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Antagonism of U-50,488H-Induced Antinociception by Ginseng Total Saponins is Dependent on Serotonergic Mechanisms

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KIM, H.-S., K.-W. OH, H.-M. RHEU AND S.-H. KIM. *Antagonism of U-50,488H-induced antinociception by ginseng total saponins is dependent on serotonergic mechanisms.* PHARMACOL BIOCHEM BEHAV **42**(4) 587–593, 1992.—Morphine-induced antinociception was prevented by pretreatment with ginseng total saponins in the tail-pinch and tail-flick tests carried out in mice. The antinociceptive effect of U-50,488H, a selective κ-opioid receptor agonist, was prevented by naloxone, a nonselective opioid receptor antagonist, in the tail-pinch but not in the tail-flick test. However, U-50,488H-induced antinociception was prevented by ginseng total saponins in the tail-flick but not in the tail-pinch test. These results indicate that nonopioid mechanisms are involved in the antagonism of U-50,488H-induced antinociception by ginseng total saponins. In addition, the antagonism of U-50,488H-induced antinociception in mice pretreated with ginseng total saponins was abolished by pretreatment with a serotonin precursor, 5-hydroxytryptophan, but not by a noradrenaline precursor, L-dihydroxyphenylalanine, in the tail-flick test. Therefore, it appears that the antagonism of U-50,488H-induced antinociception by ginseng total saponins is dependent on serotonergic mechanisms.

U-50,488H-induced antinociception Antagonism Ginseng total saponins Serotonergic mechanisms

U-50,488H displays antinociceptive actions in a variety of assays (thermal, pressure, and irritant) in mice and rats (10). Since different opioids exhibit different specificities toward different receptor types, tolerance development to 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. U-50,488H-induced antinociception appears to be mediated by the so-called κ-opioid receptor (24).

Of special interest is the work of VonVoigtlander et al. in mice, which showed that the depletion of serotonin with *p*-chlorophenylalanine (PCPA) slightly reduced morphine-induced antinociception but resulted in a marked antagonism of U-50,488H analgesic potency in the tail-flick (TF) and hot plate assays (25). And, the antagonism by PCPA was abolished by pretreatment with the serotonin precursor, 5-hydroxytryptophan (5-HT), suggesting that the serotonergic system could be involved in the opioid receptor subtypes (25). The association of serotonin with pain pathways has been widely studied and reviewed (11,15), as has the more controversial relationship of the serotonergic system and morphine analgesia (14).

Kim et al. 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 (7). Similar findings were obtained with reserpine (20). There has been another report that the daily administration of ginseng extract for 5 days decreased the serotonin levels in the brain stem, as well as cerebral cortex, in rats (17).

For these reasons, it is of interest to test whether GTS prevents the U-50,488H-induced antinociception and also whether antagonism of U-50,488H-induced antinociception by GTS is dependent on serotonergic mechanisms.

METHOD

Animals

Male mice of the ICR strain weighing 12–15 g were purchased. They were kept at an ambient temperature of 22 ± 1°C and given normal laboratory diet and tapwater ad lib. After their weights increased to 18–20 g, they were employed for these experiments.

Drugs and Administration Schedule

The following compounds were used: morphine-HCl (Dae-Won Pharm. Co., Korea), naloxone-HCl (Sigma, St.

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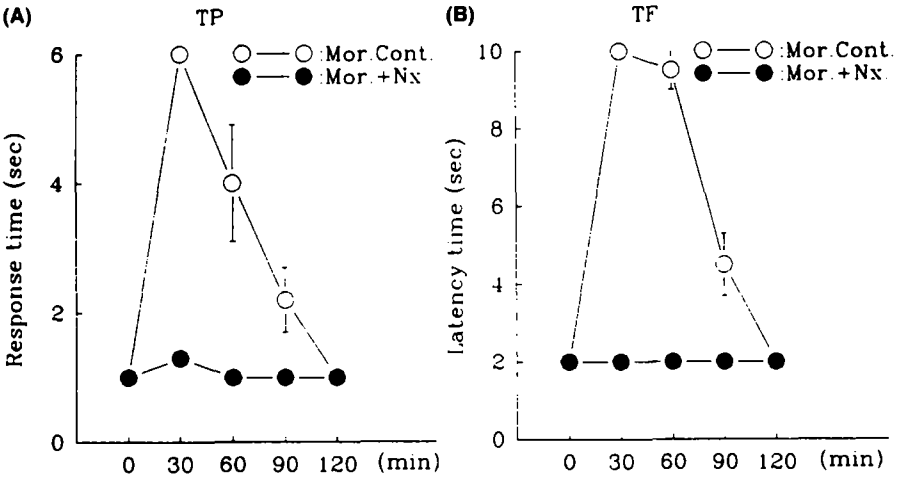

FIG. 1. Antinociceptive effect of morphine 5 mg/kg and its antagonism by naloxone in the (A) TP and (B) TF tests. Naloxone (Nx) 2 mg/kg or saline was injected 10 min prior to morphine (Mor) 5 mg/kg. Antinociceptive effect was measured by TP or TF method every 30 min for 120 min after morphine injection. Each point is the mean ± SE of at least 10 mice.

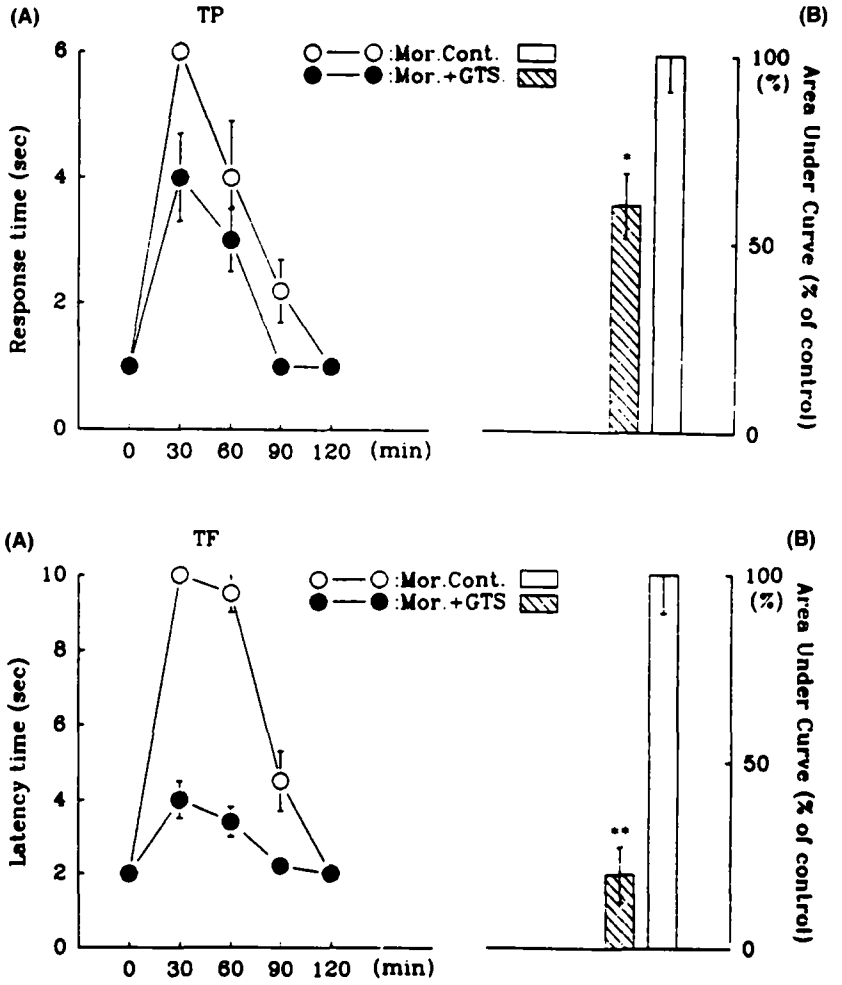

FIG. 2. Antinociceptive effect of morphine and its antagonism by GTS in the TP (upper panel) and TF (lower panel) tests. GTS 100 mg/kg or saline was injected 3 h in the TP test and 4 h in the TF test prior to morphine (Mor) 5 mg/kg. Antinociceptive effect was measured by TP or TF method every 30 min for 120 min after morphine. (A) Changes in the TP response and in the TF latencies; (B) data in panel (A) transformed into area under time latency (or response) curve (AUC) after subtraction of basal values. $*p < 0.05$, $**p < 0.01$ (values are significantly lower than the control values as determined by Student's t-test).

Louis, MO), U-50,488H (Sigma), L-dihydroxyphenylalanine (L-DOPA, Sigma), 5-HT (Sigma), and GTS [saponins mixture containing at least 10 glycosides known as ginsenosides from *Panax ginseng* C. A. Meyer, extracted and purified by Namba et al.'s method (16) and supplied by Korea Ginseng and Tobacco Research Institute]. L-DOPA and 5-HT as suspensions in CMC and other drugs as solutions in saline were administered in a volume of 0.1 ml/10 g body weight. Naloxone 2 mg/kg was injected IP 10 min prior to morphine 5 mg/kg (SC) or U-50,488H 30 mg/kg (SC). GTS was injected IP 3 h prior to administration of morphine or U-50,488H in the tail-pinch (TP) test and 4 h prior to in the TF test, because the maximal antagonisms at these time intervals were observed through preliminary experiments, respectively. Thirty or 100 mg/kg L-DOPA or 5-HT was injected IP 30 min prior to injection of U-50,488H.

Measurement of Antinociception

The antinociceptive effects of analgesics were determined by using the TP method by a modified Haffner's method (19) and using the TF method as described by D'Amour and Smith (4). The TP responses to mechanical stimulation and the TF latencies to thermal stimulation were determined before and at 15- or 30-min intervals after drug injection for a period of 60 or 120 min in these experiments. The basal responses for the TP test and the basal latencies for the TF test were found to be approximately 1 and 2 s, respectively. A cutoff time of 6 s for the TP test and 10 s for the TF test was used to prevent injury to the tail. The antinociceptive effects were calculated as area under the curve (AUC) by plotting the changes in response (or latency) time (s) on the ordinate and the intervals (min) on the abscissa. The data were expressed as percent of the effect obtained in control animals.

Statistics

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

RESULTS

Morphine-induced antinociception was completely prevented by pretreatment with naloxone in both the TP and

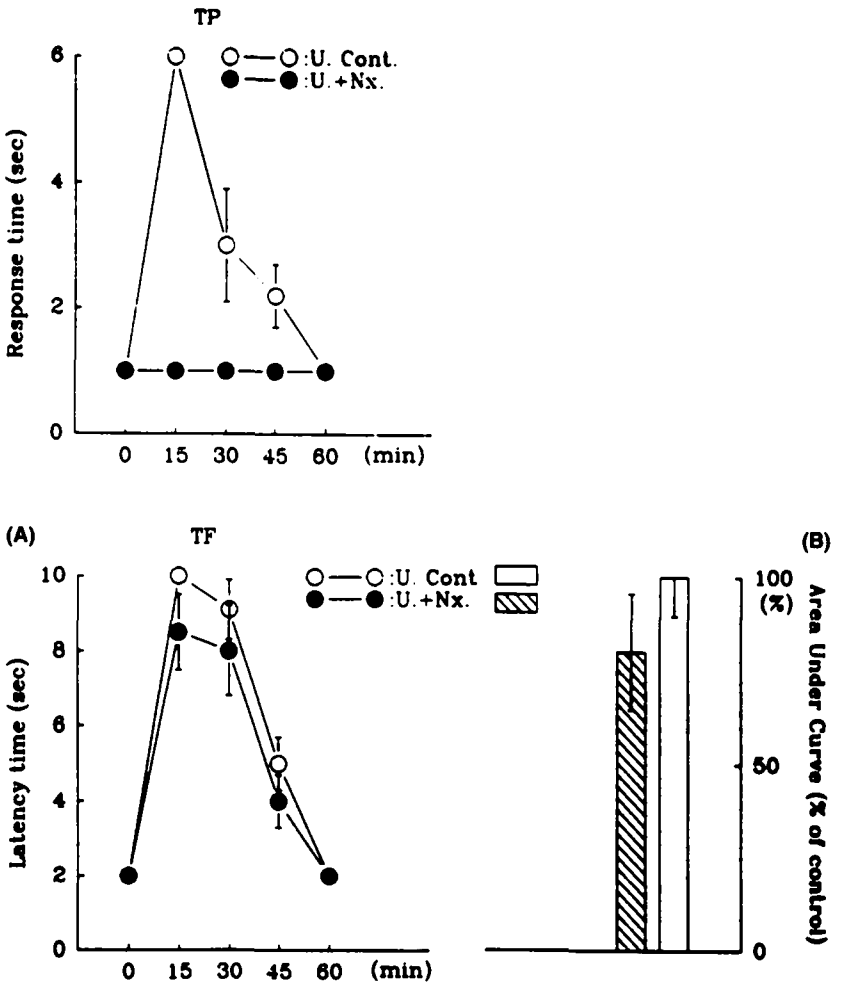

FIG. 3. Antinociceptive effect of U-50,488H and effect of naloxone in the TP (upper panel) and TF (lower panel) tests. Naloxone 2 mg/kg or saline was injected 10 min prior to U-50,488H (U) 30 mg/kg. Antinociceptive effect was measured by TP or TF method every 15 min for 60 min after U-50,488H. For other details, see Fig. 2.

TF tests (Fig. 1). In preliminary experiments, the effect of morphine alone and the combined effects of morphine and GTS were investigated at various time intervals. The maximal antagonistic effects were observed 3 h prior to administration of morphine in the TP test and 4 h prior to administration of morphine in the TF test. The antinociceptive effect of morphine 5 mg/kg was lowered to about 60% in the TP test and 20% in the TF test by GTS 100 mg/kg. The antagonistic effect of GTS was more effective in the TF test than in the TP test (Fig. 2).

U-50,488H-induced antinociception was completely suppressed by naloxone 2 mg/kg in the TP but not in the TF test (Fig. 3). U-50,488H-induced antinociception was significantly prevented by GTS 100 mg/kg in the TF test but not in the TP test (Fig. 4).

When L-DOPA or 5-HT was injected 30 min prior to administration of U-50,488H to mice pretreated with GTS, L-DOPA 30 and 100 mg/kg did not abolish the antagonism of U-50,488H-induced antinociception by GTS (Fig. 5), but 5-HT 30 and 100 mg/kg abolished the antagonism of U-50,488H-induced antinociception by GTS up to the normal level (Fig. 6). However, although GTS at 100 mg/kg alone produced an antinociceptive effect it was not observed 3 h in the TP test and 4 h in the TF test after administration of GTS. L-DOPA and 5-HT did not affect U-50,488H-induced antinociception in mice (data not shown).

DISCUSSION

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 (6). However, GTS separated from ginseng extract prevented morphine-induced antinociception and also inhibited the development of morphine-induced tolerance (8). Therefore, it is proposed that GTS is able to antagonize morphine-induced antinociception.

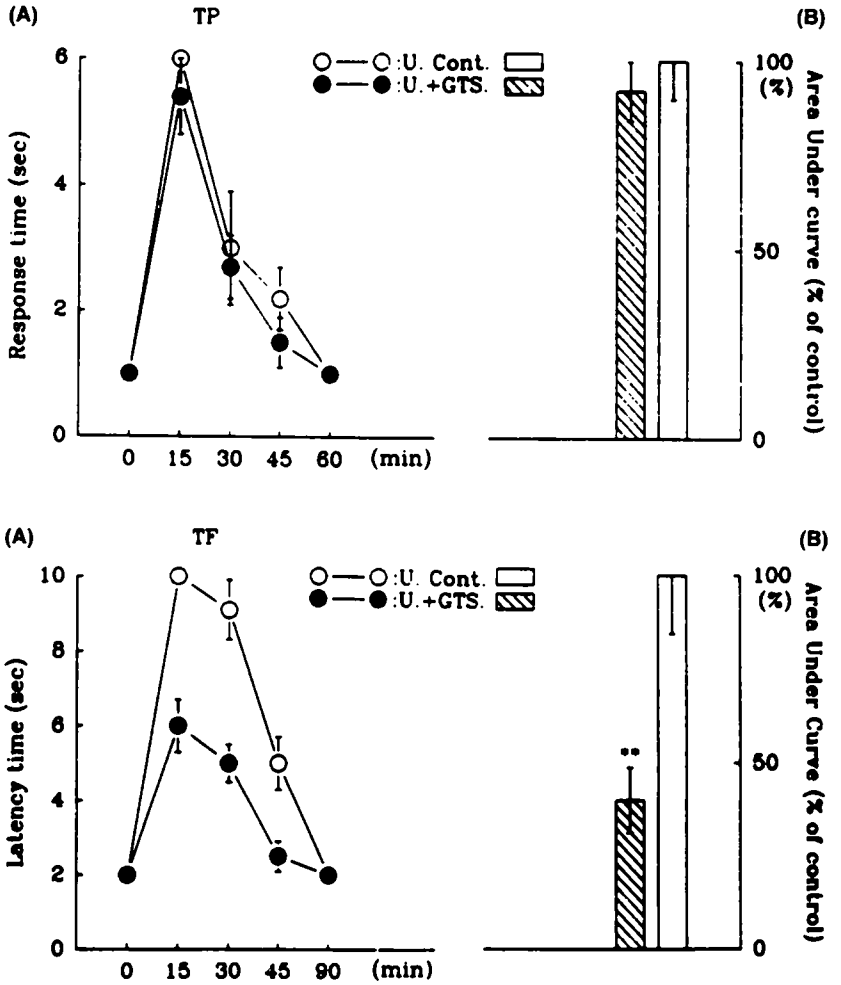

FIG. 4. Antinociceptive effect of U-50,488H and effect of GTS in the TP (upper panel) and TF (lower panel) tests. GTS 100 mg/kg or saline was injected 3 h in the TP test and 4 h in the TF test prior to U-50,488H 30 mg/kg. Antinociceptive effect was measured by TP or TF method every 15 min for 60 min after U-50,488H. For details, see Fig. 2. $^{**}p < 0.01$.

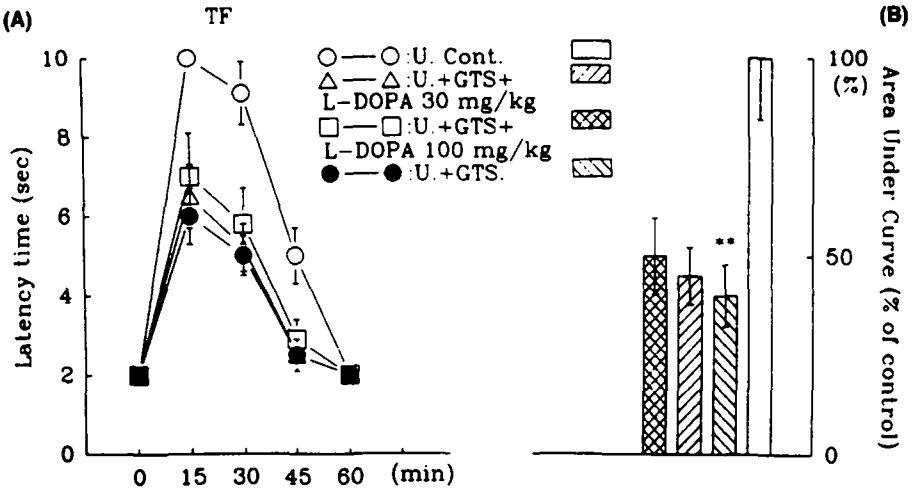

FIG. 5. Antagonism of U-50,488H-induced antinociception in the TF test by GTS and its effect of L-DOPA. GTS 100 mg/kg was injected 3 h prior to U-50,488H 30 mg/kg. L-DOPA 30 or 100 mg/kg was given 30 min prior to U-50,488H. Antinociceptive effect was measured by TP or TF method every 15 min for 60 min after U-50,488H. For other details, see Figs. 2 and 4. $^{**}p < 0.01$, compared with that of U-50,488H.

The antinociceptive effect of morphine 5 mg/kg was completely prevented by naloxone in the TP and TF tests. But, U-50,488H-induced antinociception was completely prevented by naloxone in the TP test but was not prevented in the TF test as Takahashi et al. observed (21). Therefore, U-50,488H-induced antinociception involves at least two mechanisms, opioid and nonopioid forms, on the basis of criteria such as reduction by naloxone (28). As the nonopioid mechanism, catecholaminergic (3,9,21), serotonergic (2,3,5), and cholinergic (12,13) mechanisms were involved in the production of various physical stress-induced analgesia. This may occur in combination with an opioid mechanism (3,9,12,21). In agreement with our findings, psychological stress-induced analgesia is completely prevented by naloxone in the TP but not in the TF test (21). These findings provide further support that two separate systems can regulate nociceptive TP and TF responses. The antinociceptive effect of U-50,488H that is antagonized by naloxone in the TP test is mediated by opioid mechanisms, but the effect not antagonized by naloxone in the TF test is mediated by nonopioid mechanisms. However, U-50,488H-induced antinociception was prevented by GTS but not by naloxone in the TF test, whereas the effect was prevented by naloxone but not by GTS in the TP test. These results indicate that the antagonistic effect of GTS on U-

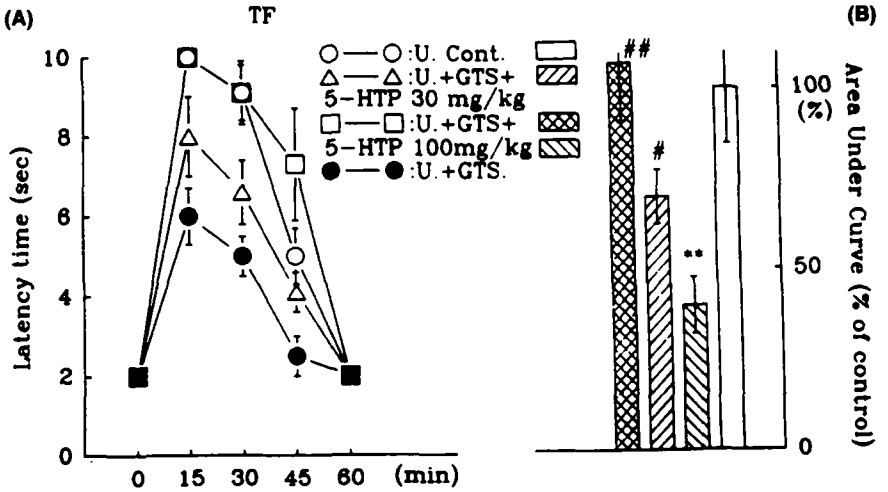

FIG. 6. Antagonism of U-50,488H-induced antinociception by GTS in the TF test and its abolition by 5-HT. GTS 100 mg/kg was injected 4 h prior to U-50,488H 30 mg/kg. 5-HT 30 or 100 mg/kg was given 30 min prior to U-50,488H. Antinociception was measured by TF method every 15 min for 60 min after U-50,488H. For other details see Figs. 2 and 4. $^{**}p < 0.01$, compared with that of U-50,488H; $\#p < 0.05$, $\#\#p < 0.01$, compared with that of GTS + U-50,488H.

50,488H-induced antinociception was substantially different from that of naloxone. Therefore, nonopioid mechanisms are implicated in the antagonism of U-50,488H-induced antinociception by GTS.

In agreement with the present study, we previously reported that catecholaminergic and serotonergic mechanisms are involved in the antagonism of morphine-induced antinociception by GTS in mice (7). The inhibitory effect of GTS in isolated guinea pig ileum and mouse vas deferens showed the direct action of GTS on smooth muscle preparations without the involvement of cholinergic or noradrenergic mechanisms on the opioid receptors (26). The inhibition of the development of morphine tolerance by GTS in guinea pig ileum was mediated through an effect on the cholinergic system without involvement of direct action on opioid receptors (27). Furthermore, recent study showed that the analgesic response to morphine was antagonized by ginseng extract, suggesting this pharmacological action was mediated by a nonopioid mechanism (18).

In terms of serotonergic mechanisms, antagonisms of morphine by reserpine and PCPA have been widely studied in the mice and rats, indicating an important contribution of serotonergic mechanisms to morphine-induced antinociception (1,20,23). 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, it suggests that κ-opioid analgesia, in contrast to μ-opioid analgesia, is manifested principally through serotonergic pathways (25). In addition, 5-HT antagonists, cyproheptadine, ketanserine, and pirenperone caused dose-related antagonism of U-50,488H in the TF test (24). None of the 5-HT antagonists significantly affected morphine analgesia. Based upon the antagonism of U-50,488H by selective 5-HT depletors and varinous 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 (17). In these experiments, GTS prevented U-50,488H-induced antinociception in the TF test and the antagonism was abolished by pretreatment with 5-HT but not by L-DOPA. This is also in agreement with the result that the loss of κ-opioid analgesia by reserpine was abolished by treatment with 5-HT. In this regard, the present results provide additional evidence that κ-opioid analgesia is dependent on serotonergic mechanisms as was demonstrated by Von-Voigtlander et al. (24).

REFERENCES

1. Berge, O. G.; Hole, K.; Ogren, S. O. Attenuation of morphine-induced analgesia by *p*-chlorophenylalanine and *p*-chloroamphetamine: Test-dependent effects and evidence for brainstem 5-hydroxytryptamine involvement. Brain Res. 271:51-64; 1983.
2. Bhattacharya, S. K.; Keshary, P. R.; Sanyal, A. K. Immobilization stress-induced antinociception in rats: Possible role of serotonin and prostaglandins. Eur. J. Pharmacol. 50:83-85; 1978.
3. Coder, T. J.; Rollman, G. B. Stress analgesia: Effects of PCPA, yohimbine and naloxone. Pharmacol. Biochem. Behav. 21:681-686; 1984.
4. D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74-79; 1941.
5. Hutson, P. H.; Tricklebank, M. D.; Curzon, G. Enhancement of footshock-induced analgesia by spinal 5,7-dihydroxytryptamine lesions. Brain Res. 237:367-372; 1982.
6. Kim, H. S.; Jang, C. G.; Lee, M. G. Antinarcotic effects of the standardized ginseng extract G115 on morphine. Planta Medica 56:158-163; 1990.
7. Kim, H. S.; Oh, K. W.; Oh, S. K. Antagonism of analgesic effect of morphine in mice by ginseng saponins. J. Korean Pharm. Sci. 16:135-138; 1986.
8. Kim, H. S.; Oh, K. W.; Park, W. G.; Yamano, S.; Toki, S. Effects of *Panax ginseng* on the development of morphine tolerance and dependence. Korean Ginseng Sci. 11:182-190; 1987.
9. Kulkarni, S. K. Heat and other physiological stress-induced analgesia: Cathecholamine mediated and naloxone reversible response. Life Sci. 27:185-188; 1980.
10. Lahti, R. A.; VonVoigtlander, P. F.; Barsuhn, C. Properties of a selective κ-agonist, U-50,488H. Life Sci. 31:2257-2260; 1982.
11. Le Bars, D. Neuronal serotonin and pain. In: Osborne, N. N.; Hamon, M., eds. New York: John Wiley and Sons; 1988; 171.
12. Lewis, J. W.; Cannon, J. T.; Liebeskind, J. C. Involvement of central muscarinic cholinergic mechanisms in opioid stress analgesia. Brain Res. 270:289-293; 1983.
13. MacLennan, A. J.; Drugan, R. C.; Maier, S. F. Long-term stress-induced analgesia blocked by scopolamine. Psychopharmacology (Berl.) 80:267-268; 1983.
14. Messing, R. B.; Lytle, L. D. Serotonin containing neurons: Their possible role in pain and analgesia. Pain 4:1-21; 1977.
15. Mohrland, J. S. Pain pathways: Potentials sites for analgesic action. In: Ledniced, D., ed. Central analgesics. New York: John Wiley and Sons; 1982:1-49.
16. Namba, T.; Yoshizaki, T.; Tominori, K.; Kobashi, K.; Mitsui, K.; Hasse, J. Fundamental studies on the evaluation of the crude drugs (1). Planta Medica 32:588-594; 1974.
17. Petkov, V. Effect of ginseng on the brain biogenic monoamines and 3',5'-AMP system. Arzneim. Forsch./Drug Res. 28:388-393; 1978.
18. Ramarao, P.; Bhargava, H. N. Antagonism of the acute pharmacological actions of morphine by *Panax ginseng* extract. Gen. Pharmacol. 21:877-880; 1990.
19. Takagi, H.; Inukai, T.; Nakama, M. A modification of Haffner's method for testing analgesics. Jpn. J. Pharmacol. 16:287-294; 1966.
20. Takagi, H.; Takashima, T.; Kimura, K. Antagonism of the analgesic effects of morphine in mice by tetrabenazine and reserpine. Arch. Int. Pharmacodyn. Ther. 149:484-492; 1964.
21. Takahashi, M.; Izumi, R.; Kaneto, H. The role of the catecholaminergic mechanism in foot shock (FS) stress- and immobilized-water immersion (IW) stress-induced analgesia in mice. Jpn. J. Pharmacol. 35:175-179; 1984.
22. Takahashi, M.; Tokuyama, S.; Kaneto, H. Implication of endogenous opioid mechanism in the production of the antinociceptive effect induced by psychological stress in mice. Jpn. J. Pharmacol. 44:283-291; 1987.
23. Tenen, S. S. Antagonism of the analgesic effect of morphine and other drugs by *p*-chlorophenylalanine, a serotonin depletor. Psychopharmacologia. 12:278-285; 1968.
24. VonVoigtlander, P. F.; Lahti, R. A.; Ludens, J. H. U-50,488H: A selective and structually novel non-mu (kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224:7-12; 1983.
25. VonVoigtlander, P. F.; Lewis, R. A.; Neff, G. L. Kappa opioid analgesia is dependent on serotonergic mechanisms. J. Pharmacol. Exp. Ther. 231:270-274; 1984.
26. Watanabe, J.; Oh, K. W.; Kim, H. S.; Takahashi, M.; Kaneto, H. A non-opioid mechanism in the inhibitory effect of ginseng

saponins on electrically evoked contractions of guinea pig ileum and mouse vas deferens. J. Pharmacobiodyn. 11:453-458; 1988.
27. Watanabe, J.; Takahashi, M.; Kaneto, H. Distinctive effect of ginseng saponins on development of morphine tolerance in guinea pig ileum and mouse vas deferens. J. Pharmacobiodyn. 11:744-748; 1988.
28. Watkins, L. R.; Mayer, D. J. Organization of endogenous opiate and non-opiate pain control systems. Science 216:1185-1192; 1982.