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Effects of Ginseng Total Saponin on Stress-Induced Analgesia

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Abstract□This study was undertaken to determine the effects of ginseng total saponin (GTS) on stress-induced analgesia (SIA) in mice. Intermittent foot shock (FS)-SIA was antagonized not by GTS but by naloxone in the tail flick FS-SIA which was not antagonized by naloxone in the T.F. test. On the other hand, GTS did not antagonize the continuous FS-SIA naloxone antagonized in the T.P. test. Also GTS antagonized psychological (PSY)-SIA which was not antagonized by naloxone in the T.F. test. However, GTS did not antagonize the PSY-SIA which naloxone antagonized in the T.P. test. Forced swimming (FSW)-SIA was not affected by both GTS and naloxone. These results suggest that the antagonisms of intermittent FS-SIA in the T.F. test, continuous FS-SIA and PSY-SIA by GTS are mediated by non-opioid mechanisms but the antagonism of intermittent FS-SIA in the T.P. test by GTS is mediated by opioid mechanism.

Key words□Stress-induced analgesia, ginseng total saponin, naloxone, antagonism, opioid, non-opioid.

Introduction

It is well established that various stressful procedures such as foot shock (FS),[1-3] immobilized water immersion (IW),[3,4] forced swimming (FSW)[4,5] and psycological (PSY) stress,[6] induce the analgesic effects in experimental animals. Some investigators have reported that stress-induced analgesia (SIA) is mediated by endogenous opioid such as endorphin, since adrenocorticotrophic hormon (ACTH) and β-endorphin are concomitantly released following stress exposure.[7,8] On the other hand, Anne *et al.* reported that SIA was mediated by neurotransmitters including noradrenaline, dopamine and serotonin in brain.[9,10]

In recent reports, it has been suggested that opioid and non-opioid forms, as defined by susceptibility to naloxone blockage or cross tolerance to morphine, are involved in the mechanisms of the production of SIA.[11,12] Kaneto *et al.* reported that endogenous opioid system is more closely associated with the mechanism of FS-SIA than IW-SIA. Non-opioid mechanism is more closely concerned in IW-SIA than in FS-SIA.[2,3] In addition, FSW-SIA is not antagonized by naloxone.

Kim *et al.* reported that GTS antagonized morphine-induced analgesia, suggesting that catecholaminergic or serotonergic mechanism was involved.[13-15]

The present study was undertaken to investigate the characteristics of the anatagonism of SIA by GTS, especially non-opioid mechanism or opioid mechanism, by comparing the results obtained from different types of the antagonisms of SIA by GTS and naloxone.

Materials and Methods

1. Animals and drugs

Male ICR mice, weighing 18~22 g, were used for all experiments. They were housed 10~15 mice in a cage. They were kept in a room temperature of 22± 2℃ and given free access to tap water and normal laboratory diet.

GTS (a gift from Korea Ginseng and Tobacco Research Institute) was dissolved in saline just prior to the experiments and administered to mice

intraperitoneally (i.p.) as a dose of 100 mg/kg. Naloxone-HCl (Sigma) was used 10 min prior to initiating stress exposure as a dose of 2 mg/kg, i.p..

2. Methods

2.1 Producing SIA

2.1.1 FS-SIA

(1) Intermittent FS-SIA

Mice were individually placed on the grids floor of inescapable operant chamber [10(D)×10(W)×30 (H) cm] divided into 9 compartments and were received unsignaled foot shock (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval) for 30 min by a scrambled electric shock generator.[1,2]

(2) Continuous FS-SIA

As in the intermittent FS stress, mice were placed on the grids floor of inescapable operant chamber [10(D)×10(W)×30(H) cm] divided into 9 compartments and were received unsignaled foot shock (2 mA, 0.2 Hz) for 3 min by a scrambled electric shock generator.

2.1.2 PSY-SIA

The chamber [32(W)×32(D)×32(H) cm] with a grid floor composed of 1.5 cm stainless steel rods placed 0.7 cm apart from each other was divided into 9 compartments [10(D)×10(W)×30(H) cm] with transparent plastic walls.

Mice were individually placed in 9 compartments. Four mice were imposed on scrambled electric shock (2 mA, 0.2 Hz) through the grids floor for 30 min as in the intermittent FS stress. Plastic plates were placed on the grids floor of 5 compartments to prevent mice in these compartments receving FS. Five mice, protected from electric shock by plastic plates, were exposed to PSY stress, seeing the responses of shocked mice, including struggling, vocalizing, defecating, urinating, and jumping (Fig. 1).

2.1.3 FSW-SIA

Mice were forced to swim in water bath [15(D)× 20(W)×20(H) cm, temp. 20± 1℃] for 3 min.

2.2 Measurement of analgesic effects

The stress induced analgesic effects were measured by a modified Haffner's method (mechanical analgesic test), a tail pinch (T.P.) test,[16] and a modified D'Amour and Smith method (thermal analgesic test), a tail flick (T.F.) test.[17]

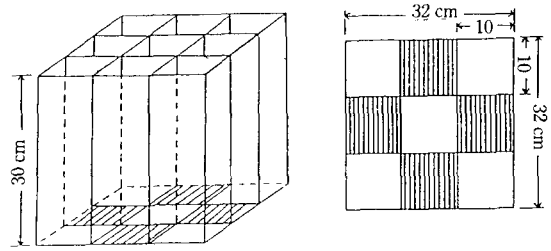

Fig. 1. Communication box to expopse animal to psycological stress. Mice which were received FS stress (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval for 30 min) were placed individually into 4 compartments (shade area). Mice which were received PSY stress were placed into 5 compartments (blank area) which were prevented from electric shock by plastic plates. Electric FS was delivered through the grids floor of shade area.

GTS 100 mg/kg was administed i.p. 3 hr in the T.P. test or 4 hr in the T.F. test prior to the exposure to each stress. The effects of GTS on each SIA were measured immediately after the termination of stress exposure every 5 min for 15 min.

Naloxone 2 mg/kg, was injected i.p. 10 min prior to the exposure to the stress. The effect of naloxone was measured immediately after the termination of stress every 5 min for 15 min in the both T.P. and T.F. tests.

Cut-off time was adopted 6 sec in the T.P. test and 10 sec in the T.F. test respectively, to avoid tail damage. The base lines of T.P. responses in different groups were around 1.0± 0.1 sec, while those of T.F. responses were 2.0± 0.2 sec.

The effects of analgesia were calculated as an area under the curve (A.U.C.) that was obtained by plotting the analgesic response time on the ordinate and the time intervals (min) on the abscissa, and were expressed as a percent of the analgesic effect obtained in control animals treated with saline.

The effects of analgesia were expressed as a percent of the analgesic effect obtained in control animals treated with saline.

3. Statistics

The data were expressed as mean± S.E. The differences in different groups were analyzed by Student's t-test.

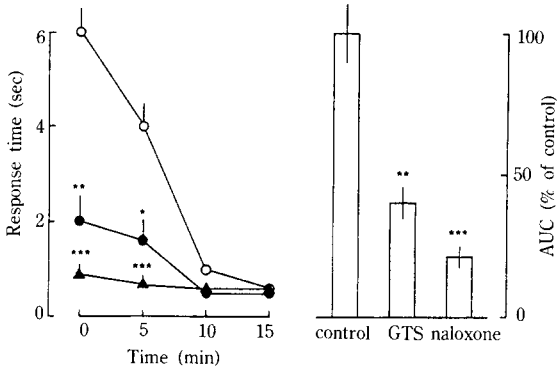

Fig. 2. Effects of GTS and naloxone on intermittent FS-SIA in the T.P. test. Mice were exposed to electric shock (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval) delivered through grids floor for 30 min. GTS 100 mg/kg was injected i.p. 3 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min priotr to the exposure to stress. The analgesia was measured by T.P. test.
○–○; control. ●–●; GTS 100 mg/kg, ▲–▲; naloxone 2 mg/kg.
*p<0.05, **p<0.01, ***p<0.001; compared with that of control.

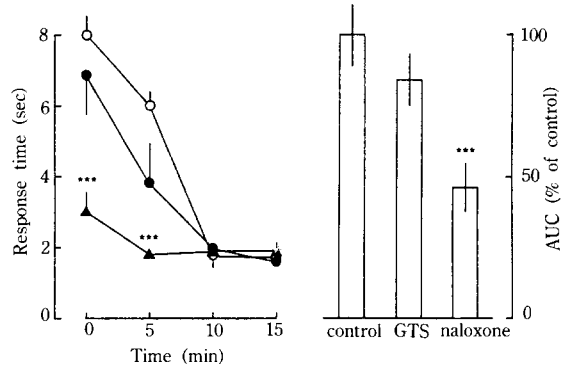

Fig. 3. Effects of GTS and naloxone on intermittent FS-SIA in the T.F. test. Mice were exposed to electric shock (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval) delivered through grids floor for 30 min. GTS 100 mg/kg was injected i.p. 4 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to the exposure to stress. The analgesia was measured by T.F. test.
○–○; control, ●–●; GTS 100 mg/kg, ▲–▲; naloxone 2 mg/kg.
***p<0.001; compared with that of control.

Results

1. Effects of GTS and naloxone on intermittent FS-SIA

The exposure to intermittent FS stress (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval for 30 min) caused short lasting potent analgesia in the T.P. and T.F. tests. The intensity of the analgesia was maximal immediately after the termination of stress exposure. At 15 min after termination of stress, the responses returned to pre-exposure state in the T. P. (Fig. 2) and in the T.F. tests (Fig. 3).

GTS (100 mg/kg, i.p.) showed different effects on intermittent FS-SIA in the T.P. test and in the T.F. tests. Intermittent FS-SIA was decreased by GTS much more in the T.P. test than that in the T.F. test. The scores calculated as percent of A.U.C. were 60 percent in the T.P. test (Fig. 2) and 10 percent in the T.F. test (Fig. 3), compared with that of control.

Naloxone (2 mg/kg, i.p.) significantly antagonized the intermittent FS-SIA in the T.P. (Fig. 2) and in the T.F. tests (Fig. 3). By AUC, naloxone antagonized intermittent FS-SIA about 80 percent in the T.P. test and 55 percent in the T.F. test, compared with that of control (Fig. 2, 3).

2. Effects of GTS and naloxone on continuous FS-SIA

As shown in Fig. 4 and 5, the exposure to continuous FS (0.2 mA, 0.2 Hz for 3 min) produced short lasting analgesia in mice. However continuous FS-SIA was lasting shoter than intermittent FS-SIA. The analgesia was maximal just after the termination of stress exposure and after 5 min, the continuous FS-SIA disappeared.

GTS (100 mg/kg, i.p.) antagonized continuous FS-SIA in the T.F. test. Pretreatment with GTS 100 mg/kg i.p. decreased the continuous FS-SIA more in T.F. test than in T.P. test (Fig. 4, 5).

By pretreatment with naloxone (2 mg/kg, i.p.), continuous FS-SIA was antagonized up to 25 percent of the analgesia of control in the T.P. test (Fig. 4). But no significant influence was observed in the T.F. test (Fig. 5).

3. Effects of GTS and naloxone on PSY-SIA

The psycological stress caused potent short lasting analgesia in the T.F. test. However, PSY-SIA in the T.P. test was less potent than that of T.F.

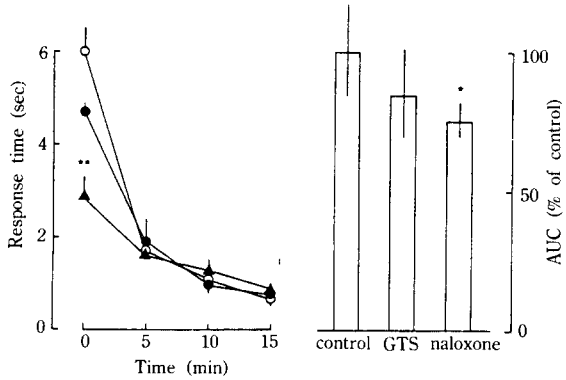

Fig. 4. Effects of GTS and naloxone on continuous FS-SIA in the T.P. test. Mice were placed in operant chamber and exposed to continuous FS stress (2 mA, 0.2 Hz, for 3 min) delivered through grids floor. GTS 100 mg/kg was injected i.p. 3 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to the the exposure to stress. The analgesic effects were measured by T.P. test.
○—○; control, ●—●; GTS 100 mg/kg, ▲—▲; naloxone 2 mg/kg.
*p<0.05, **p<0.01; compared with that of control.

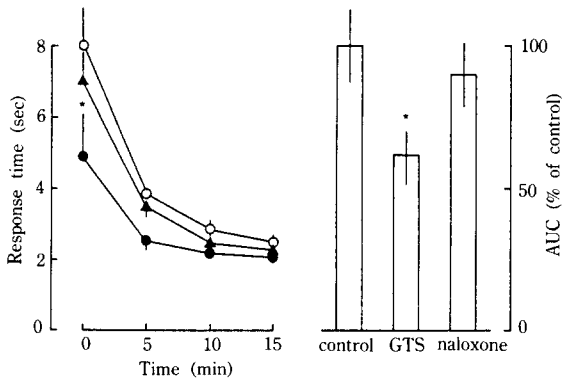

Fig. 5. Effects of GTS and naloxone on continuous FS-SIA in the T.F. test. Mice were placed in operant chamber and exposed to continuous FS stress (2 mA, 0.2 Hz, for 3 min) delivered through grids floor. GTS 100 mg/kg was injected i.p. 4 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to exposure to stress. The analgesic effects were measured by T.F. test.
○—○; control, ●—●; GTS 100 mg/kg, ▲—▲; naloxone 2 mg/kg.
*p<0.05; compared with that of control.

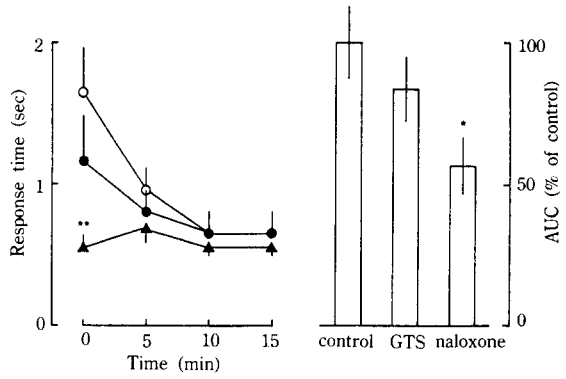

Fig. 6. Effects of GTS and naloxone on PSY-SIA in the T.P. test. Mice which were received FS stress (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval for 30 min) were individually placed into 4 compartments and mice which were received PSY-stress were placed into 5 compartments which were prevented from electric shock by plastic plates. Electric foot shock was delivered through the grids floor. Mice placed on the plastic plates were exposed to PSY-stress. GTS 100 mg/kg was injected i.p. 3 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to the exposure to PSY stress. The analgesia was measured by T.P. test.
○—○; control, ●—●; GTS 100 mg/kg, ▲—▲; naloxone 2 mg/kg.
*p<0.05, **p<0.01; compared with that of control.

test. GTS (100 mg/kg, i.p.) significantly antagonized the PSY-SIA in the T.F. test, compared with that of control as shown in Fig. 6 and 7.

Naloxone 2 mg/kg i.p. antagonized PSY-SIA in T. P. test (Fig. 6). However naloxone 2 mg/kg i.p. did not antagonize PSY-SIA in T.F. test (Fig. 7).

4. Effects of GTS and naloxone on FSW-SIA

FSW stress (immerged in 20℃ water for 3 min) showed short lasting analgesic effect in both T.P. and T.F. tests (Fig. 8, 9). As shown in Fig. 14 and 15, GTS (100 mg/kg, i.p.) has no significant effect on FWS-SIA in both tests.

Naloxone (2 mg/kg, i.p.) did not antagonize FSW-SIA in both T.P. and T.F. tests (Fig. 8, 9).

Discussion

It is well known that stress exposure produces

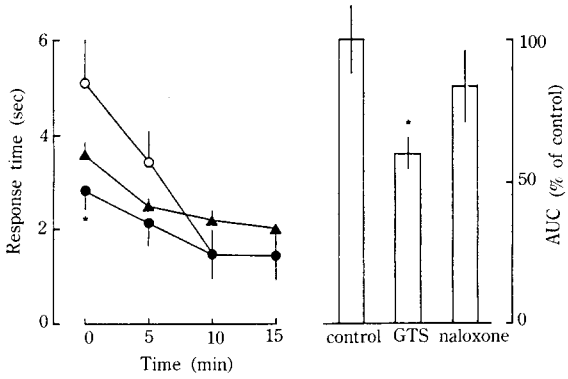

Fig. 7. Effects of GTS and naloxone on PSY-SIA in the T.F. test. Mice which were received FS stress (2 mA, 0.2 Hz, 1 sec duration, 5 sec interval for 30 min) were individually placed into 4 compartments and mice which were received PSY-stress were placed into 5 compartments which were prevented by plastic plates. Electric foot shock was delivered through the grids floor. Mice placed on the plastic plates were exposed to PSY-stress. GTS 100 mg/kg was injected i.p. 4 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to the exposure to PSY stress. The analgesia was measured by T.F. test.
○–○; control, ●–●; GTS 100 mg/kg, ▲–▲; naloxone 2 mg/kg.
*$p<0.05$; compared with that of control.

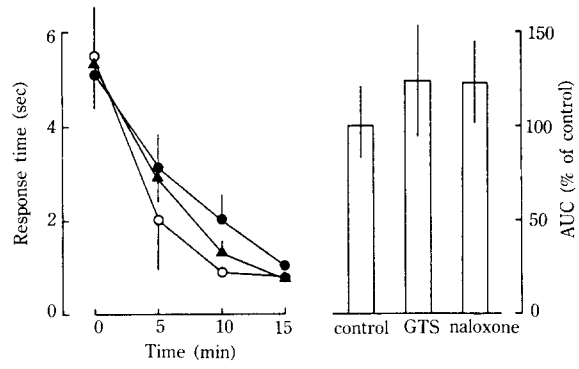

Fig. 8. Effects of GTS and naloxone on FSW-SIA in the T.P. test. Mice were exposed to FSW stress for 3 min. GTS 100 mg/kg was injected i.p. 3 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to the exposure to stress. The analgesia was measured by T.P. test.
○–○; control, ●–●; GTS 100 mg/kg, ▲–▲; naloxone 2 mg/kg.

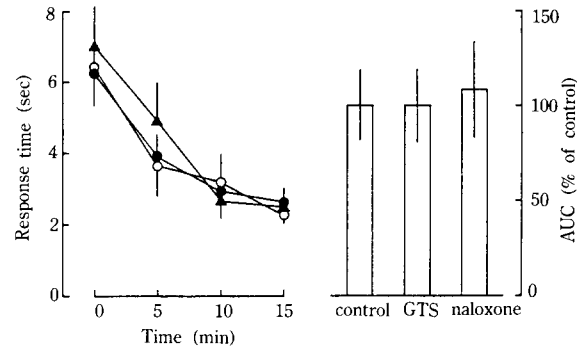

Fig. 9. Effects of GTS and naloxone on FSW-SIA in the T.F. test. Mice were exposed to FSW stress for 3 min. GTS 100 mg/kg was injected i.p. 4 hr prior to and naloxone 2 mg/kg was injected i.p. 10 min prior to the exposure to stress. The analgesia was measured by T.F. test.
○–○; control, ●–●; GTS 100 mg/kg, ▲–▲; naloxone 2 mg/kg.

analgesia in rodents. Previous investigations of the effects of various shock stress revealed that two mechanisms, opioid which is blocked by naloxone and non-opioid which is not blocked by naloxone, are involved in SIA.[18,19]

In the present study, GTS antagonized intermittent FS-SIA in the T.P. test but not in the T.F. test. Naloxone antagonized intermittent FS-SIA in both T.P. and T.F. test. Intermittent FS-SIA is mainly mediated by opioid mechanism in both T.P. and T.F. tests, whereas continuous FS is mediated by non-opioid mechanism in the T.F. test, and opioid in the T.P. test because continuous FS-SIA is antagonized by GTS in the T.F. test, but not by naloxone. It has been reported that morphine tolerant rats displayed cross tolerance to only the naloxone sensitive rats in intermittent FS-SIA.[18] Naloxone antagonized the PSY-SIA in the T.P. test, but not in the T.F. test. Therefore, these results suggest that the antagonisms of intermittent FS-SIA in the T.F. test, continuous FS-SIA and PSY-SIA by GTS are mediated, by non-opioid mechanisms, but the antagonism of intermittent FS-SIA in the T.F. test by GTS is mediated by opioid mechanism.

Lal *et al.* reported that FWS-SIA was not antagonized by naloxone.[5] In accordance with their experiments, the antagonism of naloxone was not observed in these experiments, and GTS did not antago-

nize FWS-SIA. Takahashi *et al.* reported that reserpine, depletor of catecholamines in the CNS, completely antagonized FWS-SIA.[4] There is a lack of antagonism of GTS in the FSW-SIA. Therefore, it can be postulated that FWS-SIA is not dependent on opiate but catacholamines.

It was reported that GTS antagonized the morphine-induced analgesia and inhibited the development of analgesic tolerance to morphine.[14,15] This antagonism and inhibition of the development of tolerance to morphine by GTS are predominently mediated by serotonergic system.[20] In addition, the electrically evoked contraction of guinea pig ileum (GPI) and mouse vas defernes (MVD) are inhibited by morphine or opioid peptides and the inhibition of contraction was reversed by naloxone. GTS inhibited the contraction of GPI and this inhibition was not reversed by naloxone. GTS inhibited the electrically evoked contraction of MVD, and this inhibition was not reversed by naloxone.[21] These results suggest that GTS inhibited the contraction of GPI and MVD through non opioid mechanism. It has been shown that hypophysectomy c · block SIA and that stress causes a rise in plasma ACTH and β-endorphin levels. It is possible that SIA is mediated in part by endogenous opioid in brain.

In terms of non-opioid mechanism, it has been reported that some stresses enhance noradrenaline turnover rate in the hypothalamus and amygdala.[8,9] Pretreatment with reserpine almost completely antagonized FS-SIA and IW-SIA.[1-3] It has shown that catacholamines or serotonin play an important role in the SIA.[22,23] Quinpazine, a serotoin agonist, increased SIA, and BC-105, a seretonin antagonist, antagonized SIA in the T.F. test. Pretreatment with p-chlorophenylalanine, a specific depletor of brain serotonin, decreased SIA. Morphine-induced analgesia, hyperthermia and catalepsy were antagonized by ginseng extract. They suggested that the antagonism of pharmacological effects of morphine by ginseng extract are mediated by non-opioid mechanism.[24] On the other hand, ginseng extract decresed serotonin levels in the brainstem and cerebral cortex.[25] Accordingly, these results suggest that the antagonism of SIA by GTS are mainly mediated by non-opioid mechanisms related to serotonin in brain.

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