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Effects of Ginseng Total Saponins on the Antinociception and the Tolerance Development of U-50,488H

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These studies were performed to investigate the acting sites of ginseng total saponins (GTS) on the U-50,488H-induced antinociception. And the possible mechanisms of the antagonistic effect on the U-50,488H-induced antinociception and the inhibitory effect of the development of tolerance to U-50,488H-induced antinociception by GTS were studied. The U-50,488H-induced antinociception was antagonized in mice pretreated with GTS intraperitoneally, intracerebrally and intrathecally. These antagonisms were reversed by the pretreatment with a serotonin precursor, 5-hydroxytryptophan (5-HTP), but not with a noradrenaline precursor, L-dihydroxyphenylalanine (L-DOPA). However, the intraplantar administration of GTS did not alter the intraplantar U-50,488H-induced antinociception. These findings suggest that U-50,488H-induced antinociception are mediated via the spinal and supraspinal sites. On the other hand, 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 pretreatment with 5-HTP, but not with L-DOPA. Therefore, the antagonism of U-50,488H-induced antinociception and the inhibition of the development of tolerance to U-50,488H-induced antinociception by GTS are dependent on serotonergic mechanisms.

Key words: U-50,488H-induced antinociception, Antagonism, Tolerance, Ginseng total saponins (GTS), Serotonergic mechanisms.

INTRODUCTION

The association of serotonin with pain pathway has been widely studied and reviewed (Taber and Latranyi, 1981; Basbaum and Fields, 1984; Roberts *et al.*, 1990). Of special interest is the work of VonVoigtlander *et al.* (1984) in mice, which showed that the depletion of serotonin with p-chlorophenylalanine (*p*CPA) slightly reduced morphine-induced antinociception, but resulted in a marked antagonism of analgesic potency of U-50,488H in the tail flick and hot plate assays. The antagonism by *p*CPA was abolished by the pretreatment with 5-HTP, suggesting that serotonergic system could be involved in the opioid receptor subtypes (Martin *et al.*, 1976; Berge *et al.*, 1983). Similarly, a κ-receptor agonist, pentazocine-induced antinociception was completely decreased by the pretreatment with *p*CPA, suggesting that serotonergic system could be involved in κ-receptor agonist-induced antinociception (Kim *et al.*, 1992).

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In addition, much work has been performed to determine whether k-agonists exert their antinociceptive actions at the spinal and/or the supraspinal sites (Mark, 1990). However, results are still controversial. Interest has been concentrated on a possible antinociceptive action of opioids outside the central nervous system. Evidences have been accumulated suggesting that opioid actions at receptors on the peripheral terminals of primary afferent nociceptors contribute to opioid-induced antinociception. U-50,488H elicited an marked antinociceptive effect in inflammated paw through κ-receptor sites in peripheral tissue (Stein *et al.*, 1988).

On the other hand, morphine-induced antinociception was prevented by the pretreatment with GTS in the tail pinch and tail flick tests (Kim *et al.*, 1986). Kim *et al.* (1992) also reported that GTS antagonized pentazocine-induced antinociception and the antagonistic effect of GTS on pentazocine-induced antinociception might be associated with the indirect modulation of serotonergic neuronal activity.

Tolerance development of U-50,488H-induced antinociception might be qualitatively different from that of morphine since U-50,488H showed no cross toleran-

ce to morphine (Bhargava *et al.*, 1989). However, the possible mechanisms are not clear. There has been demonstrated that the daily administration of ginseng extract for 5 days decreased the serotonin level in the brainstem as well as cerebral cortex in rats (Petkov, 1978).

For these reasons, these studies were performed to investigate the acting sites of GTS on the U-50,488H-induced antinociception and the possible mechanisms of the antagonistic effect on the U-50,488H-induced antinociception. And it is of interest to test whether GTS inhibits the development of tolerance to U-50,588 H-induced antinociception and the inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception is dependent on serotonergic mechanisms.

MATERIALS AND METHODS

Materials

Male mice of the ICR strain (Sam-Yuk), weighing 12-15 g were used in these studies. They were kept in an ambient temperature of 22±1℃ and given normal laboratory diet (Sam-Yang) and tap water *ad libitum*. After their weights increased to 18-20 g, they were subjected to the experiments. Each group contains 10-15 mice. U-50,488H, L-DOPA, 5-HTP and prostaglandin E_2 (PGE_2) were purchased from Sigma Chemical Co. (St. Louis, MO). And GTS [saponin mixture containing at least 10 glycosides known as ginsenosides from *Panax ginseng*, extracted and purified by Namba *et al.*'s (1974) method] was supplied from Korea Ginseng & Tobacco Research Institute (Taejeon).

Drug Administrations

The U-50,488H 30 mg/kg dissolved in saline was injected subcutaneously (SC), and L-DOPA as suspension in CMC or 5-HTP dissolved in saline was administered intraperitoneally (IP) in a volume of 0.1 ml/10 g of body weight. GTS was administered as follows.

Systemic injection: GTS was injected IP in a volume of 0.1 ml/10 g of body weight. GTS dissolved in saline was injected 3 hrs prior to U-50,488H administration.

Intracerebral injection: GTS was injected intracerebrally (IC) with 40 μg/body as described by Haley and McCormick (1957). GTS was injected 2 hrs prior to U-50,488H administration. The solution was injected with a volume of 10 μl/body in mice.

Intrathecal injection: GTS was injected intrathecally (IT) 40 μg/body as described by Janice and Wilcox (1980). GTS was injected 2 hrs prior to U-50,488H administration. The solution was injected with a volume of 10 μl/body in mice.

Intraplantar injection: PGE_2 (100 ng/paw) was dissolved in 10 ml absolute ethanol and diluted with 90 ml saline. U-50,488H (20 μg/paw) was injected intraplatarly 2.5 hrs after PGE_2 injection. PGE_2 (100 ng/paw) was injected intraplantary to a hindpaw of rat to induce hyperalgesia. GTS (100 and 200 μg/paw) was also injected intraplantarly 2 hrs after PGE2 injection. The intraplantar injection volume of each drug was 100 μl.

Measurement of Antinociception

A tail flick (TF) method as described by D'Amour and Smith (1941) was used for measurements of U-50,488H-induced antinociception. The TF latencies to thermal stimulation was determined before and at 15 min intervals after the U-50,488H administration for a period of 60 min in these experiments. The basal latencies for the TF test was found to be approximately 2 sec. Cut-off times of 10 sec for the TF test was used to prevent any injuries of the tail. The antinociceptive effects were calculated as the area under the curve (AUC) by plotting the changes in latency time (sec) on the ordinate and the intervals (min) on the abscissa. The data were expressed as a percentage of the effect obtained from the control animals.

Measurement of Peripheral Antinociception

A modification of the Randall-Sellito rats paw pressure test was used to measure U-50,488H-induced peripheral antinociception (Duart *et al.*, 1990). Hyperalgesia was induced by intraplantar injection of PGE_2 (100 ng/paw) to a hindpaw of rat. Three hrs after the administration of PGE2, noxious pressure was applied to the hindpaw. In this test, constant pressure of 20 mmHg was applied to the hindpaw and was discontinued when the animal showed paw withdrawal, and the latency to respond was measured. The latency obtained when PGE_2 alone was administered was taken as a control. GTS 100 and 200 μg/paw were injected intraplantarly 2 hrs after PGE_2 injection. U-50,488H (20 μg/paw) was injected intraplantarly 2.5 hrs after PGE2 injection. In order to identify the systemic antinociceptive effect of U-50, 488H, U-50,488H was administered to the contralateral paw.

Assessment of Analgesic Tolerance

To assess tolerance development, antinociceptive effect of U-50,488H measured at 24 hrs after the final administration of U-50,488H on 11th day, was expressed as a percentage of the antinociceptive effect obtained with single administration of

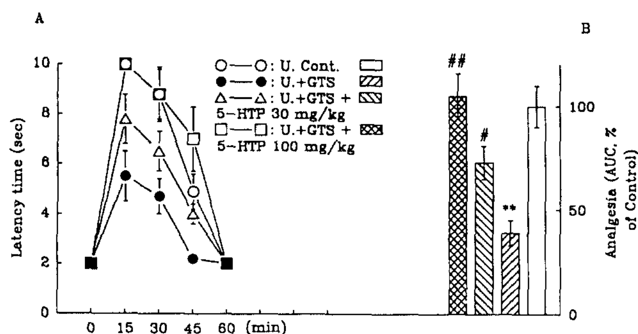

Fig. 1. Antagonism of U-50,488H-induced antinociception by IP GTS and its reversal by 5-HTP. GTS was injected IP at 3 hrs prior to the administration of U-50,488H. The 30 or 100 mg/kg of 5-HTP was given IP 30 min prior to U-50,488H 30 mg/kg. The U-50,488H-induced antinociception was measured by the TF method every 15 min for 60 min after U-50,488H administration. Panel A shows the changes in the TF latencies after subtraction of basal values, and panel B shows the data in panel A transformed into the area under the time latency curve (AUC).
**P<0.01, compared with that of U-50,488H.
#P<0.05, ##P<0.01, compared with that of GTS%U-50,488H. Values are significantly different from the control values as determined by ANOVA test.

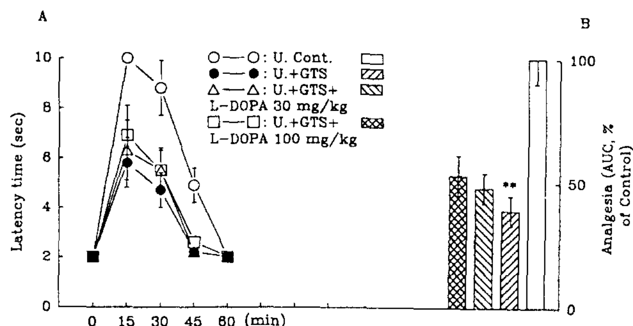

Fig. 2. Antagonism of U-50,488H-induced antinociception by IP GTS and no reversal by L-DOPA. GTS was injected IP 3 hrs prior to the administration of U-50,488H. The 30 or 100 mg/kg of L-DOPA was given IP 30 min prior to U-50,488H 30 mg/kg. The U-50,488H-induced antinociception was measured by the TF method every 15 min for 60 min after U-50,488H administration.
**P<0.01, compared with that of U-50,488H. For other details, refer Fig. 1.

U-50,488H. GTS was administered IP at 3 hrs prior to every U-50,488H injection.

Statistical Analysis

The results were expressed as the means± S.E.. Differences between the individual mean values in various groups were analyzed by ANOVA test.

RESULTS

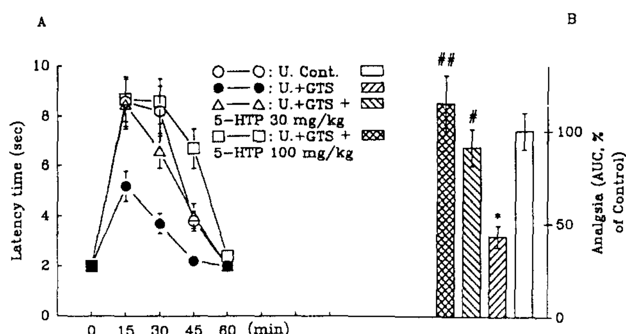

Fig. 3. Antagonism of U-50,488H-induced antinociception by IC GTS and its reversal by 5-HTP. GTS was injected IC 2 hrs prior to the administration of U-50,488H. The 30 or 100 mg/kg of 5-HTP was given IP 30 min prior to U-50,488H 30 mg/kg. The U-50,488H-induced antinociception was measured by the TF method every 15 min for 60 min after U-50,488H administration.
*P<0.05, compared with that of U-50,488H.
#P<0.05, ##P<0.01, compared with that of GTS%U-50,488H. For other details, refer Fig. 1 and Fig. 2.

In the preliminary experiments, the analgesic effect of U-50,488H alone and the combined effect of U-50,488H and GTS were tested at various time intervals. The maximal antagonistic effects by GTS were observed at 3 hrs prior to IP administration and 2 hrs prior to IC and IT administration of U-50,488H, respectively. Therefore, GTS was pretreated at 3 or 2 hrs prior to U-50,488H administration in all of the experiments.

The U-50,488H-induced antinociception was antagonized 35% by the systemic (IP) GTS-pretreated group compared with U-50,488H alone. The reduced U-50,488H-induced antinociception by pretreatment with 100 mg/kg of GTS was restored in mice pretreated with 30 and 100 mg/kg of 5-HTP, but not with L-DOPA (Fig. 1, Fig. 2). The U-50,488H-induced antinociception was antagonized approximate 40% in the group pretreated with IC GTS. The reduced U-50,488H-induced antinociception by pretreatment with 40 μg/body of IC GTS was more predominantly restored in mice pretreated with 5-HTP than L-DOPA (Figs. 3 and 4). The U-50,488H-induced antinociception was antagonized 45% by the group pretreated with IT GTS. The reduced U-50,488H-induced antinociception by pretreatment with 40 μg/body of IT GTS was restored in mice administered with 5-HTP, but not with L-DOPA (Figs. 5 and 6). Pretreatments with 5-HTP reversed the antagonisms of U-50,488H-induced antinociception by IP, IT and IC administration of GTS, but not with L-DOPA. However, the intraplantar administration of GTS did not alter the intraplantar U-50,488H-induced antinociception (Fig. 7).

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

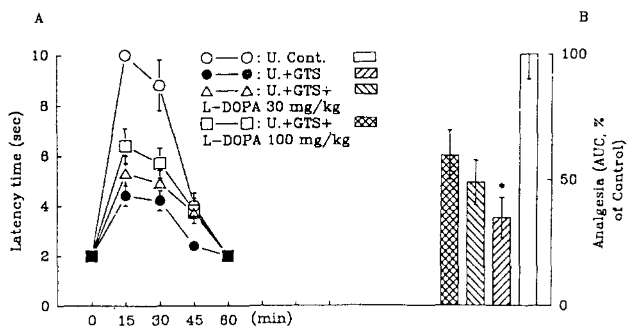

Fig. 4. Antagonism of U-50,488H-induced antinociception by IC GTS and no reversal by L-DOPA. GTS was injected IC 2 hrs prior to the administration of U-50,488H. The 30 and 100 mg/kg of L-DOPA were given 30 min prior to U-50,488H 30 mg/kg. The U-50,488H-induced antinociception was measured by the TF method every 15 min for 60 min after U-50,488H administration.
*P<0.05, compared with that of U-50,488H. For other details, refer Fig. 1 and Fig. 2.

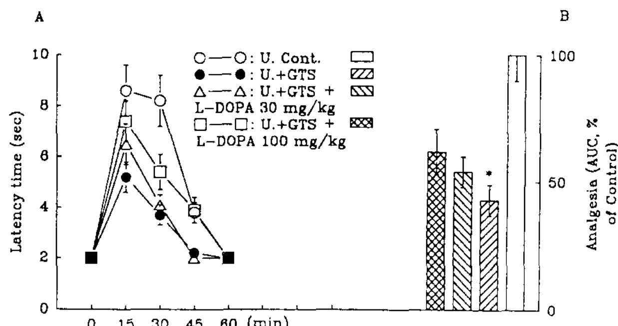

Fig. 6. Antagonism of U-50,488H-induced antinociception by IT GTS and no reversal by L-DOPA. GTS was injected IT 2 hrs prior to the administration of U-50,488H. The 30 and 100 mg/kg of L-DOPA were given 30 min prior to U-50,488H 30 mg/kg. The U-50,488H-induced antinociception was measured by the TF method every 15 min for 60 min after U-50,488H administration.
*P<0.05, compared with that of U-50,488H.
#P<0.05, compared with that of GTS+U-50,488H. For other details, refer Fig. 1 and Fig. 2.

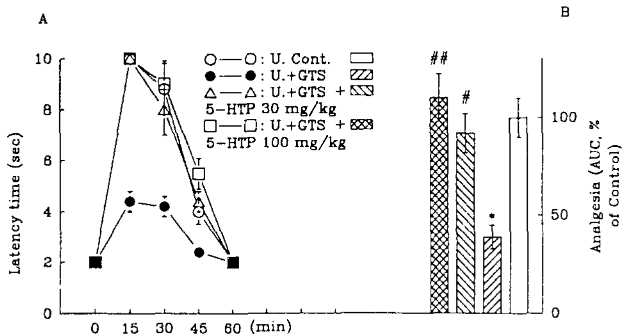

Fig. 5. Antagonism of U-50,488H-induced antinociception by IT GTS and its reversal by 5-HTP. GTS was injected IT 2 hrs prior to the administration of U-50,488H. The 30 and 100 mg/kg of 5-HTP were given 30 min prior to U-50,488H 30 mg/kg. The U-50,488H-induced antinociception was measured by the TF method every 15 min for 60 min after U-50,488H administration.
*P<0.05, compared with that of U-50,488H.
#P<0.05, ##P<0.01, compared with that of GTS%U-50,488H. For other details, refer Fig. 1 and Fig. 2.

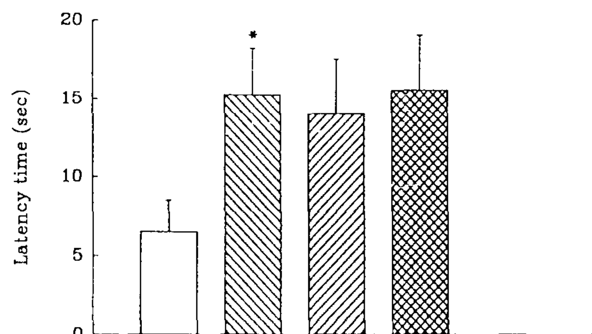

Fig. 7. No antagonism of U-50,488H-induced peripheral antinociception by GTS. PGE2 100 ng/paw was injected intraplantarly (i.pl.). U-50,488H 20 mg/paw was injected i.pl. 2.5 hours after PGE2 injection. GTS (100 and 200 mg/paw) was injected i.pl. 2 hours after PGE2 injection. Noxious pressure was applied to the hindpaw 3 hours after PGE2. □: PGE2 control, ▨: PGE2+U-50,488H, ▨: PGE2+U-50,488H+GTS (100 mg /paw), ▩: PGE2+U-50,488H+GTS (200 mg/paw).
*P<0.05, compared with that of U-50,488H control.

with that of single administration. However, the 100 and 200 mg/kg of GTS inhibited the development of tolerance to U-50,488H-induced antinociception (Fig. 9). The inhibitory effect of tolerance to U-50,488H-induced antinociception by GTS was reversed by 5-HTP, but not by L-DOPA.

DISCUSSION

Since different opioids exhibit different specificities toward different receptor subtypes, tolerance development to U-50,488H-induced antinociception might be qualitatively different from that of morphine. The U-50,488H-induced antinociception is known to be mediated by the κ-opioid receptor (VonVoigtlander *et al.*, 1982). Takahashi *et al.* (1987) reported that U-50,488H-induced antinociception was completely prevented by naloxone in the TP test, but not in the TF test. They demonstrated that the antagonism of U-50,488H-induced antinociception by naloxone was similar to that of psychological stress-induced analgesia by naloxone, suggesting that the κ-receptor participates partially via the mechanism by which psychological stress-induced analgesia was mediated. Panerai *et al.* (1984) recognized that the analgesic effect mediated via κ-opioid receptor was rather resistant to naloxone

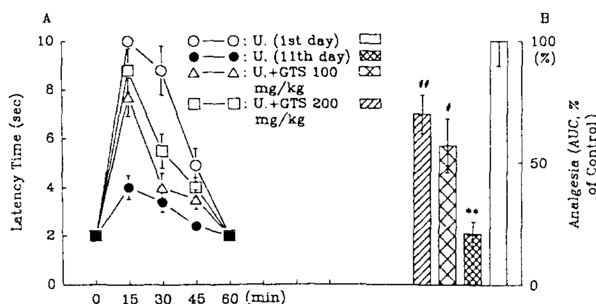

Fig. 8. Inhibitory effect of GTS on the development of tolerance to U-50,488H-induced antinociception in the TF test. The U-50,488H 30 mg/kg was injected SC once a day for 10 days. Saline or GTS 100 and 200 mg/kg were injected IP 4 hours prior to U-50,488H administration. The development of tolerance to U-50,488H-induced antinociception was measured 24 hrs after the final U-50,488H administration. *P<0.05, **P<0.01 compared with that of U-50,488H. For other details, refer Fig. 1 and Fig. 2.

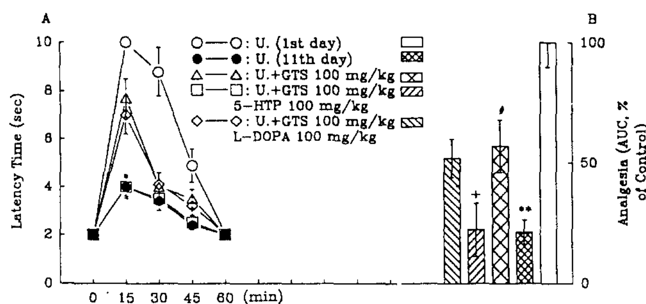

Fig. 9. Inhibitory effects of GTS on the development of tolerance to U-50,488H-induced antinociception and its reversal by 5-HTP or L-DOPA. U-50,488H 30 mg/kg was injected SC once a day for 10 days. Saline or GTS 100 mg/kg was injected IP 4 hours prior to U-50,488H administration. The 100 mg/kg of 5-HTP or L-DOPA was injected IP 30 min prior to every U-50,488H administration. The measurement of antinociception was made on 11th day.
**P<0.05, 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 U-50,488H%GTS. For other details, refer Fig. 1 and Fig. 2.

antagonism.

Antagonisms of morphine actions by reserpine and *p*CPA have been widely studied in the mouse and rat, indicating an important contribution of serotonergic mechanism to morphine-induced antinociception (Takagi *et al.*, 1964; Tenen, 1968; Berge *et al.*, 1983). Recently, it was reported that U-50,488H-induced antinociception was highly dependent upon serotonin (5-HT) in the mouse TF test, whereas morphine analgesia was minimally reliant on 5-HT under the same conditions, suggesting that κ-opioid analgesia, in contrast to μ-opioid analgesia, is manifested principally through serotonergic pathway (VonVoigtlander *et al.*, 1983).

On the other hand, Kim *et al.* (1992) reported that the antinociceptive effect of U-50,488H was antagonized by naloxone in the TP but not in the TF test, and U-50,488H-induced antinociception was antagonized by GTS in the TF test but not in the TP test. These results indicate that the antagonistic effect of U-50,488H-induced antinociception by GTS was substantially different from that by naloxone. In previous studies, Kim *et al.* (1986, 1992) had reported that GTS antagonized the analgesic effects of morphine and pentazocine, respectively. The antagonistic effect of GTS on the pentazocine-induced analgesia was reversed by pretreatment with 5-HTP, but not with L-DOPA. These results provided an evidence that GTS antagonized κ-receptor-mediated analgesia by serotonergic mechanisms.

Ginseng extract decreased serotonin levels in the brainstem and cerebral cortex (Petkov, 1978). In these experiments, the similar results were obtained that the antagonism of U-50,488H-induced antinociception by GTS was reversed by 5-HTP, but not L-DOPA, in agreement with the result that the loss of κ-opioid analgesia by *p*CPA was abolished by the pretreatment with 5-HTP. In this regards, the present result provides additional evidence that κ-opioid analgesia is dependent on serotonergic mechanisms as demonstrated by Von-Voigtlander *et al.* (1983).

The question of the sites at which κ-opioid receptor agonists act in inducing antinociception has engen dered much controversy. For many years, it was assumed that κ-receptors were located in the dorsal horn of spinal cord and so κ-receptor binding drugs produced spinal analgesia. However, in the brain, structures rich in κ-receptor also include the periacqueductal gray and thalamus. Indeed, the finding that IC application of nor-binaltophimine (κ-opioid receptor antagonist) attenuates the action of a systemically applied k-agonist suggests that additional sites of action exist in the brain (Millan and Czlonkowski, 1989). Intraplantar GTS failed to antagonize intraplntar U-50,488H-induced antinociception. This result indicates that GTS does not affect the peripheral U-50,488H-induced antinociception. In addition, the IC and IT ginseng total saponin also antagonized the antinociceptive action of systemically applied U-50,488H. Correspondingly, these experiments showed additional evidence indicating the spinal and supraspinal sites of action for κ-receptor agonists in the production of antinociception.

On the other hand, these sites are involved in the integration of ascending noxious information and are also linked to the descending inhibitory system in the central nervous system. Accordingly, it is suggested that the antagonistic action of GTS might be due to their inhibition of the activation of descending inhibitory serotonergic system. In these experiments, GTS also

inhibited the development of tolerance to U-50,488H-induced antinociception. The inhibitory effect of the development of tolerance to U-50,488H-induced antinociception by GTS was reversed by 5-HTP, not but L-DOPA. Therefore, these results suggest that the inhibitory effect of the development of tolerance to U-50,488H by GTS are dependent on serotonergic mechanisms.

In conclusion, these studies demonstrated that the sites of action of U-50,488H might exist both in the spinal cord and brain because systemically applied U-50,488H-induced antinociception was antagonized by IP, IC and IT pretreatments with GTS without antagonizing the antinociceptive effect at the peripheral site. The antagonism of U-50,488H-induced antinocicep tion and the inhibitory effect of the development of tolerance to U-50,488H by GTS are dependent on serotonergic mechanisms.

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