by treatment with 5-HTP. In this regard, the previous results provide additional evidence that κ-opioid analgesia is dependent on serotonergic mechanisms as demonstrated by VonVoigtlander *et al.*[3] In these experiments, GTS also inhibited the development of tolerance to U-50,488H-induced antinociception, and the inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception was reversed by 5-HTP, but not by L-DOPA. Accordingly, these results suggest that the inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception is also dependent on serotonergic mechanism.

These findings taken together with the previous and present results suggest that both the antagonism and the development of tolerance to U-50,488 H are mediated by the serotonergic system as demonstrated by Ho and Takemori.[18]

요 약

인삼사포닌의 전처치에 의한 U-50,488H 진통력의 길항작용이 serotonin의 전구물질인 5-HTP에 의해 소실되었고 noradrenaline의 전구물질인 L-DOPA에 의해서는 소실되지 않았다는 결과를 발표하였다. 본 실험에서는 U-50,488H의 진통성 내성형성에 대한 인삼사포닌의 효과를 검토하였다. 인삼사포닌은 U-50, 488H의 내성형성을 억제하였으며, 이러한 내성형성 억제효과가 serotonin의 전구물질인 5-HTP에 의해 소실되었고 noradrenaline의 전구물질인 L-DOPA에 의해서는 소실되지 않았다. 이와 같은 결과는 U-50, 488H 진통성 내성형성에 대한 인삼사포닌의 억제효과는 serotonin에 의존적이라고 사료된다.

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