Antinarcotic Effect of *Panax ginseng*

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Abstract□ The analgesic effect of morphine was antagonized and the development of tolerance was suppressed by the modification of the neurologic function in the animals treated with ginseng saponins.

The activation of the spinal descending inhibitory systems as well as the supraspinal structures by the administration of morphine was inhibited in the animals treated with ginseng saponins intracerebrally or intrathecally.

The development of morphine tolerance and dependence, and the abrupt expression of naloxone induced abstinence syndrom were also inhibited by ginsenoside Rb_1, Rb_2, Rg_1 and Re. These results suggest that ginsenoside Rb_1, Rb_2, Rg_1 and Re are the bioactive components of panax ginseng on the inhibition of the development of morphine tolerance and dependence, and the inhibition of abrupt abstinence syndrome. In addition, further research on the minor components of *Panax ginseng* should be investigated.

A single or daily treatment with ginseng saponins did not induce any appreciable changes in the brain level of monoamines at the various time intervals and at the various day intervals, respectively.

The inhibitory or facilitated effects of ginseng saponins on electrically evoked contractions in guinea pig ileum (μ-receptor) and mouse vas deferens (δ-receptor) were not mediated through opioid receptors.

The antagonism of a κ receptor agonist, U-50, 488H was also not mediated through opioid receptors in the animals treated with ginseng saponins, but mediated through serotonergic mechanisms.

Ginseng saponins inhibited morphine 6-dehydrogenase which catalyzed the production of morphinone from morphine, and increased hepatic glutathione contents for the detoxication of morphinone.

This result suggests that the dual action of the above plays an important role in the inhibition of the development of morphine tolerance and dependence.

Introduction

A folk medicine prescribed by seven herbal drugs including *Panax ginseng* has been used as an antidote in the treatment of morpine tolerant-dependent patients. Therefore, the present study was undertaken to investigate the possible mechanisms of ginseng saponins on the antagonism of morphine analgesia and the inhibition of the development of tolerance.

So, we would like to show you our experiments, titled as follow;

1. Antagonism of morphine analgesia on mechanical and thermal nociceptions by systemic (i.p.), intracerebral(i.c.) and intrathecal(i.t.) treatment with ginseng total saponins (GTS).
2. Inhibition of the development of morphine tolerance.
3. Ginsenosides as bioactive components on the inhibition of the development of morphine tolerance.
4. Brain monoamines levels in mice treated with a single or daily treatment with GTS.
5. Non-opioid mechanism (in the inhibitory effects of ginseng saponins) on the μ-, δ- and κ-receptors.
6. Increase in the hepatic glutathione contents which are closely related with degree of detoxication of morphinone (a novel and active metabolite of morphine during chronic morphinization).
7. Inhibition of morphine 6-dehydrogenase which catalyzes the production of morphinone from

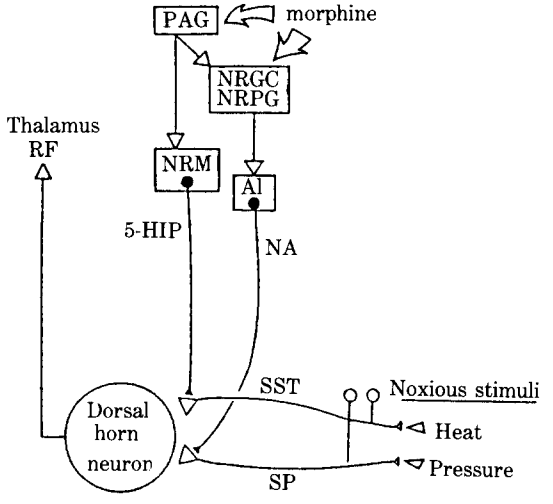

Fig. 1. Pain transmission and inhibition.
PAG, periaqueductal gray matter; NRGC, nucleus reticularis gigantocellularis; NRPG, nucleus reticularis paragigantocelluaris; NRM, nucleus raphe magnus; RF, reticular formation; NA, noradrenaline; 5-HT, 5-hydroxytryptamine (seroptonin); SST, somatostatin; SP, substance P.

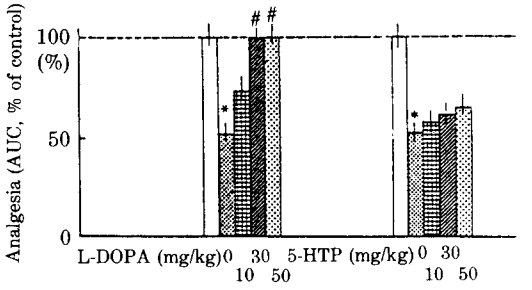

Fig. 2. Antagonism of morphine (Mor) analgesia in the pinch test by GTS and its reversal by L-DOPA or 5-HTP.
GTS 100 mg.kg was injected i.p.3 hrs prior to the administration of Mor 5 mg/kg (s.c.). L-DOPA or 5-HTP (each 10, 30 and 50 mg/kg, i.p.) were given 30 min prior to the administration of Mor.
*. $p<0.05$; **, $p<0.01$; compared ith that of saline+Mor #, $p<0.05$; ##, $p<0.01$; compared with that of GTS+Mor
□, Mor control;
▨, GTS+Mor;
■, GTS+10 mg/kg, of L-DOPA or 5-HTP+Mor
▨, GTS+30 mg/kg, of L-DOPA or 5-HTP+Mor
▨, GTS+50 mg/kg, of L-DOPA or 5-HTP+Mor

morphine.

1. Antagonism of morphine analgesia on mechanical and thermal nociceptions by i.p., i.c. and i.t. treatments with GTS

Fig. 1 shows the pain transmission and spinal descending inhibitory systems. The analgesic action of morphine is the result of direct or indirect inhibition of pain trasmission in the spinal dorsal horn. The analgesic action of systemic morphine in analgesic doses is primarily mediated by the activation of the descending inhibitory systems. Recent studies have shown that descending inhibitory systems consist in part of the noradrenergic and serotonergic systems[1]. The noradrenergic descending inhibitory system in mechanical nociception (tail pinch test) and serotonergic system in thermal nociception (tail flick test) play more important roles in the production of morphine analgesia, respectively. For this reason, we are going to tell whether the analgesic action of morphine in analgesic doses on mechanical and thermal nociceptions is antagonized by 1) i.p. (100 mg/kg of GTS), 2) i.c.[2] (40 μg/body) and 3) i.t.[3] (40 ug/body) pretreatments with GTS and its reversal by L-DOPA or 5-HTP to check the acting sites of antagonism and the roles of spinal descending inhibitory systems in the production of antagonism.

The analgesic action of morphine 5 mg/kg was determined by the tail pinch or tail flick method and calculated as percent of the control by the AUC method and compared with that of the mice pretreated with GTS.

Fig. 2 shows antagonism of morphine analgesia in the tail pinch test by GTS and morphine injected systemically in mice, and its reversal by L-DOPA or 5-HTP.

The inset white column represents the analgesic activity of morphine 5 mg/kg as 100%. The parallel cut out lines column also represents the antagonized activity of morphine 5 mg/kg by the pretreatments with GTS 100 mg/kg and the others, the suppressions of antagonism by 10, 30 and 50 mg/kg of L-DOPA or 5-HTP.

In the tail pinch test (Fig. 2), analgesic action of morphine 5 mg/kg administered subcutaneously (s.c.) was antagonized by 50% with the i.p. pretreat-

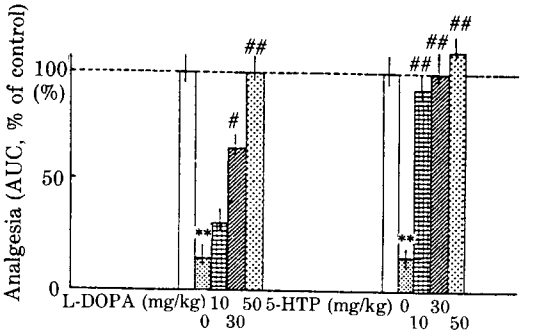

Fig. 3. Antagonism of morphine analgesia in the tail flick test by GTS and its reversal by L-DOPA or 5-HTP. GTS 100 mg/kg (i.p.) was injected 4 hrs prior to the administration of morphine 5 mg/kg (s.c.). L-DOPA or 5-HTP (each 10, 30 and 50 mg/kg, i.p.) were given 30 min prior to the adminstration to morphine. For other details, refer to Fig. 2.

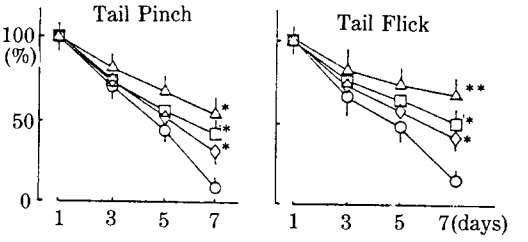

Fig. 4. Effects of GTS, PD and PT on the development of morphine tolearance.
Mice wee pretreated daily with 100 mg/kg of GTS (□−□), PD (◇−◇) and PT (△−△) i.p. 3 hrs in the tail pinich test or 4 hrs in the tail flick test prior to the administration of morphine (10 mg/kg, s.c.) for 7 days. Control group (○−○) received saline instead of ginseng saponins.
*, $p<0.05$; **, $p<0.01$; compared with that of saline + morphine

ment with GTS 100 mg/kg, but the suppression of morphine antagonism by L-DOPA was more predominant than that of 5-HTP.

In the tail flick test (Fig. 3), analgesic action of morphine was antagonized by about 80% with the i.p. pretreatment with GTS 100 mg/kg, and its reversal by 5-HTP was more predominant than that of L-DOPA.

All of the antagonized patterns of morphine by i.c. or i.t. pretreatments with GTS and their reversals by L-DOPA or 5-HTP are almost the same as those of i.p. treatments with GTS in the tail pinch and tail flick tests, respectively. Therefore, we can summarize the results as follows;

1) The analgesic action of systemic morphine in analgesic doses on mechanical and thermal nociceptions was antagonized by i.p., i.c. and i.t. pretreatments with GTS.
2) L-DOPA in the tail pinch test and 5-HTP in the tail flick test, respectively played more important roles in the suppression of morphine antagonism by GTS.
3) The above results suggest that GTS inhibits the activation of the spinal descending inhibitory noradrenergic and serotonergic systems as well as the activation of the supraspinal structures.

2. Inhibition of the development of morphine tolerance

Fig. 4 shows effects of GTS, protopanaxadiol saponins (PD) and protopanaxatriol saponins (PT) on the development of morphine tolerance. Tolerance to morphine 10 mg/kg in mice was estimated on the 1st, 3rd, 5th and 7th days for 7 days. The effects were expressed daily as percent of the effect obtained from the original groups.

Daily injection of morphine 10 mg/kg for 7 days caused development of tolerance rapidly and the effect of morphine disappeared after 7 days. But the development of tolerance to morphine 10 mg/kg in mice treated with GTS, PD and PT daily was inhibited in both of the tail pinch and tail flick tests. The inhibitory effect was in the order of PT, GTS and PD.

3. Ginsenosides as bioactive components on the inhibition of the development of morphine tolerance and dependence

To investigate the bioactive components of *Panax ginseng*, 100 mg/kg of each ginseniside Rb_1, Rb_2, Rg_1 and Re was administered to mice i.p. once a day 1 hr prior to the last administration of morphine. To induce morphine tolerance and dependence, morphine hydrochloride 40 mg/kg was administered s.c. to mice every 8 hrs for a period of 6 days by Way and his coworker's method[4].

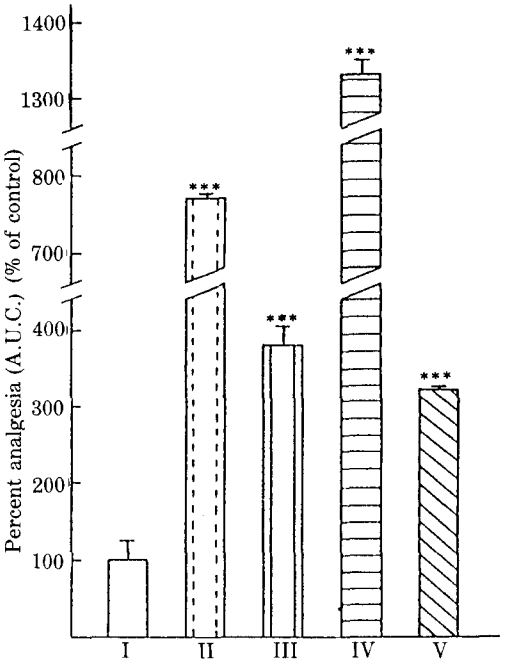

Fig. 5. Effects of ginsenoside Rb_1, Rg_1 and Re on the development of morphine tolerance in mice.
Ginsenoside Rb_1, Rg_1 and Re (each 100 mg/kg) was administered i.p. in mice 1 hr prior to the morphine treatment k(10 mg/kg, s.c.). ***, $p<0.001$
I, morphine + saline; II, morphine + Rb_1; III, morphine + Rb_2; IV, morphine + Rg_1; V, morphine + Re.

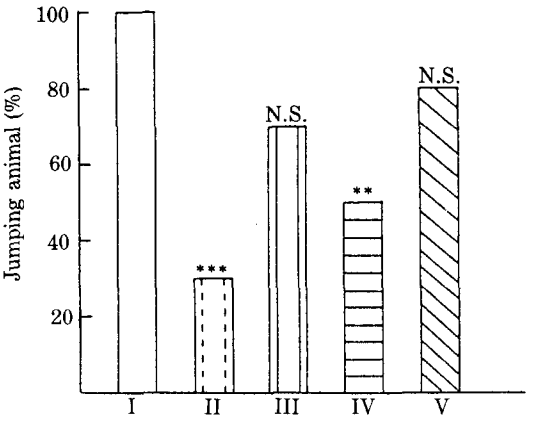

Fig. 6. Effects of ginsenoside Rb_1, Rb_2, Rg_1 and Re on the development of morphine dependence in the mice by the naloxone-induced jumping response.
**, $p<0.01$; ***, $p<0.001$; N.S., non-significant
For other details, refer to Fig. 5.

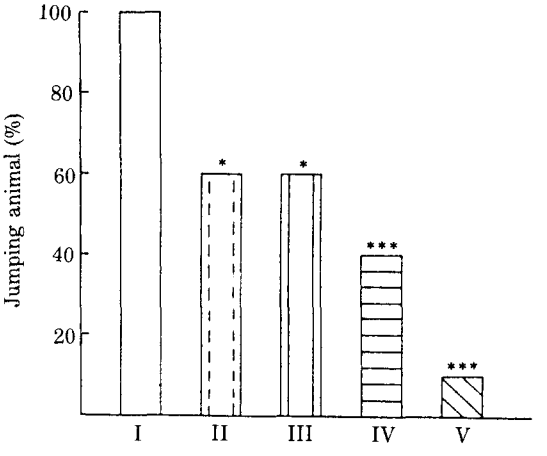

Fig. 7. Effects of ginsenoside Rb_1, Rb_2, Rg_1 and Re on the abrupt inhibitions of morphine dependence in the mice.
Naloxone 4 mg/kg was injecterd s.c. in the morphine-dependent mice for jumping response.
*, $p<0.05$; ***, $p<0.001$
For other details, refer to Fig. 5.

1) Inhibition of analgesic tolerance development

The analgesia of each group calculated as the AUC to morphine 10 mg/kg was observed by 7.7 in Rb_1 100 mg/kg, 3.8 in Rb_2 100 mg/kg, 13.3 in Rg_1 100 mg/kg and 3.2 times in Re 100 mg/kg as compared with that of the morphine control group (Fig. 5).

2) Inhibition of naloxone-induced jumping response

Rb_1 and Rg_1 (each 100 mg/kg) produced significant inhibition of naloxone-induced jumping response by 70% and 50% respectively, but not significant 30% in Rb_2 100 mg/kg, 20% in Re 100 mg/kg and 0% inhibition in morphine control group (Fig. 6).

3) Ginsenosides as bioactive components on the abrupt inhibitions of naloxone induced jumping response

To test the abrupt inhibitory effects of the ginsenosides, 100 mg/kg of each ginsenosides was injected i.p. in morphine dependent mice 30 min prior to the naloxone-induced jumping response.

The inhibitory effect (%) of ginsenosides 100 mg/kg in morphine dependent mice was determined by counting the decreased number of naloxone-

induced jumping response for 30 min after the injection of naloxone 4 mg/kg (s.c.) and calculated as percent of the control. All of the ginsenosides tested here showed significant abrupt inhibitions of naloxone-induced jumping response: both of Rb_1 and Rb_2 by 40%, Rg_1 by 60% and Re by 90%, respectively (Fig. 7).

These results suggest that the ginsenoside Rb_1, Rb_2, Rg_1 and Re are the bioactive components of *Panax ginseng* on the inhibition of the development of morphine tolerance and dependence, and the abrupt inhibition of abstinence syndrome. In addition, further research on minor components of *Panax ginseng* should be investigated continuously.

4. Changes of brain biogenic monomines levels in mice treated with a single or daily treatment with GTS

Fig. 8 shows effects of GTS, a single dose of 100 mg/kg, on the changes of brain monoamines in mice. The present studies were undertaken to determine whether the antagonism of morphine analgesia by the treatment with GTS and the inhibition of the development of morphine tolerance by daily treatment with GTS were responsible for the changes of the brain biogenic monoamines levels.

The brain biogenic monoamines contents in mice treated with a single dose of GTS 100 mg/kg were determined at the various time intervals of 0, 2, 4, 8 and 24 hrs after the treatment with GTS using HPLC with an electrochemical detector[5,6]. Data were expressed as a percent of the contents in naive mice.

The brain levels of noradrenaline, dopamine and serotonin were not modified in mice treated with a single dose of GTS, when estimated at various time intervals.

Fig. 9 shows effects of GTS on the changes of brain levels of noradrenaline, dopamine and serotonin during daily administration of GTS in mice. GTS 100 mg/kg was injected i.p. daily for 5 days. Brain biogenic monoamines were determined at various day intervals of the 1st, 3rd and 5th days 24 hrs after the final administration. The brain monoamines levels were not modified by daily treatment

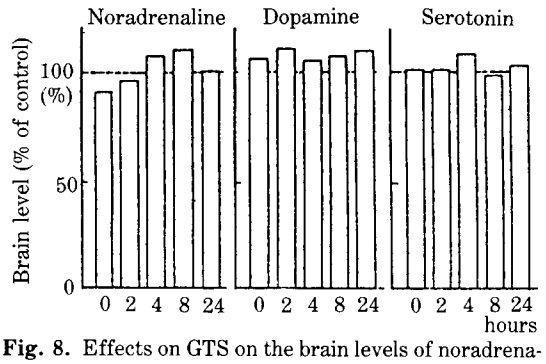

Fig. 8. Effects on GTS on the brain levels of noradrenaline, dopamine and serotonin in mice.
The brain levels of noradrenaline, dopamine and serotonin were estimated at various time intervals, after a single i.p. administration of GTS 100 mg/kg. Data were shown as a percent of content in naive mice (noradrenaline, 0.34 ± 0.02; dopamine, 1.04 ± 0.13; serotonin, 0.52 ± 0.08 μg/g wet tissue). Five mice were used at least.

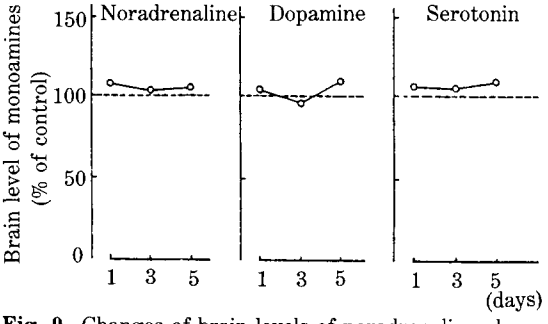

Fig. 9. Changes of brain levels of noradrenaline, dopamine and serotonin during daily adminstration of GTS in mice.
GTS 100 mg/kg was injected i.p. daily for 5 days. Brain biogenic monamines were measured 24 hrs after the final administration. Five mice were used at least.

with GTS for 5 days.

A single or daily treatment with GTS not only antagonized the analgesic effect of morphine but also inhibited the development of morphine tolerance without any appreciable changes in monoamines levels of the whole brain.

The present results are consistent with studies by Kaneto *et al.* reported that daily treatment with a small dose of reserpine 0.1 mg/kg, which did not affect any appreciable changes in brain catecholamines contents, could effectively block the develop-

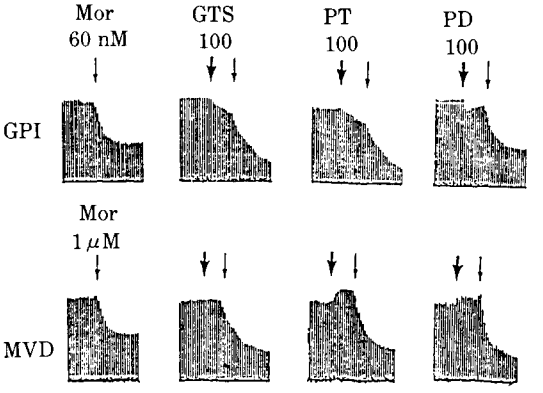

Fig. 10. Effects of ginseng saponins on morphine-induced inhibitions of contrations of GPI and MVD preparations.
Morphine (Mor) and ginseng saponins were at the slim and bold arrows indicated, respectively.

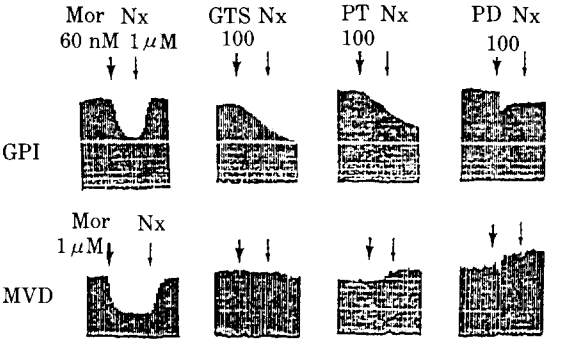

Fig. 11. No antagonism of naloxone (Nx) on the inhibitions or the facilitations of the contrations of GPI or MVD preparations induced by ginseng saponins.
Morphine (Mor) or ginseng saponins was added at the bold arrow indicated, followed by naloxone at the slim arrow.

ment of morphine tolerance. Meanwhile, the antagonism of morphine analgesia by the pretreatment with a small dose of reserpine 0.1 mg/kg was also identified in our studies.

Accordingly, these results clearly indicate that a newly equilibrate state of neurologic function in mice can be an underlying common mechanism in the groups treated with GTS and a small dose of reserpine as Kaneto *et al.* reported.

5. Non-opioid mechanism (in the inhibitory effects of ginseng saponins) on the μ-, δ- and κ-receptors

Isolated guinea pig ileum (GPI) has been used as *in vitro* model for the opioid μ-receptor. It has been well known that electrically evoked contraction of GPI is mediated by acetylcholine, and the release of acetylcholine is regulated presynaptically by opioid μ-receptor. For this reason, the effect of ginseng saponins on the electrically evoked contraction of GPI has been determined. GPI longitudinal muscle strips were set up in a 10 m*l* organ bath filled with Krebs-Hensleit solution and stimulated with platinum ring electrodes. The contractions were recorded through an isotonic tranducer.

Isolated mouse vas deferens (MVD) has been used as *in vitro* model for the opioid δ-receptor. It has been well established that electrically evoked contraction of MVD is mediated by noradrenaline, and the release of noradrenaline is regulated presynaptically by opioid δ-receptor. For this reason, the studies of ginseng saponins on electrically evoked contraction of MVD have been carried out.

Morphine inhibited the electrically induced contractions of GPI and MVD preparations in a concentration-dependent manner. The IC_{50} of the drug was 60 nM and 1 μM for GPI and MVD, respectively. The inhibitory effect of morphine was arithmatically increased by pretreatment with 100 μg/m*l* of these saponins in the GPI preparation, while the effect was apparently potentiated in the MVD preparation (Fig. 10).

In both GPI and MVD preparations, 1 μM naloxone completely reversed morphine induced inhibitions of the contractions. However, neither the inhibition of the contractions by GTS, PT and PD, in the GPI preparation, nor the facilitation of the contractions by PT and PD, in the MVD preparation was reversed by 1 uM naloxone (Fig. 11).

U-50,488H has been known as a selective and pure κ receptor agonist *in vitro* and *vivo*. For this reason, the present study was undertaken to investigate the antagonistic mechanism of U-50,488H analgesia *in vivo* by naloxone and GTS in the tail pinch and tail flick tests, respectively.

U-50,488H 30 mg/kg produced analgesia in the both tests. The analgeasic effect of U-50,488H was

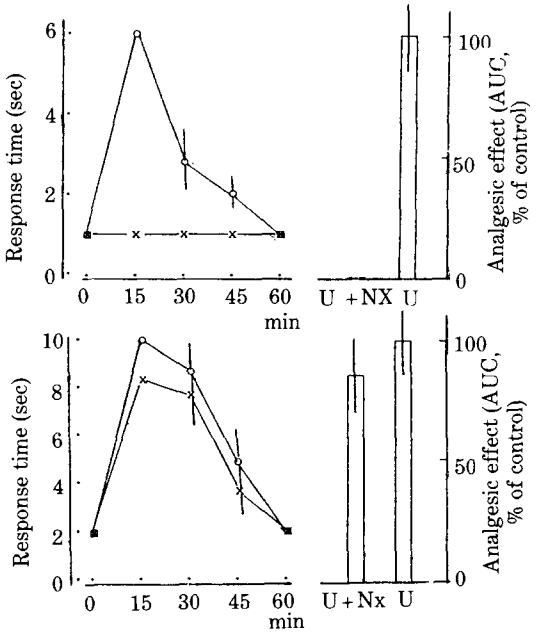

Fig. 12. Analgesic action of U-50,488H (U) and its naloxone (Nx) antagonism in tail pinch (upper) and tail flick (lower) tests.
Analgesic effect was measured by tail pinch or tail flick method every 15 min for 60 min after U-50,488H injection. Nx (2 mg/kg, * — *) or saline (○—○) was injected i.p. 10 min prior to the injection of U-50,488H 30 mg/kg. Each point is the mean ± S.E. of least 10 mice.

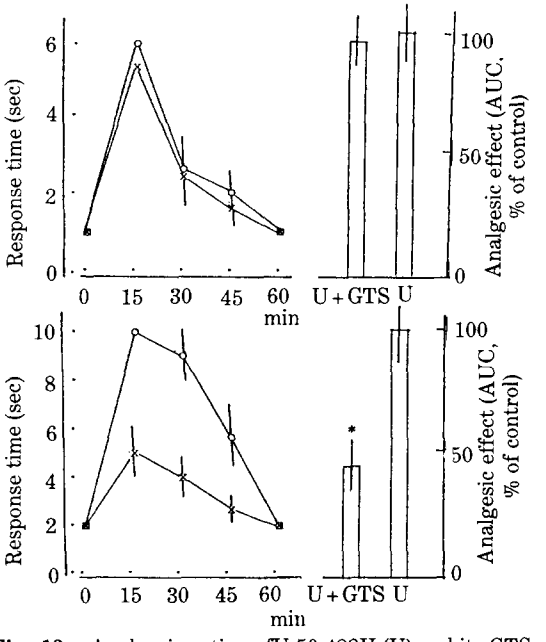

Fig. 13. Analgesic action ofU-50,488H (U) and its GTS antagonism in tail pinch (upper) and tail flick (lower) tests.
Analgesic effect was measured by tail pinch or tail flick method every 15 min for 60 min after U-50,488H injection. GTS (100 mg/kg, x-x) or saline (○—○) was injected i.p. 3 hrs in tail pinch test and 4 hrs in tail flick test prior to the injection of U-50,488H 30 mg/kg. Each point is the mean ± S.D. of at least 10 mice. *, $p<0.05$.

completely suppressed by naloxone 2 mg/kg only in the tail pinch test, but the inhibition of the analgesia by naloxone was not observed in the tail flick test (Fig. 12). One hundred mg/kg of GTS significantly antagonized the effect of U-50,488H analgesia only in the tail flick, but not in the tail pinch test (Fig. 13). The antagonism of U-50,488H analgesia was not reversed at all by L-DOPA (30 and 100 mg/kg) in the tail flick test (Fig. 14). But the antagonism of U-50,488H was reversed up to the normal level by 5-HTP 30 and 100 mg/kg dose-dependently (Fig. 15).

The present results suggest that the antagonism of U-50,488H by GTS is not mediated through opioid κ receptor but mediated through serotonergic mechanisms.

6. Increase in the hepatic glutathione contents which are closely related with degree of detoxication of morphinone (a novel and active metabolite of morphine during chronic morphinization)

The inhibitory effects of GTS, PD and PT on the hepatic glutathione level observed. Other additional groups of mice that had received the same morphine and ginseng saponins as described before, were used to determine the hepatic glutathione levels by the modified Ellman's method[7].

The glutathione contents in mice treated with only GTS, PD and PT are generally a little bit higher than that of the saline control group. But most of the values of the glutathione contents in ginseng treated groups show higher than that of the morphine control group.

Schole *et al.* reported that ginseng extract increased the hepatic glutathione level in rats as we

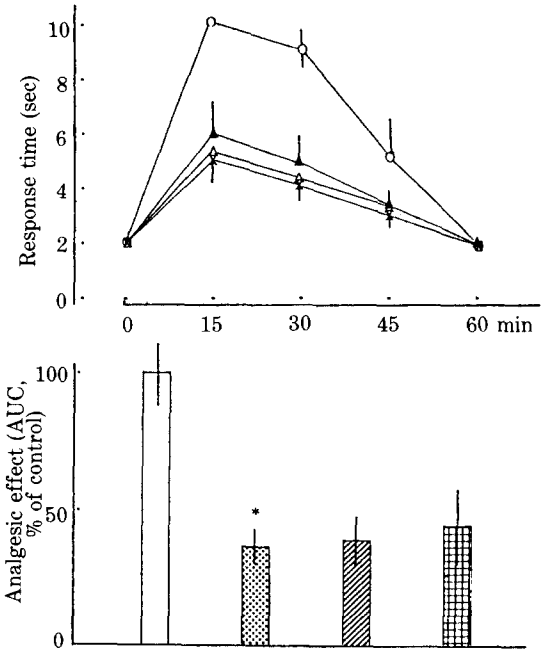

Fig. 14. Antagonism of U-50,488H anlgesia in the tail flick test by GTS and its reversal by L-DOPA. GTS 100 mg/kg was injected i.p. 4 hrs prior to the adminstration of U-50,488H (30 mg/kg, i.p.). L-DOPA (30 and 100 mg/kg, i.p.) were given 30 min prior to the adminstration of U-50,488H. The analgesia was measured by the tail flick method every 15 min after U-50,488H injection for 60 min.
U-50,488H:○-○; □
GTS+U-50,488H; *-* ; ▨
GTS+L-DOPA 30 mg/kg+U-50,488H:△-△; ▨
GTS+L-DOPA 100 mg/kg+U-50,488H:▲-▲; ■

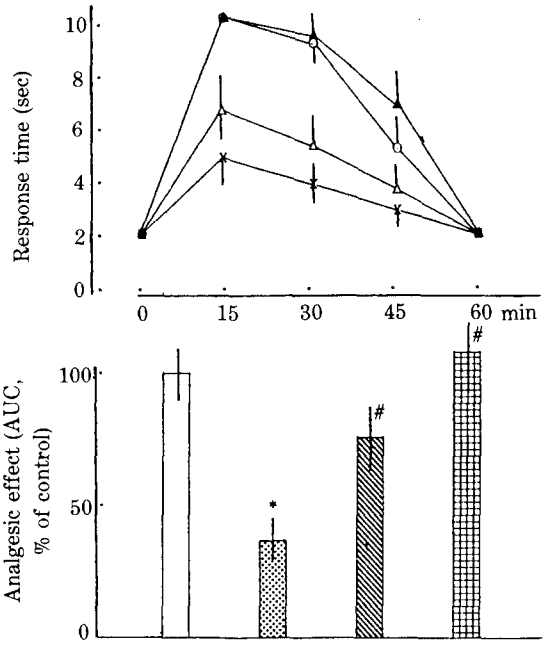

Fig. 15. Antagonism of U-50,488H analgesia in the tail flick test by GTS and its reversal by 5-HIP. GTS 100 mg/kg was injected i.p. 4 hrs prior to the adminstration of U-50,488H (30 mg/kg, i.p.). 5-HIP (30 and 100 mg/kg, i.p.) were given 30 min prior to the adminstration of U-50,488H. The analgesia was measured by the tail flick method every 15 min after U-50,488H injection for 60 min.
*, $p<0.05$; compared with that of saline+U-50,488H
#, $p<0.05$; compared with that of GTS+U-50,488H
U-50,488H:○-○; □
GTS+U-50,488H: *-* ; ▨
GTS+5-HIP 30 mg/kg+U-50,488H:△-△; ▨
GTS+5-HIP 100 mg/kg+U-50,488H:▲-▲; ■

observed by the similar increase in ginseng saponins treated mice[8]. The present results show that ginseng saponins inhibited the reduction of hepatic glutathione levels by daily injection of morphine for 6 days.

7. Inhibition of morphine 6-dehydrogenase which catalyzes the production of morphinone from morphine

The inhibitory effects of GTS, PD and PT on guinea pig liver morphine 6-dehydrogenase were investigated. Morphine 6-dehydrogenase was prepared and assayed by Yamano's method[9]. PT functioned as effective inhibitor. We found that PT was a little more effective than naloxone. The enzyme was inhibited about 50% by 0.01% PT (corresponding) to approximately 0.125 mM based on an aveaverage M.W. 800) at the physiological conditions (pH 7.4).

Morphine 6-dehydrogenase catalyzes the production of morphinone from morphine. A part of the produced morphinone is non-enzymatically conjugated with non protein-SH at the opioid receptor

to produce tolerance and the rest goes to form the morphinone-glutathione conjugate for detoxication and excretion. Ginseng saponins increased the production of hepatic non protein-SH lebels, for the formation of morphinone-glutathione conjugate and its excretion while it inhibited the activity of morphine 6-dehydrogenase.

This result suggests that the dual action of the above plays an important role in the inhibition of the development of morphine tolerance and dependence.

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