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Effects of Ginseng Total Saponins on the Analgesia and Tolerance Development of Pentazocine

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Abstract□This study examined the influence of ginseng total saponins (GTS) on the analgestic action and tolerance development of pentazocine in mice. Pentazocine prolonged the latency to response in the tail flick rather than in the tail pinch test. The analgesic effect of pentazocine was antagonized by naloxone and completely eliminated by pretreatment with *p*-chlorophenylalanine (*p*CPA). GTS prevented the pentazocine-incuced analgesia and inhibited the development of tolerance to pentazocine. The antagonistic effect of GTS on the pentazocine-induced analgesia was abolished by 5-HTP, but not by L-DOPA. These results suggest that GTS inhibits the analgesic action of pentazocine by the interaction with serotonergic neuron.

Key words□pentazocine, analgesia, antagonism, tolerance, ginseng total saponins, serotonergic mechanisms

Introduction

Opiate drugs produce analgesia through distinct classes of opioid receptors at different neuroanatomical regions. Several studies have addressed the possible neural mechanisms underlying μ-and κ-opioid receptor-mediated analgesia.[1-3] Pentazocine is an analgesic of the benzomorphan series with mixed narcotic agonist-antagonist properties. It acts as an agonist on the κ-receptor sites, but as an antagonist on the μ-receptor sites. Thus, it has been considered that pentazocine-induced analgesia is mainly mediated by κ-receptor sites.[4-6]

The association of serotonin with pain pathways has been widely studied and reviewed.[7-9] In the tail flick assay using mouse, depletion of serotonin with *p*CPA results in elimination of U-50, 488H-, a selective κ-receptor agonist, induced antinociception. And this effect is reversed by pretreatment with serotonin precursor, 5-hydroxytryptophan (5-HTP). However, morphine-induced antinociception is only slightly decreased by *p*CPA and reserpine.[6] It is conclued that κ-receptor mediated antinociception, unlike μ-receptor mediated effect, appears to be strongly dependent on serotonergic mechanisms.[10]

Kim *et al.*[11] have reported that GTS inhibited morphine-induced analgesia and the development of tolerance to and dependence on morphine. GTS also prevented U-50,488H-induced analgesia. The antagonism of U-50,488H-induced analgesia by GTS was abolished by pretreatment with 5-HTP.[12] There has been another report that the daily administration of ginseng extract for 5 days decreased the serotonin levels in the brainstem as well as cerebral cortex in rat.[13]

It is of interest to test how GTS influences on pentazocine-, a mixed agonist-antagonist, induced analgesia. In the present experiments, we studied the effect of GTS on the pentazocine-induced analgesia and the development of tolerance to pentazocine. Furthermore, we added an experiment to probe the involvement of monoamines in the effect of GTS on the pentazocine-induced analgesia.

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Materials and Methods

ICR male mice weighing 22~25 g were used in all experiments. They were allowed *ad libitum* access to laboratory chow and tap water and housed in a group of 10~15 under controlled temperature (20~22℃) and humidity (60%).

The following compounds were used: Pentazocine (Dae-Won Pharm. Co., Korea), GTS (supplied by Korea Ginseng & Tobacco Research Institute), naloxone hydrochloride (Sigma, USA), 5-HTP (Sigma), L-3,4-dihydroxyphenylalanine (L-DOPA, Sigma) and *p*CPA (Sigma). Pentazocine, GTS, naloxone and 5-HTP were dissolved in saline and L-DOPA, in saline containing *d*-HCl and *p*CPA was suspended in aqueous 0.3% tween 80. Pentazocine was injected subcutaneously (s.c.) and the other drugs, intraperitoneally (i.p.) in a volume of 0.1 ml/10 g of body weight. Naloxone was injected 10 min prior to pentazocine. GTS was injected 2 hrs prior to pentazocine. *p*CPA 200 mg/kg was given 72 hrs and 5-HTP or L-DOPA, 30 min before the administration of pentazocine.

1. Measurement of analgesic effect

Analgesic effects were measured by a modified Haffner's method,[14] a tail pinch test using a 1.0± 0.1 sec pre-drug time and a 6 sec cut-off time, and a D'Amour and Smith method,[15] a tail flick test using a 2.0± 0.2 sec pre-drug time and a 10 sec cut-off time. It was measured every 30 min for 120 min or every 20 min for 100 min after pentazocine administration. The analgesic effects measured by both tests were calculated as area under the curve (AUC) by plotting the changes in response time (sec) on the ordinate and the intervals (min) on the abscissa.[16] The data were expressed as percent of the effect obtained in control animals.

2. Assessment of analgesic tolerance

Pentazocine 30 mg/kg was administered to mice once a day for 7 days and GTS was administered 2 hrs prior to every pentazocine injection. The development of tolerance to pentazocine was evidenced by measuring the analgesic effect of pentazocine 30 mg/kg on day 8 by the tail flick test.

3. Statistics

The data were expressed as mean± S.E. and analyzed by the Student's t-test.

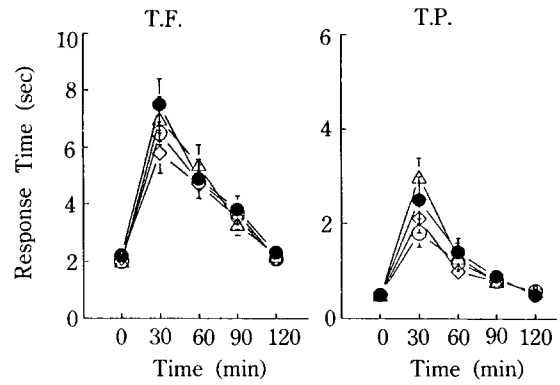

Fig. 1. Analgesic effect of pentazocine in control animals.

Pentazocine 15 (○), 30 (●), 45 (△) and 60 (◇) mg/kg was injected s.c. to mice. The analgesic effect of pentazocine was measured by the tail flick test (T.F.) and the tail pinch test (T.P.) every 30 min for 120 min after pentazocine administration.

Results

As shown in Fig. 1, pentazocine showed more potent analgesic effect in the tail flick than in the tail pinch test. The analgesic effect of pentazocine appeared to be the most potent in 30 mg/kg treatment group but at the higher doses, 45 and 60 mg/kg, the effect was rather decreased. Thus, for further experiments, pentazocine was fixed at 30 mg/kg and analgesic effect of pentazocine was measured by the tail flick test.

Naloxone inhibited pentazocine-induced analgesia in a dose-dependent manner (Fig. 2). Pentazocine-induced analgesia was completely abolished by prior administration of the mice with *p*CPA (Fig. 3).

GTS dose-dependently decreased the analgesic effect of pentazocine (Fig. 4). The analgesic effect was decreased by about 50% by 100 mg/kg of GTS. When we investigated the combined effects of pentazocine and GTS at various time intervals, the maximal inhibitory effect of GTS was observed 2 hrs prior to pentazocine administration (data not shown).

Fig. 5 and 6 show the effects of 5-HTP and

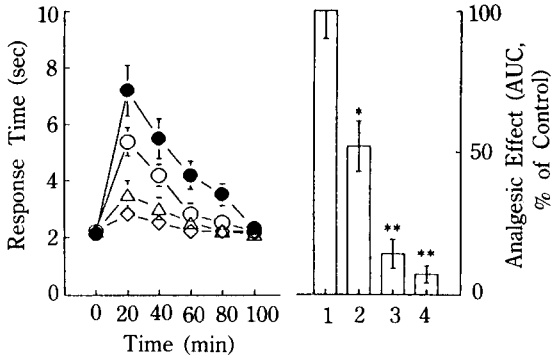

Fig. 2. Effect of naloxone on the analgesic action of pentazocine.

Saline (1, ●) or naloxone 0.4 (2, ○), 2 (2, △) and 4 (4, ◇) mg/kg (i.p.) was injected 10 min prior to the administration of pentazocine 30 mg/kg (s.c.). The analgesic effect pentazocine was measured by the tail flick test every 20 min for 100 min after pentazocine administration. *p>0.05, **p>0.01, compared with that of pentazocine control.

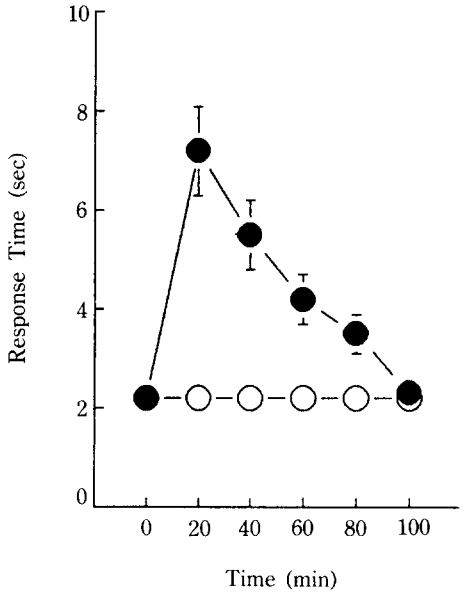

Fig. 3. Blockade by *p*CPA of the analgesic effect of pentazocine.

Saline (●) or *p*CPA 200 mg/kg (○) (i.p.) was given 72 hrs prior to the administration of pentazocine 30 mg/kg (s.c.). For other details, refer to Fig. 2.

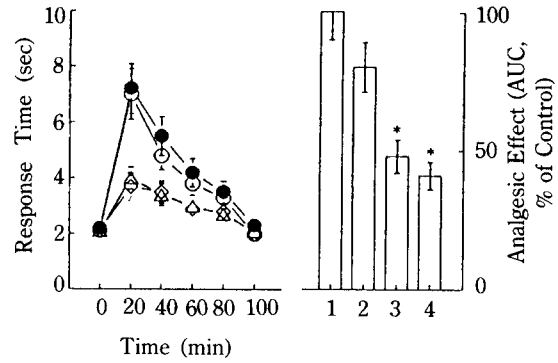

Fig. 4. Antagonism of pentazocine-induced analgesia by GTS.

Saline (1, ●) or GTS 50 (2, ○), 100 (3, △) and 200 (4, ◇) mg/kg(i.p.) were given 2 hrs prior to the administration of pentazocine 30 mg/kg (s.c.). For other details, refer to Fig. 2. *p>0.01, compared with that of pentazocine control.

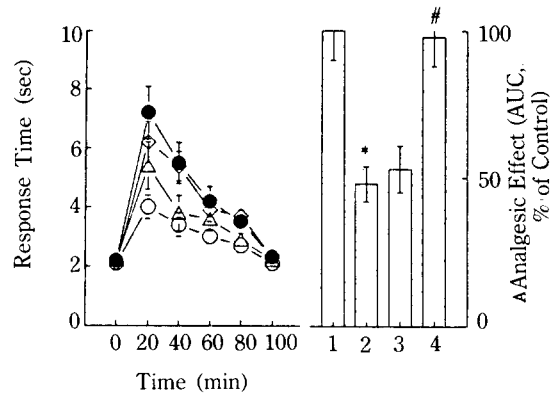

Fig. 5. Reversal of antagonistic effect of GTS on the pentazocine-induced analgesia by 5-HTP.

GTS 100 mg/kg was injected i.p. 2 hrs prior to the administration of pentazocine 30 mg/kg (s.c.). Saline or 5-HTP (i.p.) were given 30 min prior to the administration of pentazocine. Pentazocine control (1, ●), pentazocine+GTS (2, ○), pentazocine+GTS+5-HTP 30 mg/kg (3, △), pentazocine+GTS+5-HTP 50 mg/kg (4, ◇). *p>0.01, compared with that of pentazocine control. #p>0.001, compared with that of GTS +pentazocine. For other details, refer to Fig. 2.

L-DOPA, respectively, on the antagonism of the analgesic effect of pentazocine by GTS. Pretreatment of animals with 5-HTP caused a dose-depen-

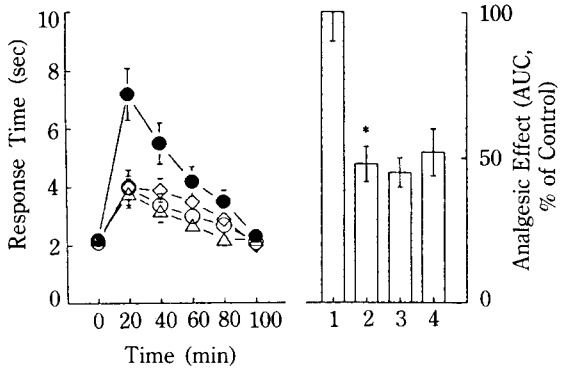

Fig. 6. Reversal of antagonistic effect of GTS on the pentazocine-induced analgesia by L-DOPA.

GTS 100 mg/kg was injected i.p. 2 hrs prior to the administration of pentazocine control (1, ●), pentazocine+GTS (2, ○), pentazocine+GTS+L-DOPA 30 mg/kg (3, △), pentazocine+GTS+L-DOPA 50 mg/kg (4, ◇). *p>0.01, compared with that of pentazocine control. For other details, refer to Fig. 2.

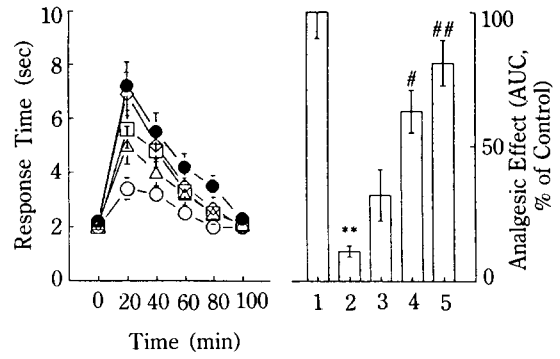

Fig. 7. Inhibitory effect of GTS on the development of tolerance to the analgesic action of pentazocine.

Pentazcine 30 mg/kg (s.c.) was injected once a day for 7 days. Saline or various concentrations of GTS (i.p.) was given 2 hrs prior to every pentazocine administration. The development of tolerance to pentazocine was evidenced by measuring the analgesic effect of pentazocine 30 mg/kg 24 hrs after the final injection. Pentazocine control (1, ●), pentazocine (7 days), (2, ○), pentazocine+GTS 50 mg/kg (7 days) (3, △), pentazocine+GTS 100 mg/kg (7 days) (4, □), pentazocine+GTS 200 mg/kg (7 days) (5, ◇). *p>0.001, compared with that of pentazocine control. #p>0.01, ##p>0.001, compared with that of consecutive pentazocine treatment (7 days). For the details, refer to Fig. 2.

dent reveral of the GTS-induced antagonism of pentazocine analgesia (Fig. 5). However, L-DOPA showed no significant effect (Fig. 6).

Pentazocine 30 mg/kg, combined with various concentrations of GTS or saline, was administered for seven consecutive days. The analgesic effect measured with 30 mg/kg pentazocine 24 hrs after the final administration was decreased to about 10 % of control effect demonstrating that tolerance had developed to the drug's analgesic effects. GTS administered 2 hrs prior to pentazocine inhibited the development of tolerance in a dose-dependent manner (Fig. 7).

Discussion

Multiple opioid receptors appear to be involved in the production of an analgesic response to the opioid agents, since different opioids exhibit different specificities toward different receptor sites.[1,17,18] Agonists at both μ-and κ-opioid receptors are antinociceptive in animals, whereas the involvement of δ-opioid receptors in antinociception is more controversial.[19-21] Tyers[2] has proposed that relative μ- and κ-receptors involvement in opiate-induced analgesia varies according to the types of nociceptive stimuli employed. He suggested that μ-selective opioid agonists are more effective than κ-selective agonists in tests in which heat is the nociceptive stimulus, whereas tests in which pressure or chemical stimuli are used do not differentiate between the two groups. However, pentazocine at comparatively high dose, which induced only slight analgesic effect, showed complete antagonism of morphine-induced analgesia by activating μ-receptor in the tail pinch test using mice.[22] In addition, Gray *et al.*[23] reported that the analgesic effect of pentazocine was significant when low and intermediate stimuli was applied, but it was gradually and markedly decreased when the intensity of stimulus was increased in the D'Amour and Smith' tail flick method. In accordance with these results, pentazocine-induced analgesia was more obviously detected in the tail flick test than in the tail pinch test in the

present experiments. Although it is difficult to compare directly the intensity of stimuli between the two different types employed, it might be supposed that the tail flick test condition used in the present study is more effective to observe pentazocine-induced analgesia.

Naloxone is a selective μ-subtype antagonist. However, in higher concentrations, it also antagonizes the action of compounds having δ- or κ-affinities.[24] The observation that naloxone antagonizes the antinociceptive effect of pentazocine is in agreement with other studies.[24 27]

Both ascending and descending noradrenergic and serotonergic pathways have been implicated in mechanisms of analgesia produced by systemic administration of opioid and non-opioid drug.[28 30] These agents affect the turnover and release of noradrenaline and serotonin in various regions of the central nervous system, and alter the various neuronal activity. It is suggested that κ-opioid-induced analgesia, in contrast to μ-opioid-induced analgesia, is manifested principally through serotonergic pathways.[10] Serotonin antagonists, cyproheptadine, ketanserin and pirenperone caused dose-related inhibition of U-50,488H-induced analgesic effect in the tail flick test.[31] It is well known that the analgesic action of pentazocine, which has κ-receptor agonistic characteristics, is closely related with the change of serotonergic neuronal activity.[7] Pentazocine-induced analgesia is decreased by depression of serotonergic neuron which is accomplished by *p*CPA or 5,6-DHT administration. Likewise, pentazocine-induced analgesia was completely abolished by the pretreatment with *p*CPA in the present study.

We had reported that GTS inhibited the analgesic effect of morphine and U-50,488H, μ- and κ- receptors specific agonists, respectively. And GTS prevented the development of tolerance to these compounds in mice.[11,12] In the present study, GTS also inhibited the analgesic effect of and the development of tolerance to pentazocine in a dose-dependent manner. The antagonistic effect of GTS on the pentazocine-induced analgesia was reversed by pretreatment with 5-HTP, but not with L-DOPA. In previous publication,[12] we had obtained the similar results that the antagonism of U-50,488H-induced analgesia by GTS was reversed by 5-HTP, but not by L-DOPA. In this regard, the present result provides the additional evidence that GTS inhibits κ-receptor-mediated analgesia by serotonergic mechanism. Acute or chronic (to 5 days) administration of GTS in mice did not induce significant change of brain levels of noradrenaline, dopamine and serotonin.[32] Together with the fact that pentazocine-induced anagesia is dependent on the serotonergic mechanism, however, we can conclude that GTS inhibits the pentazocine-induced analgesia and the development of tolerance by indirect modulation of serotonergic neuronal activity.

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