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# A Non-opioid Mechanism in the Inhibitory Effect of Ginseng Saponins on Electrically Evoked Contractions of Guinea-Pig Ileum and Mouse vas Deferens

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Both ginseng total saponins (GTS) and one of its constituents, protopanaxatriol saponins (PT), inhibited the electrically evoked contractions of guinea-pig ileum (GPI) in a concentration dependent manner in a range of 1—100 $\mu$g/ml, and this effect was irreversible at high concentrations of the saponins. Protopanaxadiol saponins (PD) had a transient and weak effect. On the other hand, in mouse vas deferens (MVD), the contractions were increased by PT and PD, however, GTS was almost without effect. The inhibitory effect of morphine was arithmetically increased by pretreatment with 100 $\mu$g/ml of these saponins in GPI preparations, while the inhibitory effect of the contractions was potentiated in MVD preparations. Neither the inhibition of contractions in the GPI preparation nor the facilitation of contractions in the MVD preparation by these ginseng saponins was reversed by 1 $\mu$M naloxone, in contrast to naloxone antagonism of morphine-induced contractions in both preparations. GTS and PT caused a dose-dependent inhibition of $BaCl_2$-induced contraction of GPI. It is concluded that the mechanism on the inhibitory or facilitated effect of ginseng saponins on electrically evoked contractions in GPI and MVD preparations may be separated from the effect of opioids, and the mechanism may be based on the direct action of the saponins on smooth muscles preparations.

**Keywords** — ginseng total saponin (GTS); protopanaxatriol (PT); protopanaxadiol (PD); electrically induced contraction; barium chrolide-induced contraction; non-opioid mechanism; guinea-pig ileum (GPI); mouse vas deferens (MVD)

## Introduction

Ginseng root has been used medically for thousands of years in Korea, China and Japan as a folk medicine. Pharmacological studies of ginseng components have been reported by a number of investigators, and so far some investigations have revealed the analgesic effects of ginseng saponins.[1,2] Recently, Kim *et al.* [3] demonstrated the inhibitory effect on the development of morphine tolerance and dependence, in addition to the antagonism of morphine analgesia, using pure red ginseng saponins. These results suggest the interactions of the actions of ginseng saponins show an opioid mechanism. Furthermore, the findings of an increase in norepinephrine in rat brain regions after repeated treatment with the ginseng extracts[4] and, as for the peripheral actions of the ginseng, the influences on cholinergic mechanism have been recognized.[2,5] On the other hand, isolated guinea-pig ileum (GPI) and mouse vas deferens (MVD) have been used as *in vitro* models for the assessment of opioid actions, specifically concerning cholinergic and catecholaminergic mechanisms, respectively. In this context, studies of ginseng saponins on electrically evoked contractions of GPI and MVD preparations have been carried out.

## Materials and Methods

**Longitudinal Muscles of GPI and MVD** — Male guinea pigs weighing 300 to 350 g were sacrificed by a blow to the head and segments (about 4 cm) of the ileum 10 to 15 cm from the ileo-cecal valve were isolated. The longitudinal muscle with myenteric plexus (GPI) was prepared as described by Rang.[6] The strips were mounted in a 10 ml organ bath filled with modified Krebs Hensleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.1 mM $KH_2PO_4$, 2.4 mM $MgSO_4$, 25 mM $NaHCO_3$, 11 mM glucose) kept at 37 °C and aerated. On the

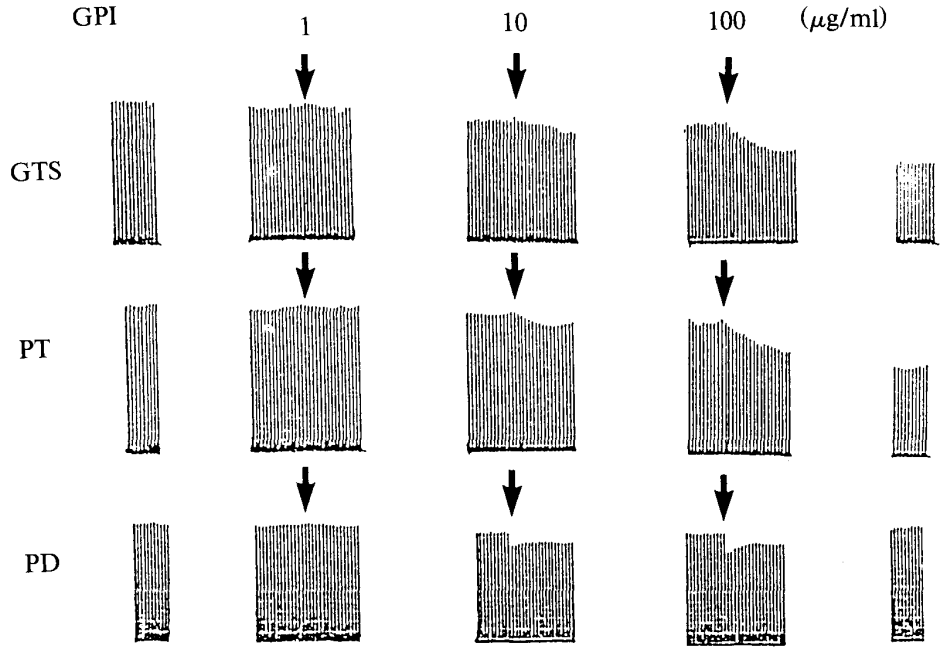


Fig. 1. Effect of Ginseng Saponins on Electrically Evoked Contractions of GPI Preparation
GTS, PT or PD was added at the arrow indicated. The values are the final concentrations of the compounds (μg/ml). For other details, see text.

other hand, MVD rapidly isolated from male dd mice weighing 30 to 35 g was mounted in a 10 ml organ bath filled with $Mg^{2+}$-free Krebs Henseleit solution kept at 37 °C.[7] These muscle strips were stimulated transmurally with square-wave electrical pulses of 80 V, 0.8 ms for GPI or 80 V, 1 ms for MVD duration at a frequency of 0.1 Hz through platinum ring electrodes. The contractions were recorded through an isotonic transducer.

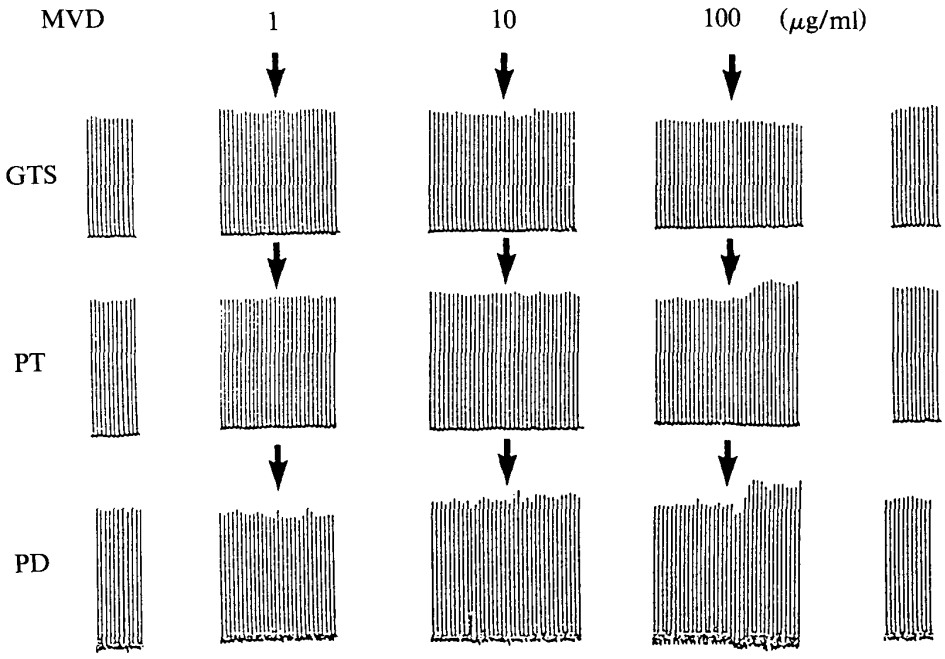


Fig. 2. Effect of Ginseng Saponins on Electrically Evoked Contractions of MVD Preparation
For abbreviations and other details, see Fig. 1 and text.

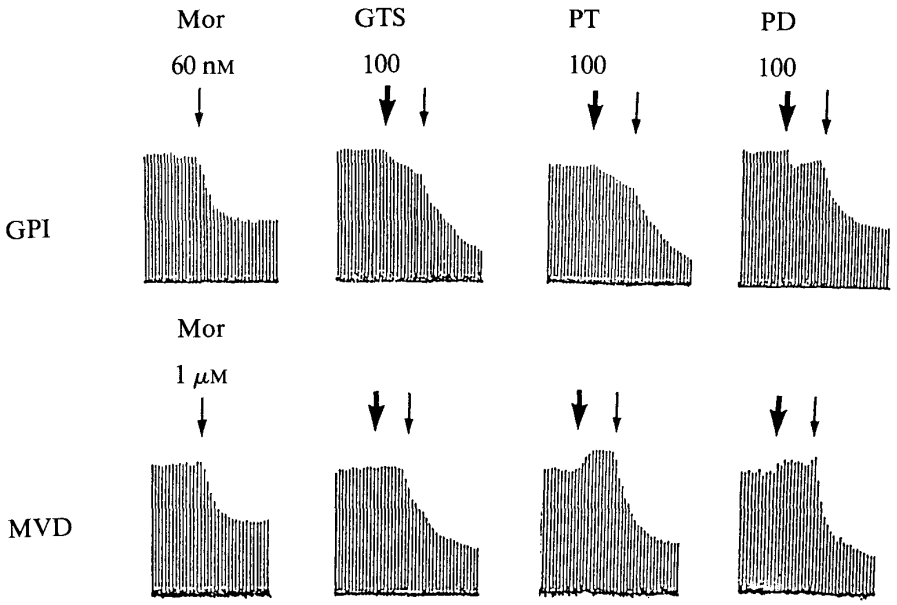


Fig. 3. Effect of Ginseng Saponins on Morphine-Induced Inhibition of the Contractions of GPI and MVD Preparations
Morphine (Mor) and ginseng saponins were added at the slim and bold arrows indicated, respectively. For abbreviations and other details, see Fig. 1 and text.

**Compounds** — Ginseng total saponins (GTS), protopanaxadiol saponins (PD) and protopanaxatriol saponins (PT) (Korean Ginseng & Tobacco Research Institute), morphine-HCl (Takeda), naloxone-HCl (Sankyo) and $BaCl_2$ (Nakarai) were dissolved in distilled water.

## Results

### Effect of Ginseng Saponins on Electrically In-

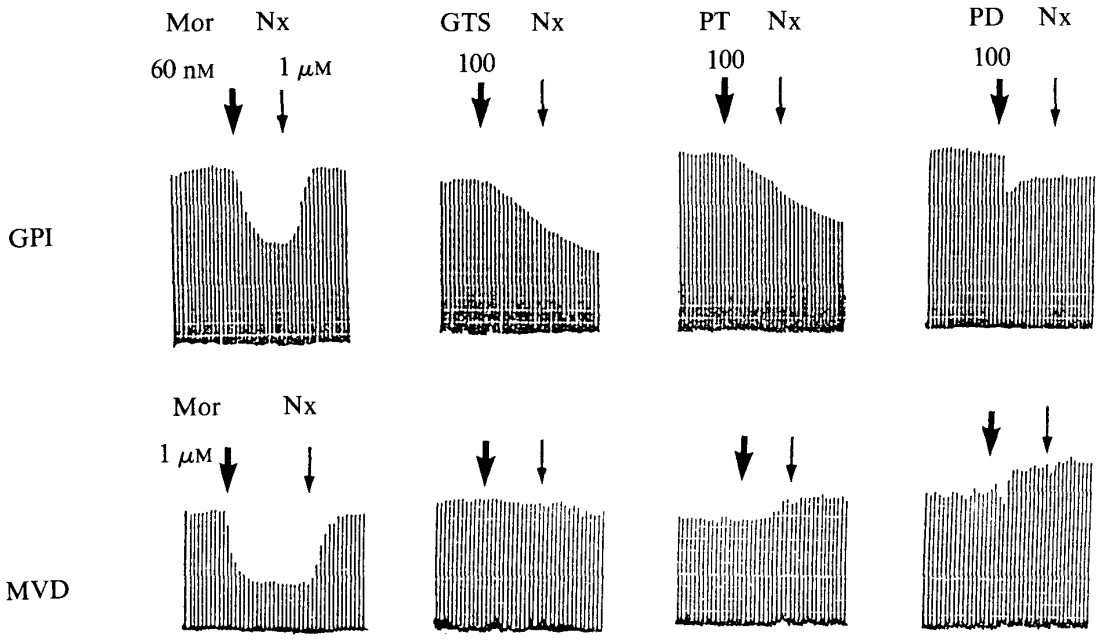


Fig. 4. No Antagonism of Naloxone on the Inhibition of or the Facilitation of the Contractions of GPI or MVD Preparation Induced by Ginseng Saponins
Morphine (Mor) or ginseng saponins were added at the bold arrow indicated, followed by naloxone (Nx) at the slim arrow. For abbreviations and other details, see Fig. 1 and text.

**duced Contractions of Longitudinal Muscle Strips of GPI**

Both GTS and PT inhibited electrically induced contractions of the GPI preparations in a concentration dependent fashion, and the contractions were reduced by 20—30% by these saponins at 100 $\mu$g/ml concentration. The effect of PD was transient and weak; about 10% inhibition was observed at the same concentration. At high concentrations of GTS and PT, the effect was irreversible and the contractions did not return to the control level even after intensive washings (Fig. 1).

**Effect of Ginseng Saponins on Electrically Evoked Contractions of MVD**

In contrast to the effect on GPI strips, the electrically induced contractions of MVD were increased in the presence of 100 $\mu$g/ml PT and PD. On the other hand, GTS, up to 100 $\mu$g/ml, was almost without effect on the contractions (Fig. 2).

**Effect of Ginseng Saponins on the Morphine-Induced Inhibition of Contractions of GPI and MVD Preparations**

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 $\mu$M for GPI and MVD, respectively. The inhibitory effect of morphine was arithmetically increased by pretreatment with 100 $\mu$g/ml of these saponins in the GPI preparations, while the effect was apparently potentiated in the MVD preparations (Fig. 3).

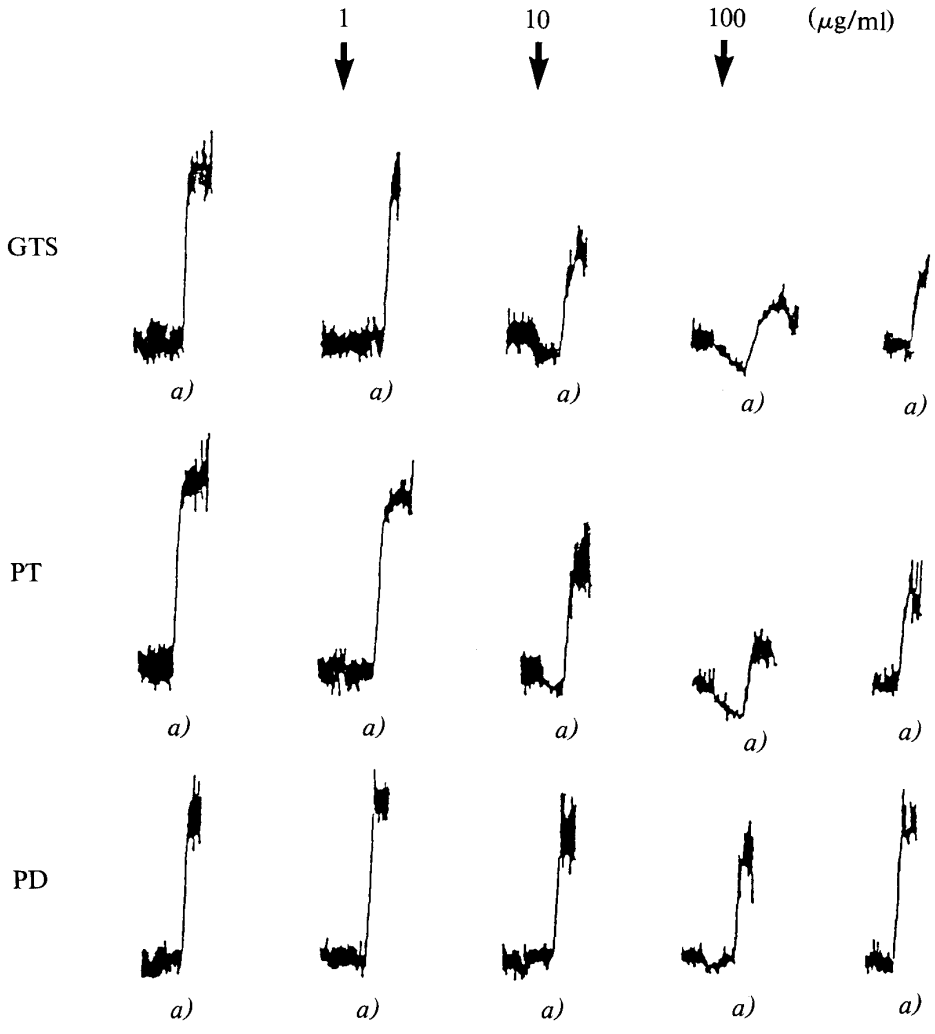


Fig. 5. Effect of Ginseng Saponins on the $BaCl_2$-Induced Contraction of GPI
*a)* Indicates the addition of $BaCl_2$ (0.1 mM). For abbreviations and other details, see Fig. 1 and text.

**Lack of Naloxone Antagonism to the Inhibitory Effect of Ginseng Saponins on the Contractions of GPI and MVD Preparations**

In both GPI and MVD preparations, 1 $\mu$M naloxone completely reversed morphine-induced inhibition of the contractions. However, neither the inhibition of the contractions by these ginseng saponins, 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 $\mu$M naloxone (Fig. 4).

**Effect of Ginseng Saponins on the $BaCl_2$-Induced Contraction of GPI Preparation**

GTS and PT inhibited the $BaCl_2$-induced contraction of GPI in a concentration dependent fashion and, in the preparations treated with high concentration of these saponins, the contraction induced by 0.1 mM $BaCl_2$ did not return to the normal level even after repeated washings of the tissues. On the other hand, the inhibitory effect of PD was much less than that of GTS and PT, and the effect disappeared after washing (Fig. 5).

## Discussion

It is well established that the electrically evoked contractions of GPI and MVD are inhibited by morphine or opioid peptides and the inhibitions reversed by naloxone, an opioid antagonist, in a dose dependent fashion. Hence, models are available for the characterization or assessment of opioid-like properties of the compounds to be tested.

The facts that ginseng components possess analgesic effects[1,2] and also affect the development of tolerance to morphine[3] stimulated our attention to investigate the effect of ginseng saponins on electrically induced contractions of GPI and MVD. GTS and PT but not PD inhibited the contractions of GPI in a concentration dependent manner. However, in the case of MVD preparation, the contractions were not affected by all components of ginseng saponins but rather facilitated by PT and PD. The inhibitory effects of ginseng saponins on the contractions of GPI were not reversed by naloxone, and naloxone was also without effect on the PT- and PD-induced facilitation of the contractions of MVD. These findings suggested that the inhibitory effect of ginseng saponins on the electrically induced contractions of GPI preparations and also their facilitated effect on the MVD may not be mediated through opioid receptors.

The inhibitory effect of morphine on the contractions was additive to the effect of ginseng saponins in the GPI preparation, while the effect of morphine was enhanced in spite of the facilitation of the contractions by ginseng saponins, in the MVD preparation. Furthermore, the inhibition of the contractions of GPI by a high concentration of GTS and PT, 100 $\mu$g/ml, was not removed even after repeated washings. The contraction induced by $BaCl_2$, which was produced by the actions on the smooth muscle directly or on the myenteric plexus in this preparation, was also inhibited in the presence of GTS and PT. Thus, ginseng saponins indirectly affected the opioid actions without mediating opioid receptors in these preparations.

Ginseng saponins suppressed the contractions of GPI but, in contrast, PT and PD facilitated the contractions of MVD. The discrepancies in the effect of ginseng saponins in GPI and MVD may be attributed to the difference in the mechanisms for electrically evoked contractions in both preparations, since the contractions of GPI preparations are mediated through the release of acetylcholine, while those of MVD through the release of norepinephrine. In fact, there have been a few reports showing that ginseng saponins extracts affect both catecholaminergic and cholinergic mechanisms; namely, they increased the norepinephrine content in the brain,[4] and also potentiated or, at higher concentration antagonized cholinergic mechanisms in the peripheral tissues.[5]

In conclusion, the inhibitory and facilitated effects of ginseng saponins on the electrically evoked contractions in GPI and MVD are not mediated through opioid receptors, but these effects can modify the inhibitory effect of morphine in these preparations.

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