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Effect of Ginseng Total Saponin on the Development of Psychic and Physical Dependence on Nalbuphine

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Abstract—This study was undertaken to estimate whether nalbuphine, a mixed agonist/antagonist opioid analgesic, produced psychic dependence. Moreover, the physical dependence liability of nalbuphine was compared with that of morphine after 7 days administrations of the drugs in mice and rats, and the effects of ginseng total saponin (GTS) on the development of physical dependence on nalbuphine were also studied. Nalbuphine did not produce psychic dependence. However, various abstinence signs precipitated by naloxone were observed in nalbuphine-dependent mice and rats. As the nature of the dependence syndrome produced by nalbuphine 30 mg/kg under these conditions seems similar to that induced by morphine 10 mg/kg, it is clear that nalbuphine possesses the substantial abuse potential. Therefore, nalbuphine may be needed to initiate more stringent controls for the prevention of nalbuphine abuse. On the other hand, GTS inhibited the development of physical dependence on nalbuphine and reduced the contents of dopamine and its metabolite in the brains of mice. Accordingly, results of this study suggest that the inhibitory effects of GTS on the development of physical dependence on nalbuphine may involve dopaminergic mechanism. GTS may be useful for the therapy of physical dependence on nalbuphine.

Keywords □ nalbuphine, psychic and physical dependence, ginseng total saponin (GTS), striatum, dopamine, DOPAC.

Nalbuphine HCl [(-)-17 (cyclobutylmethyl)-4,5 α-epoxy-morphinan-3,6 α,14-triol hydrochloride], a mixed opioid agonist/antagonist, was introduced as a potent analgesic for moderate pain to severe pain (Beever and Feise, 1978; Miller, 1980). The dependence liability of nalbuphine was considered to be low when used within the therapeutic dose range (Jasinski *et al.*, 1970; Jasinski and Mansky 1972). However, chronic administration of nalbuphine produces physical dependence which resembles that produced by pentazocine, since it has elements of both morphine and nalorphine dependence (Jasinski and Mansky 1972; Schmidt *et al.*, 1985). It is also estimated that nalbuphine has an abuse potential which approximates that of pentazocine. Cases of the abuse potential of nalbuphine have been reported as a popular substance for abuse (Hoover, 1985; McGarity, 1984). Nevertheless, unlike pentazocine and butorphanol, nalbuphune is not controlled under the Controlled Substance Act. Although it is thought this drug is needed to initiate more stringent controls, yet little information is available concerning psychic and physical dependence on nalbuphine.

Central catecholaminergic neurons are known to be involved in the development of tolerance to and physical dependence on opiates (Herz *et al.*, 1974). There is substantial evidence suggesting that central dopaminergic neurons play a key role in the mechanism of opioid withdrawal as well as reinforcement. In particular, precipitation of withdrawals in opioid-dependent rats by administration of opioid antagonist such as naloxone causes an elevation in the dopamine level and an increase in turnover of dopamine in the brain of mice and rats (Iwamoto *et al.*, 1973). Some neuropharmacological investigations suggested an involvement of the mesolimbic and mesocortical dopaminergic system in the neuronal mechanism mediating reinforcement (Kalivas *et al.*, 1988).

Ginseng extract has a variety of effects on the activity of the central nervous system. Ginsenosides isolated from ginseng saponin exhibited a sedative effect and inhibited the uptake of neurotransmitters such as norepinephrine and dopamine (Tsang *et al.*, 1985). In-

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terestingly, Ramarao and Bhargava (1990) reported that morphine-induced analgesia, hyperthermia and catalepsy were antagonized by ginseng extract showing anti-narcotic effects. Furthermore, withdrawals induced by morphine were suppressed by pretreatment with ginseng extract or ginseng saponin (Kim *et al.*, 1987; Kim *et al.*, 1990).

Therefore, the psychic and physical dependence liabilities on nalbuphine were re-evaluated in this experiment because of the abuse potential of nalbuphine. In addition, these experiments were undertaken to estimate the effects of ginseng total saponin on the development of physical dependence on nalbuphine in the mouse.

Materials and Methods

Materials

Male Sprague-Dawley rats weighing 251~300 g and male ICR mice weighing 20~25 g in groups of 10~20 were used. They were kept in a room with an ambient temperature of 21± 2℃ and a 12−12 hours light-dark cycle for a week before the experiment. Nalbuphine hydrochloride was donated by Je-Il Pharm. Co.. Morphine hydrochloride and naloxone hydrochloride were purchased by Dae-Won Pharm. Co. and by Sigma Co., respectively. Ginseng total saponin (GTS, characterized saponin mixture quantitatively containing at least 11 ginsenosides from Panax ginseng, extracted and purified by Namba *et al.*'s methods) was a gift from the Korea Ginseng and Tobacco Research Institute. GTS (100 mg/kg or 200 mg/kg) was dissolved in saline and administered to rats and mice (0.1 m*l*/10 g) intraperitoneally (i.p.). Nalbuphine (10 mg/kg or 30 mg/kg) or morphine (10 mg/kg) was administered subcutaneously (s.c.).

Measurement of psychic dependence

The conditioned place preference (CPP) apparatus made by modified Mucha *et al.*'s method (1982) was used for the development of psychic dependence on nalbuphine. It consisted of two square-base plexiglass compartments (15×15×15 cm), one with a white and the other with a black plastic box joined by a gray tunnel (3×3×7.5 cm) which could be closed by guillotine doors. To provide tactile difference between compartments, the white compartment had a rough floor and the black compartment had a smooth floor. Removal of the guillotine doors during the pre-testing and the final testing phase allowing animals free access to all two compartments were recorded for 15 min using an infrared detector connected to a computer.

The control mice received i.p. injection of saline immediately before exposure to the white or black compartment. Nalbuphine dissolved in saline was given immediately before the mice were placed in the white compartment.

Phase I (Pre-testing phase) : On day 1, the mice were pre-exposed to the test apparatus for 5 min. The guillotine doors were raised and each animal was allowed to move freely between the two compartments. On day 2, the time spent by the mouse in each of the two compartments was recorded for 15 min.

Phase II (Conditioning phase) : On days 3, 5 and 7, the mice were injected with nalbuphine before being confined in the white compartment, the non-preferred side, for 40 min. On days 4, 6 and 8, the mice were injected with saline before being confined in the black compartment, the preferred side, for 40 min.

Phase III (Testing phase) : On day 9, the guillotine doors were raised. The mice were placed in the tunnel of the central parts and the time spent by the mice in the two compartments was recorded for 15 min. These scores were calculated by changes of the testing phase and the pre-testing phase in the white compartment.

Measurement of physical dependence

Nalbuphine (10 or 30 mg/kg) or morphine (10 mg/kg) was administered s.c. to mice or rats once a day for 7 days. GTS (100 or 200 mg/kg) was administered i.p. once a day for 7 days, 2 hours prior to nalbuphine. On day 8 after 7 days of administration, rats or mice were weighed and placed in one gallon mayonnaise jar. A piece of filter paper was placed on the bottom of the jar to aid visualization of the withdrawal-associated period. Rats or mice were challenged with naloxone 1 mg/kg i.p.. Naloxone-precipitated withdrawal signs were observed for 30 min on a quantal basis (i.e., the number of animals exhibiting more than 2 episodes of teeth chattering, rearing, wet shakes, forepaw tremors and licking penis; and the number of animals showing a single episode of jumping, yawning, ptosis, and diarrhea). Body weight was measured before and 1 hour after the administration of the opioid antagonist. In addition, the total number of abstinence signs (jumping and forepaw tremors) was recorded for a 30-min period to assess the severity of these signs (Oh *et al.*, 1992).

Determination of dopamine and 3,4-dihydroxyphenyl acetic acid (DOPAC) contents in the mouse striatum

On day 8 after repeated administration of GTS (100 or 200 mg/kg) for 7 days, the subjects were sacrificed

and brains were removed and dissected as follows. The brain was placed on a cold block on its dorsal surface, and an initial coronal slice was taken directly anterior to the hypothalamus. The striatum was then removed from the caudal surface of this slice, based on its distinct morphological appearance. The striatum included tissue dorsal to the anterior commissure, ventral to the corpus callosum and medial to the external capsule (Heffner *et al.*, 1980).

The tissues were stored in liquid nitrogen until the time of assay. The tissue was then homogenized in 10 volumes of 0.4 N perchloric acid. The samples were centrifuged at 14,000×g for 20 min at 4℃. The supernatant was assayed on a Bioanalytic Systems (West Lafayette, IN) liquid chromatography system equipped with a C-18 reverse phase (Biophase ODS 5 mm) column. The LC4A amperometric detector was set at a range of 100 nA with a +0.72 V potential between the glassy carbon electrode and the Ag-AgCl reference electrode. The sample was delivered via a high-pressure (Rheodyne) valve fitted with a 20-μl sample loop. The mobile phase consisted of 0.1 M citric acid, 0.2 M sodium phosphate dibasic and methanol (10%). All of the sample was assayed for dopamine and DOPAC contents. Qualification was accomplished by comparison with external standards (Jakubovic *et al.*, 1987).

Statistics

Data were expressed as mean± S.E. The significances of the changes obtained in the CPP test and dopamine contents were also evaluated by two-tailed Student's t-test and ANOVA. In addition, quantal (all or none) data from the behavioral studies on the experimental groups and saline controls were compared by the Chi-square test (number of animals exhibiting each abstinence sign) and Student's t-test (mean± SE of total number of occurrences of each abstinence sign) for the study of physical dependence.

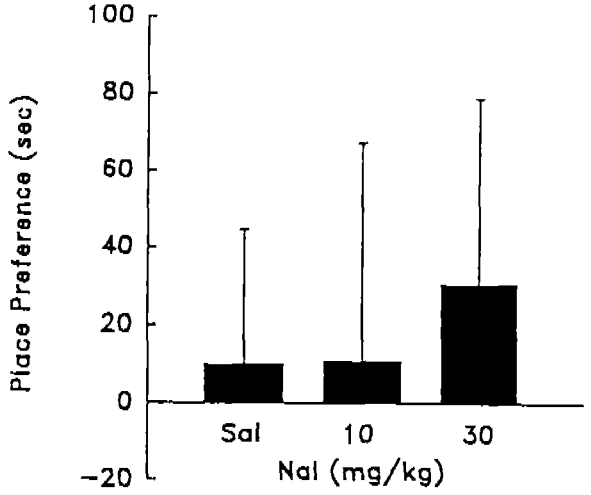

Fig. 1. Nalbuphine (Nal)-induced conditioned place preference (CPP).
Mice were injected with saline or nalbuphine (10 or 30 mg/kg) before being confined in the black or white compartment for 40 min. The scores were calculated by changes of the testing phase (15 min) and the pre-testing phase (15 min) in the white compartment. Vertical bars represent the S.E.

Results

Psychic dependence on Nalbuphine

Nalbuphine (10 or 30 mg/kg) was administered to mice. These doses of nalbuphine were not shown to produce conditioned place preference in this experiment (Fig. 1). This result is in agreement with a former report that nalbuphine does not show any conditioned place preference (McGarity, 1984).

Table I. Naloxone-precipitated abstinence signs in morphine- or nalbuphine-dependent rats

Abstinence signs	Saline	Morphine (10 mg/kg)	Nalbuphine (10 mg/kg)	Nalbuphine (30 mg/kg)
Jumping	0/10[a]	2/10	0/10	1/10
Teeth chattering	1/10	9/10*	2/10	5/10
Weet shakes	10/10	10/10	10/10	10/10
Licking penis	1/10	9/10*	6/10	5/10
Weight loss (3%>)	0/10	8/10*	4/10	4/10
Yawning	0/10	0/10	1/10	0/10
Ptosis	1/10	5/10	2/10	6/10
Forepaw tremors	1/10	9/10*	7/10*	7/10*
Diarrhea	0/10	3/10	2/10	2/10

[a] Numerator values are number of animals displaying this behavior; denominator values are the total number of animals per group.
* $P<0.05$, (Values are significantly different from the control values as determined by the Chi-test.)

Table II. Naloxone-precipitated abstinence signs in morphine- or nalbuphine-dependent mice

Abstinence signs	Saline	Morphine (10 mg/kg)	Nalbuphine (10 mg/kg)	Nalbuphine (30 mg/kg)
Jumping	0/10	5/10	2/8	7/18
Teeth chattering	0/10	5/10*	0/8	0/18
Wet shakes	0/10	10/10	2/8	6/18
Rearing	10/10	10/10	8/8	18/18
Licking penis	1/10	5/10*	2/8	6/18
Weight loss (3%>)	0/10	8/10*	4/8	4/18
Yawning	0/10	0/10	1/8	0/18
Ptosis	0/10	4/10	2/8	8/18
Forepaw tremors	1/10	10/10*	8/8*	17/18*
Diarrhea	0/10	3/10	0/8	0/18

* $P<0.05$, (Values are significantly different from the control values as determined by the Chi-test.)

Table III. Effects of GTS on naloxone-precipitated abstinence signs in nalbuphine-dependent mice

Abstinence signs	Treatment with GTS (mg/kg)		
	0	100	200
Jumping	7/18 (15± 3.1)[a]	5/15 (10.2± 2.7)	2/8 (6.5± 2.2)*
Teeth chattering	0/18	0/15	0/8
Weet shakes	6/18	6/15	2/8
Rearing	10/18	10/15	6/8
Licking penis	6/18	3/15	2/8
Weight loss (3%>)	2/18	0/15	0/8
Yawning	0/18	0/15	0/8
Ptosis	8/18	4/15	2/8
Forepaw tremors	17/18 (8.9± 2.5)[a]	9/15 (4.1± 1.2)*	3/8# (3.5± 1.0)*
Diarrhea	0/18	0/15	0/8

(Values are significantly lower than the control values as determined by the Chi-test.)
[a] Values are mean± SE of the total number of occurrences of rearing.
* P<0.05, (Values are significantly lower than the control values as determined by the Student's t-test.)

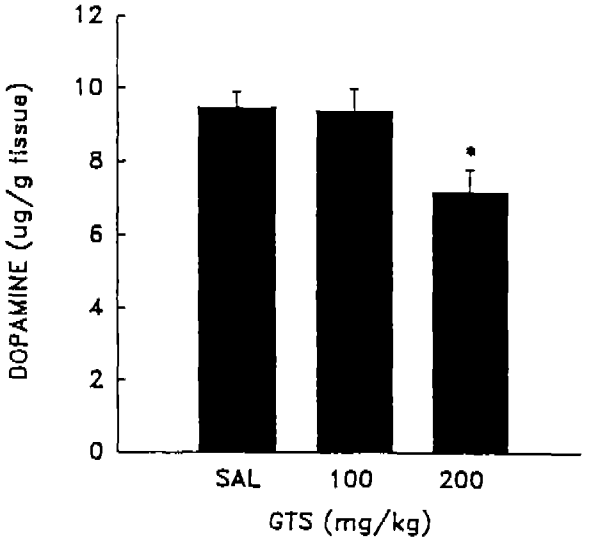

Fig. 2. Striatal dopamine contents after GTS treatment. GTS (100 or 200 mg/kg) was administered once a day for 7 days. On day 8 after repeated administration of GTS, mice were sacrificed and brains were removed and dissected. *P<0.05, (Values are significantly different from saline-treated controls as determined by Student's t-test). Vertical bars represent the S.E.

Inhibitory effects of GTS on nalbuphine physical dependence

In the nalbuphine-dependent rats or mice, various abstinence signs precipitated by naloxone were observed. The symptoms observed after naloxone were qualitatively similar to those in morphine treated rats and mice (Table I, Table II).

On the other hand, GTS reduced naloxone-precipitated forepaw tremors on a quantal basis (all or none, by Chi-test) in the nalbuphine dependent mice. The incidences of nalbuphine abstinence signs (jumping and forepaw tremors) were significantly reduced by GTS (Table III).

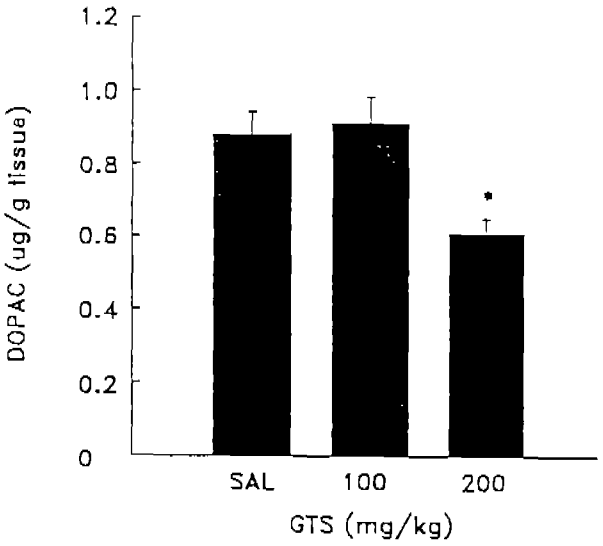

Fig. 3. Striatal DOPAC contents after GTS treatment. GTS (100 or 200 mg/kg) was administered once a day for 7 days. On day 8 after repeated administration of GTS, mice were sacrificed and brains were removed and dissected. *P<0.05, (Values are significantly different from saline-treated controls as determined by Student's t-test). Vertical bars represent the S.E.

Effects of GTS on striatal concentrations of dopamine and DOPAC

After 7 days administration, GTS 100 mg/kg did not reduce any striatal contents of dopamine but GTS 200 mg/kg reduced in comparison with saline (Fig. 2). Furthermore, DOPAC contents were reduced by GTS 200 mg/kg (Fig. 3).

Discussion

To date, there have been numerous reports demonstrating a conditioned place preference with morphine or heroin. Other opioid agonists, etorphine and levorphanol also produced place preferences (Hoffman, 1989). The results obtained with kappa agonists appeared to be more complicated. U-50, 488H consistently showed an aversion. Other kappa agonists have been shown to produce conditioned place preferences slightly (Mucha and Herz, 1985). Nalbuphine virtually did not produce psychomimetic adverse effects, even at 9 times the therapeutic analgesic dose (Peachey 1987). In agreement with a former report, nalbuphine did not show any conditioned place preference in this ex-

periment.

Pentazocine was reported to possess a lower abuse potential than that of morphine (Jasinski *et al.*, 1970) and nalbuphine to possess a low abuse potential similar to that of pentazocine (Jasinski and Mansky, 1972). Steinfels *et al.* (1982) suggested that the abuse potentials of the mixed agonist-antagonists analgesics including pentazocine, nalbuphine and butorphanol, were analogous to those of rats with a history of morphine addiction. Schmidt *et al.* (1985) suggest that nalbuphine can be expected to precipitate severe withdrawals in opioid-dependent subjects. A primary dependence study reported that a severe physical dependence syndrome developed in man in which large doses of nalbuphine were administered for a prolonged period of time (Preston *et al.*, 1989). These studies show that a substantial degree of physical dependence can be developed in mice or rats which receive large doses of this compound by s.c. administration for 7 days. Furthermore, the nature of the dependence syndrome produced by nalbuphine 30 mg/kg under these conditions seems similar to that induced by morphine 10 mg/kg. Under these conditions, nalbuphine was also shown to have a substantial physical dependence liability. In the present study, there were no overt differences in terms of behavioral signs in the withdrawal syndromes induced by naloxone in morphine- or nalbuphine-repeated animals.

In addition, body weight loss was also determined for mice and rats throughout the duration of these administration. Morphine administration consistently reduced body weight during the administration period. However, this effect was not observed in nalbuphine-treated animals (data not shown).

Clearly, there had been very few cases of abuse with the antagonist analgesics reported until 1987 (Peachey 1987). However, mixed agonist/antagonist analgesics (pentazocine, butorphanol and nalbuphine) abuse has been gradually increased in the USA after 1987 and has been also increased in Korea among teenagers since 1990. Nevertheless, unlike pentazocine and butorphanol, nalbuphune is not controlled under the Controlled Substance Act. Accordingly, nalbuphine may be needed to initiate more stringent controls.

Nalbuphine is a synthetic morphian with structural characteristics similar to the mixed opioid agonist/antagonist, butorphanol. In vivo nalbuphine acts as an agonist or an antagonist at both mu and kappa opioid receptors depending upon the assay and the parameters employed. Because of mu agonist actions of nalbuphine (Walker and Young, 1993), naloxone precipitates withdrawal in nalbuphine dependent animals and in morphine-dependent animals. It is suggested that nalbuphine-induced withdrawals are mediated by mu opioid receptors, because nalbuphine showed morphine like-withdrawals that might be mediated by the mu agonist.

Kim *et al.*, (1987; 1990) reported that ginseng inhibited the development of physical dependence on morphine. It is suggested that these effects might involve the modulation of noradrenergic and dopaminergic systems. However, the mechanisms have remained unclear. Much work has been in an attempt to define the mechanisms involved in the development of tolerance and physical dependence to narcotic analgesics. Several neurotransmitters, including serotonin, norepinephrine, dopamine and acetylcholine have been implicated in the production of physical dependence upon these drugs (Takemori, 1974, Ary *et al.*, 1977). Guinutsos *et al.* (1974) have suggested that dopamine plays an important role in the withdrawals. Especially, the expression of abstinence signs may be partially due to an increase of the dopamine level in the brain. GTS inhibited the development of physical dependence on nalbuphine and reduced the contents of dopamine and DOPAC in the striatum of mouse. Accordingly, the results of this study suggest that the inhibitory effects of GTS on the development of physical dependence on nalbuphine may involve dopaminergic mechanisms. On the other hand, GTS inhibited the tyrosine hydroxylase activity using PC12 cell (unpublished data). The above results can provide an evidence that GTS may reduce contents of the dopamine ant it metabolites. Finally, GTS may also be useful for the therapy of physical dependence on nalbuphine.

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