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# Effects of Ginsenosides Injected Intrathecally or Intracerebroventricularly on Antinociception Induced by D-Pen$^{2,5}$-enkephalin Administered Intracerebroventricularly in the Mouse

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**Abstract** : The effect of total saponin fraction of Ginseng injected intrathecally (i.t.) or intracerebroventricularly (i.c.v.) on the antinociception induced by D-Pen$^{2,5}$-enkephalin (DPDPE) administered i.c.v. was studied in ICR mice in the present study. The antinociception was assessed by the tail-flick test. Total saponin fraction at doses 0.1 to 1.0 μg, which administered i.t. alone did not affect the latencies of tail-flick threshold, attenuated dose-dependently the inhibition of the tail-flick response induced by i.c.v. administered DPDPE (10 μg). However, total saponin fraction at doses 1 to 20 μg, which administered i.c.v. alone did not affect the latencies of the tail-flick response, did not affect i.c.v. administered DPDPE (10 μg)-induced antinociception. The duration of antagonistic action of total saponin fraction against DPDPE-induced antinociception was lasted at least for 6 hrs. Various doses of ginsenosides Rd, but not $Rb_2$, Rc, $Rg_1$, and $Rb_1$ and Re, injected i.t. dose-dependently attenuated antinociception induced by DPDPE administered i.c.v. Our results indicate that total saponin fraction injected spinally appears to have antagonistic action against the antinociception induced by supraspinally applied DPDPE. Ginsenoside Rd appears to be responsible for blocking i.c.v. administered DPDPE-induced antinociception. On the other hand, total ginseng fraction, at supraspinal sites, may not have an antagonistic action against the antinociception induced by DPDPE.

**Key words** : Total saponin fraction, ginsenosides, D-Pen$^{2,5}$-enkephalin, antinociception.

## Introduction

It has been documented that the antinociceptive effects induced by supraspinally applied morphine and D-Pen$^{2,5}$-enkephalin (DPDPE) are mediated by the stimulation of μ- and δ-opioid receptors, respectively. This contention is supported by the findings that β-funaltrexamine (μ-opioid receptor antagonist) selectively antagonizes the antinociception induced by morphine, but not DPDPE.[1] Additionally, ICI174,864 (δ-opioid receptor antagonist) reduces DPDPE-, but not morphine-, induced antinociception.[2] Furthermore, one intracerebroventricular (i.c.v.) injection of morphine or DPDPE induces acute tolerance to its own distinctive opioid receptor and does not induce cross-tolerance to another opioid agonists with different opioid receptor specificities.[3]

The action of total saponin fraction in the production of antinociception induced by opioids such as morphine and pentazocin, or stress have been previously studied. The systemic administration of total saponin fraction attenuates the antinociceptive effects induced by morphine and pentazocin administered systemically.[4,5] Furthermore, total saponin fraction reduces stress-induced antinociception.[6] Recently, we have reported that total saponin fraction injected intrathecally (i.t.), but not i.c.v., effectively at-

tenuates the antinociception induced by morphine administered supraspinally.[7] However, the effect of total saponin fraction on DPDPE-induced antinociception has not yet been characterized. Furthermore, the responsible ginsenosides of the total saponin fraction for antagonistic action against DPDPE-induced antinociception has not also been characterized. The present study was then designed to characterize the role of total saponin fraction and several ginsenosides injected i.c.v. or i.t. in the regulation of antinociception induced by i.c.v. administered DPDPE.

## Materials and Methods

### 1. Experimental animals

Male ICR mice weighing 23~25 g were used for all the experiments. The animals were housed 5 per cage in a room maintained at 22±0.5℃ with an alternating 12h light-dark cycle. Food and water were available ad libitum. Animals were used only once.

### 2. Assessment of antinociception

Antinociception was determined by the tail-flick test.[8] For the measurement of the latency of the tail-flick response, mice were gently held with one hand with the tail positioned in the apparatus (EMDIE Instrument Co., Maidens, VA., USA, Model TF[6]) for radiant heat stimulation. The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of heat stimulus in the tail-flick test was adjusted so that the animal flicked its tail after 3 to 5 s. The inhibition of the tail-flick response was expressed as "percent maximal possible effect (%MPE)" which was calculated as $[(T_1-T_0)/(T_2-T_0)]\times 100$, where $T_0$ and $T_1$ were the tail-flick latencies before and after the injection of opioid agonist and $T_2$ was the cutoff time, which was set at 10 s for the tail-flick test.

### 3. Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection

Intracerebroventricular injections were made according to the procedure of Haley and McCormick[9] and i.t. injections followed the method described by Hylden and Wilcox[10] using a 25-μl Hamilton syringe with a 30 gauge needle. I.c.v. and i.t. injection volumes were 5 μl and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space or in the spinal cord. The dye injected i.c.v. was found to be distributed through the ventricular spaces and some dye reached the ventral surface of the brain and upper cervical portion of the spinal cord. The dye injected i.t. was distributed over a short distance (about 1 cm) both rostrally and caudally and no dye was found in the brain. The success rate for the injections was consistently found to be over 95%, before the experiments were done.

### 4. Experimental protocol

In the first group of experiments, mice were pretreated i.t. with either saline or various doses (from 0.1 to 1 μg) of total saponin fraction, $Rb_1$, $Rb_2$, Rc, Rd, Re, and $Rg_1$ 30 min prior to i.c.v. administration of a fixed dose of DPDPE (10 μg). In the second group of experiments, mice were pretreated i.c.v. with either saline or various doses (from 1 to 20 μg) of total saponin fraction 30 min prior to i.c.v. administration of a fixed dose of DPDPE (10 μg). In the third group of experiments, mice were pretreated i.c.v. or i.t. with either saline or total saponin fraction 0.5, 1, 2, 3, 4, or 6 hrs prior to i.c.v. administration of a fixed dose of DPDPE (10 μg). In the time-course studies, the tail-flick response was tested 10, 20, 30, 45 and 60 min after i.c.v. injection of DPDPE. In the dose dependence studies, the tail-flick response was tested 20 min after i.c.v. injection of morphine. This time chosen was based on the preliminary time-course studies that inhibition of the tail-flick response reached a maximum after the injection of DPDPE.

### 5. Statistics

The data were presented as the mean±S.E.M. Statistical analysis was carried out by one-way analysis of variance (ANOVA) with post-hoc test.

p Values of less than 0.05 were considered to indicate statistical significance.

**6. Drugs**

DPDPE was purchased from Peninsula Laboratory (Belmont, Calif., USA). Total saponin fraction, $Rb_1$, $Rb_2$, Rc, Rd, Re, and $Rg_1$ were obtained from Korea Ginseng and Tobacco Research Institute (Taejeon, Korea). All drugs used for injection were dissolved in sterile saline (0.9% NaCl solution).

## Results

**1. Effects of total saponin fraction injected i.t. and i.c.v. on the inhibition of the tail-flick responses induced by DPDPE given i.c.v.**

Group of mice were pretreated i.t. or i.c.v. with 5 μl of saline or various doses of total saponin fraction 30 min before i.c.v. administration of DPDPE. As shown in Fig. 1, DPDPE (10 μg) injected i.c.v. alone caused a profound inhibition of the tail-flick response. The inhibition of the tail-flick responses induced by DPDPE was attenuated dose-dependently by total saponin fraction (0.1~1 μg) injected i.t. (Fig. 1). Total saponin fraction at 0.1 to 1 μg given i.t. alone did not have any significant effect on the latencies of the tail-flick response (Fig. 1b). On the other hand, the same doses of total saponin fraction injected i.c.v. did not affect the inhibition of the tail-flick response induced by DPDPE administered i.c.v. (data not shown). Furthermore, even higher doses (1 to 20 μg) of total saponin fraction injected i.c.v. did not alter the inhibition of the tail-flick response induced by DPDPE administered i.c.v. (Figs. 2a and b).

To examine the duration of antagonistic action of total saponin fraction against antinociception induced by supraspinally administered DPDPE, the time of pretreatment with total saponin fraction was varied. As shown in Fig. 3a, total saponin fraction (1 μg) pretreated i.t. up to 6 hr significantly attenuated the inhibition of the tail-flick response induced by DPDPE administered i.c.v. However, total saponin fraction (20 μg) pretreated i.c.v. up to 6 hr did not affect the inhibition of the tail-flick response induced by DPDPE administered i.c.v. (Fig. 3b).

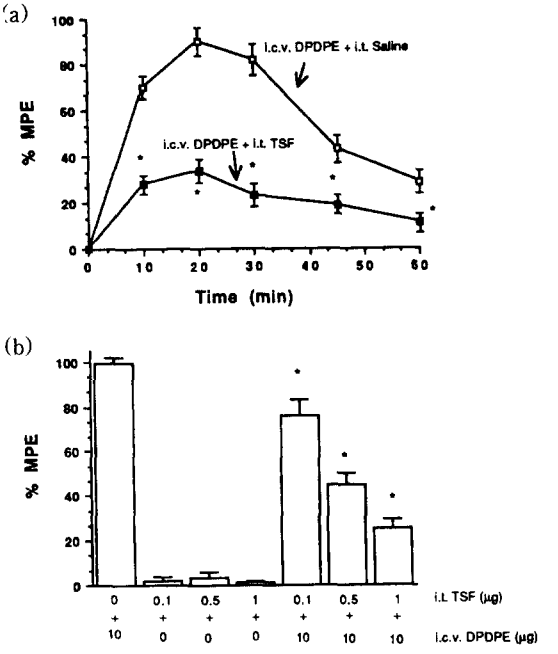


**Fig. 1.** Time-course (a) and dose-dependent (b) experiments examining the effect of total saponin fraction (TSF) injected intrathecally (i.t.) on inhibition of the tail-flick response induced by DPDPE administered intracerebroventricularly (i.c.v.). (a) Mice were pretreated i.t. with either saline (5 μl) or total saponin fraction (1 μg) 30 min before i.c.v. administration of DPDPE (10 μg). The tail-flick response was measured at 10, 20, 30, 45, and 60 min after DPDPE injection. (b) Various doses (0.1, 0.5, and 1 μg) of total saponin fraction was pretreated i.t. 30 min before i.c.v. administration of DPDPE (10 μg) and the tail-flick response was measured at 30 min after DPDPE injection. The vertical bars denote the standard error of the mean. The number of animal used for each group was 8-10. *, $p<0.05$ compared to the group of mice treated with saline plus DPDPE.

**2. Effects of ginsenosides injected i.t. on the inhibition of the tail-flick responses induced by DPDPE given i.c.v.**

Group of mice were pretreated i.t. with 5 μl of saline or various doses of ginsenosides (0.1~1 μg) 30 min before i.c.v. administration of morphine.

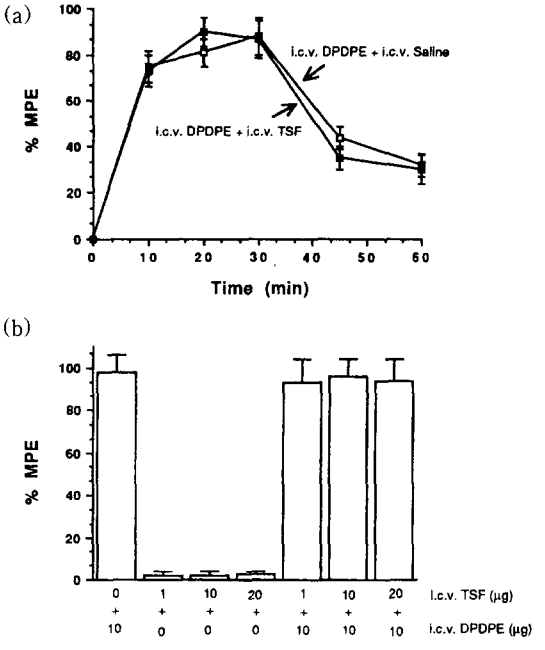


**Fig. 2.** Time-course (a) and dose-dependent (b) experiments examining the effect of total saponin fraction (TSF) injected intratcerebroventricularly (i.c.v.) on inhibition of the tail-flick response induced by DPDPE administered i.c.v. (a) Mice were pretreated i.c.v. with either saline (5 μl) or total saponin fraction (20 μg) 30 min before i.c.v. administration of DPDPE (10 μg). The tail-flick response was measured at 10, 20, 30, 45, and 60 min after DPDPE injection. (b) Various doses (1, 10, and 20 μg) of total saponin fraction was pretreated i.c.v. 30 min before i.c.v. administration of DPDPE (10 μg) and the tail-flick response was measured at 30 min after DPDPE injection. The vertical bars denote the standard error of the mean.

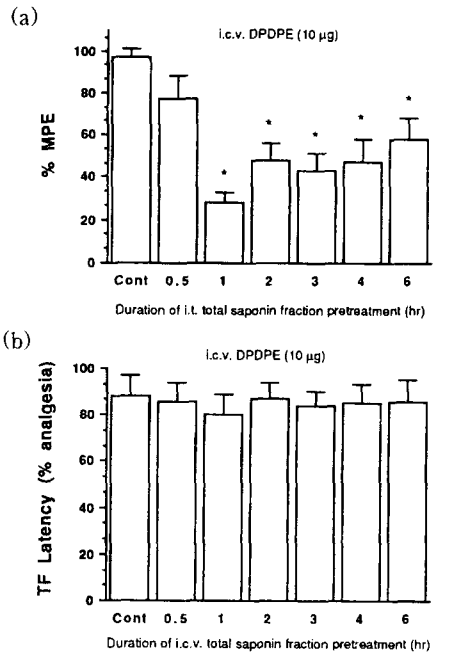


**Fig. 3.** Time dependence of effect of total saponin fraction (TSF) injected intrathecally (i.t.) and intracerebroventricularly (i.c.v.) on inhibition of the tail-flick response induced by DPDPE administered i.c.v. Mice were pretreated i.t. (a) or i.c.v. (b) with either saline (5 μl) or total saponin fraction (1 μg) 0.5, 1, 2, 3, 4, or 6 hrs before i.c.v. administration of DPDPE (10 μg). The tail-flick response was measured at 30 min after DPDPE injection. The vertical bars denote the standard error of the mean. The number of animal used for each group was 8-10. *, $p<0.05$ compared to the group of mice treated with saline plus DPDPE.

As shown in Fig. 4, DPDPE (10 μg) injected i.c.v. alone caused a profound inhibition of the tail-flick response. The inhibition of the tail-flick responses induced by DPDPE was attenuated dose-dependently by ginsenosides Rd injected i.t. (Fig. 4b). However, ginsenosides $Rb_1$, $Rb_2$, Rc, $Rg_1$, and Re did not affect the inhibition of the tail-flick responses induced by DPDPE (data not shown). Ginsenosides $Rb_1$, $Rb_2$, Rc, Rd, $Rg_1$ and Re at 0.1 to 1 μg given i.t. alone did not have any significant effect on the latencies of the tail-flick response (Fig. 4 and data not shown).

## Discussion

To determine the action of total saponin fraction against DPDPE-induced antinociception, the effect of total saponin fraction injected i.t. or i.c.v. on inhibition of the tail-flick response induced by DPDPE was examined in the present study. We found that i.t. injection of total saponin fraction dose-dependently attenuated inhibition of the tail-flick response induced by DPDPE administered i.c.v. Our results suggest that total saponin fraction injected spinally, but not supraspinally, reduces the antinociception induced by

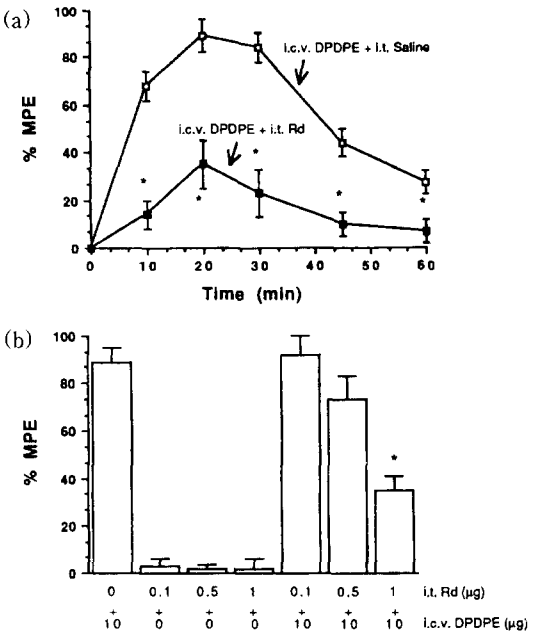


**Fig. 4.** Time-course (a) and dose-dependent (b) experiments examining the effect of $R_d$ injected intrathecally (i.t.) on inhibition of the tail-flick response induced by DPDPE administered intracerebroventricularly (i.c.v.). (a) Mice were pretreated i.t. with either saline (5 μl) or Rd (1 μg) 30 min before i.c.v. administration of DPDPE (10 μg). The tail-flick response was measured at 10, 20, 30, 45, and 60 min after DPDPE injection. (b) Various doses (0.1, 0.5, and 1 μg) of $R_d$ was pretreated i.t. 30 min before i.c.v. administration of DPDPE (10 μg) and the tail-flick response was measured at 30 min after DPDPE injection. The vertical bars denote the standard error of the mean. The number of animal used for each group was 8-10. *, $p < 0.05$ compared to the group of mice treated with saline plus DPDPE.

DPDPE administered supraspinally. However, the effect of total saponin fraction injected i.t. on antinociception induced by DPDPE administered spinally were not characterized.

The pretreatment with total saponin fraction at the dose of 1 μg for 0.5, 1, 2, 3, 4, and 6 hrs effectively attenuated i.c.v. administered DPDPE-induced inhibition of the tail-flick response, suggesting that the antagonistic action of total saponin fraction against DPDPE-induced antinociception was lasted at least for 6 hrs. Our results were in part in line with the previous findings that the systemic or spinal pretreatment ginseng total saponin for 4 or 6 hrs attenuated systemically or supraspinally injected morphine-induced antinociception.[5,7]

To find ginsenosides responsible for antagonism of total saponin fraction against morphine-induced antinociception, the effects of various kinds of ginsenosides ($Rb_1$, $Rb_2$, Rc, Rd, Re, and $Rg_1$) on inhibition of the tail-flick response induced by morphine administered i.c.v. were examined. Among several ginsenosides, only Rd effectively attenuated the inhibition of the tail-flick response induced by DPDPE administered i.c.v. We found in previous study that Rd injected i.t. also effectively antagonizes the antinociception induced by morphine administered i.c.v.[7] The antagonistic effect of Rd was equally potent with that of total saponin fraction. Although the reasons for this finding is not currently clear, some ginsenosides in total saponin fraction may have opposing effects for the regulation of antinociception.

We and others have previously hypothesized that the antinociception induced by morphine and DPDPE given supraspinally is mediated by the stimulation of μ- and δ-opioid receptors, respectively, and the activation of descending noradrenergic or serotonergic pathway and subsequently stimulation of noradrenergic or serotonergic receptors in the spinal cord for the production of antinociception.[11,15] The results of the present study raise the possibility that total saponin fraction or ginsenosides such as Rd may modulate supraspinally administered DPDPE-induced antinociception by several actions. First, total saponin fraction and ginsenoside, Rd may modulate, presynaptically, the release of neurotransmitter from descending neurons activated by DPDPE administered supraspinally. Second, total saponin fraction and ginsenoside, Rd may modulate, postsynaptically, the actions of neurotransmitters released by descending neurons activated by DPDPE administered supraspinally. To examine this hypothesis, the effects of total saponin fraction or ginsenosides on antinociception induced by

serotonin injected i.t. should be assessed. Third, total saponin fraction and ginsenoside, Rd may modulate the antinociception induced by DPDPE administered supraspinally via nitric oxide pathway. Xu and Tseng[16] recently reported that the activation of nitric oxide system in the spinal cord by either L-arginine or 3-morpholino-sydnonimine attenuates antinociception induced by opioid administered i.c.v. Since ginseng exerts the pharmacological action by promoting nitric oxide release in some vascular tissues[17,18], the role of nitric oxide system in the spinal cord on the antagonistic action of total saponin fraction or ginsenoside against DPDPE-induced antinociception should be further investigated.

The present studies with total saponin fraction or several types of ginsenosides were carried out at the doses that total saponin fraction and ginsenosides injected i.t. or i.c.v. alone did not affect baseline pain sensitivity in the tail-flick test. Thus, total saponin fraction or ginsenosides may modulate the production of antinociception when the descending system is activated by DPDPE administered supraspinally.

## 요 약

척수강 혹은 척수상부로 투여된 total saponin fraction의 척수상부로 투여한 D-Pen$^{2,5}$-enkephalin (DPDPE)에 의한 진통작용에 대한 영향을 검색하여 보았다. 척수강으로 투여한 total saponin fraction은 효과적으로 DPDPE의 진통작용을 유의하게 억제시켰으나 DPDPE의 진통작용은 척수상부로 투여한 total saponin fraction에 의하여 억제되지 않음을 관찰하였다. Total saponin fraction의 DPDPE의 진통작용에 대한 억제 작용은 적어도 6시간동안 계속됨을 발견하였다. 더 나아가 여러 가지 ginsenosides중 척수강으로 투여한 Rd가 효과적으로 척수상부로 투여한 DPDPE의 진통작용을 억제함을 관찰하였다. 이와 같은 결과는 척수로 흡수된 total saponin fraction이나 Rd는 척수상부로 투여된 DPDPE의 진통작용을 효과적으로 억제하는 작용이 있음을 시사한다.

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