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Distinctive Effect of Ginseng Saponins on Development of Morphine Tolerance in Guinea-Pig Ileum and Mouse vas Deferens

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Studies on the effect of ginseng saponins on the development of tolerance to morphine have been carried out using isolated preparations of guinea-pig ileum (GPI) and mouse vas deferens (MVD). Incubation of GPI preparation with morphine resulted in the development of tolerance to the inhibitory effect of morphine on the electrically evoked contractions. Ginseng total saponins and one of the constituents, protopanaxatriol saponin, suppressed the development of morphine tolerance in a concentration dependent manner in GPI preparation, though another constituent, protopanaxadiol saponin, did not affect the tolerance development substantially. In the MVD preparation, the development of tolerance to the morphine effect was observed as well, but none of the ginseng saponins affected it. It has been well established that electrically evoked contractions of GPI and MVD are mediated by acetylcholine and norepinephrine, respectively, and presumably their release is regulated presynaptically by opioid receptors. The fact that ginseng saponins suppressed the development of morphine tolerance only in the GPI preparation suggest that the inhibitory effect is mediated through an effect on the cholinergic system, without the involvement of direct action on opioid receptors.

Keywords — morphine; tolerance; ginseng saponin; guinea-pig ileum; mouse vas deferens

Introduction

Much attention has been paid to ginseng saponins by a number of investigators because of their multiple pharmacological actions. Recently, Kim *et al.* demonstrated that administration of ginseng saponins antagonized morphine analgesia and inhibited the development of morphine tolerance and dependence in mice.[1] Although there is no agreement on the mode of their actions on the development of morphine tolerance, there have been a few reports showing that ginseng saponin extracts affect both catecholaminergic and cholinergic mechanisms; namely, they increased the norepinephrine content in the brain,[2,3] and also potentiated, or at higher concentration antagonized, cholinergic mechanisms in the peripheral tissues.[4] These findings raise the possibility that the inhibitory effect of ginseng saponins on the development of morphine tolerance *in vivo* is attributable to an effect on the mechanisms mediated by neurotransmitters. On the other hand, isolated guinea-pig ileum (GPI) and mouse vas deferens (MVD) have been widely used as *in vitro* models for the assessment of acute and chronic opioid actions, since they have distinctive advantages, for example, they seem to be easily liable to develop morphine tolerance[5,6] and provide much simpler models for investigating the mechanism involved in the development of morphine tolerance. Furthermore, it is considered that electrically evoked contractions of GPI and MVD preparations are mediated by cholinergic[7] and adrenergic [8] mechanisms, respectively. Accordingly, it is of interest to use these isolated preparations to understand the mechanism by which the tolerance to morphine develops, considering the difference in neurotransmission mechanism of these preparations. The aims of present studies are to reproduce the inhibitory effect of ginseng saponins on the development of the morphine tolerance *in vivo* and to elucidate the possible role of neurotransmitters in the underlying mechanisms.

Materials and Methods

Preparation of Longitudinal Muscles of GPI and MVD — Male guinea-pigs weighing 300—350 g were sacrificed by a blow to the head and segments (about 4 cm) of ileum 10 to 15 cm

from the ileo-cecal valve were isolated. The longitudinal muscle with myenteric plexus was prepared as described by Paton.[7] MVD was isolated from male ddY mice weighing 30 to 35 g. Both preparations were mounted in a 10 ml organ bath filled with Krebs-Henseleit 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) for GPI or Mg^{2+} free Krebs-Henseleit solution for MVD, respectively, kept at 37 °C. 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, at a frequency of 0.1 Hz through platinum ring electrodes. The contractions were recorded through an isotonic transducer.

Development and Assessment of Morphine Tolerance — After 30 min equilibration, the tissues were left in contact with 10 μM morphine for 90 min at 37 °C. After incubation, the tissues were washed several times with Krebs-Henseleit solution at 10 min intervals until the electrically stimulated contractions returned to the preincubation level. Control preparations were incubated with vehicle or ginseng saponins.

Effect of Ginseng Saponins on Tolerance Development — In order to assess the effect of ginseng saponins on the development of morphine tolerance, ginseng saponins (1—10 μg/ml) were included in the incubating solution and the IC_{50} values of control (C) and morphine tolerant preparations (T) were determined; the tolerance ratio (T/C) was calculated.

Compounds — Ginseng total saponins (GTS), protopanaxadiol saponins (PD) and protopanaxatriol saponins (PT) (gifts from Prof. Kim), and morphine-HCl (Takeda) were dissolved in water before use.

Statistical Analysis — Statistical comparison between the results of different treatments was made by using Student's *t*-test.

Results

Incubation of GPI preparations with 10 μM morphine for 90 min resulted in the development of tolerance to the inhibitory effect of morphine (Fig. 1). In GPI preparations, the presence of 10 μg/ml GTS or PT with morphine suppressed the development of morphine tolerance completely or partially (Fig. 1). The sensitivity to morphine was unaffected by incubation with 10 μg/ml GTS or PT alone. As shown in Table I, the inhibitory effect of GTS and PT on the development of morphine tolerance was concentration-dependent.

Meanwhile, PD failed to suppress the tolerance development (Fig. 1). the ratio of IC_{50} (T/C) for 1 — 10 μg/ml PD, as in the case of GTS and PT, was decreased concentration-

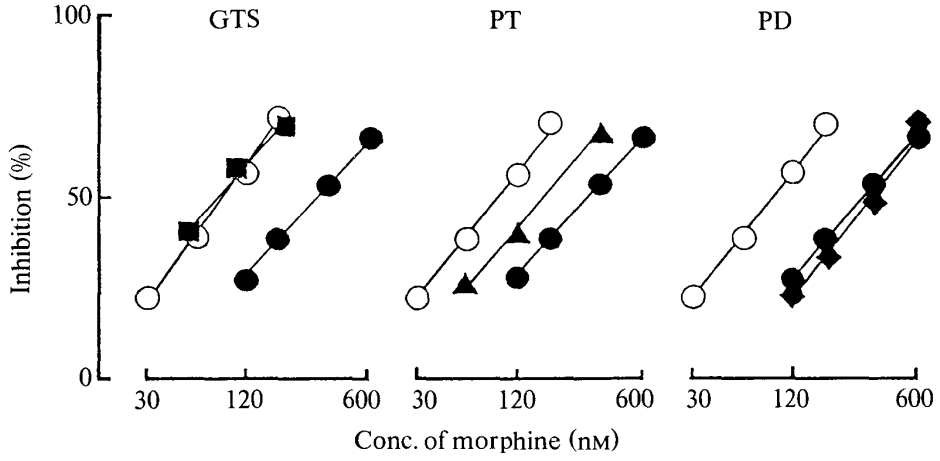

Fig. 1. Inhibitory Effect of Ginseng Saponins on the Development of Morphine Tolerance in GPI Preparation

GPI preparation was incubated with vehicle (○), 10 μM morphine (●) or morphine + 10 μg/ml GTS (■), PT (▲) or PD (◆). Abscissa: Concentration of morphine added. Ordinate: Inhibition (%) by morphine of electrically evoked contractions. For other details, refer to the text.

TABLE I. Effect of Ginseng Saponins on the Development of Morphine Tolerance in Isolated Longitudinal Muscle of GPI

Drug	(μg/ml)	Morphine IC_{50} (nM) Control (C)	*n*	Mor-tolerant (T)	*n*	T/C
Vehicle		90±10	12	270±27	11	3.0
GTS	1	91±12	3	269±66	4	3.0
	3	89± 9	3	225±21	3	2.5
	10	81±15	3	87± 8 *a)*	4	1.1
PT	1	100± 8	3	294±18	4	2.9
	3	95± 6	3	253±27	3	2.7
	10	92± 5	3	152± 8 *b)*	4	1.7
PD	1	108±13	3	405±50 *c)*	4	3.8
	3	120±13	3	306±25	3	2.6
	10	171±15	3	285±48	4	1.8

GPI preparations were incubated with 1—10 μg/ml ginseng saponins in the presence and absence of 10 μM morphine (mor-tolerant and control, respectively). The degree of tolerance was expressed as the ratio of IC_{50} values of control and mor-tolerant preparations (T/C). *n*: Number of experiments. Significantly different from mor-tolerant without ginseng saponins, *a)* $p < 0.001$, *b)* $p < 0.01$, *c)* $p < 0.05$. Each value is the mean ± S.E.

dependently; however, the IC_{50} value of morphine in the control preparation of GPI was markedly increased by 10 μg/ml PD alone and there was no significant difference between the IC_{50} values of morphine in the tolerant preparations from the vehicle alone group, 270 nM, and the 10 μg/ml PD treatment group, 285 nM (Table I).

In the MVD preparation, the development of tolerance to the morphine effect was observed as well, but none of the ginseng saponins affected it (Fig. 2 and Table II).

Discussion

In agreement with previous findings,[5,6] isolated preparations of GPI and MVD, exposed to 10 μM morphine for 90 min, readily developed tolerance to the inhibitory effect on electrically evoked contractions *in vitro*. As for the influence of ginseng saponins constituents in GPI preparations, GTS and PT but not PD inhibited the tolerance development. Although the ratio of IC_{50} (T/C) for PD was decreased concentration-dependently, the IC_{50} value of the naive control was equivalent to that of the

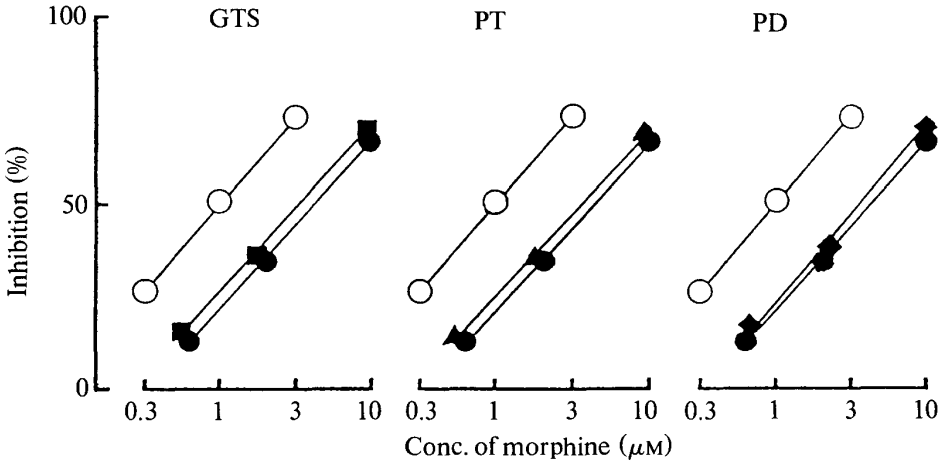

Fig. 2. Lack of Inhibitory Effect of Ginseng Saponins on the Development of Morphine Tolerance in MVD Preparation
MVD preparation was incubated with vehicle (○), 10 μM morphine (●) or morphine + GTS (■), PT (▲) or PD (◆). For other details, refer to Fig. 1 and the text.

TABLE II. Effect of Ginseng Saponins on the Development of Morphine Tolerance in Isolated MVD

Drug	(μg/ml)	Morphine IC_{50} (μM) Control (C)	*n*	Mor-tolerant (T)	*n*	T/C
Vehicle		1.2±0.1	12	4.3±0.7	12	3.6
GTS	1	1.3±0.1	3	3.9±0.4	6	3.0
	3	1.2±0.2	3	3.6±0.3	3	3.0
	10	0.9±0.2	3	3.5±0.3	3	3.9
PT	1	1.6±0.2	3	4.5±0.3	6	2.8
	3	1.4±0.2	3	4.2±0.5	3	3.0
	10	0.9±0.1	3	4.1±0.3	4	4.6
PD	1	0.9±0.2	3	4.0±0.3	6	4.6
	3	1.6±0.3	3	3.4±0.2	3	2.1
	10	1.7±0.2	3	3.6±0.5	3	2.1

MVD preparations were incubated with 1—10 μg/ml ginseng saponins in the presence and absence of 10 μM morphine (mor-tolerant and control, respectively). The degree of tolerance was expressed as the ratio of IC_{50} values of control and mor-tolerant preparations (T/C). *n:* Number of experiments. Each value is the mean ± S.E.

10 μg/ml PD group. This fact may suggest that the apparent increase in IC_{50} in the presence of PD is due to the decrease in sensitivity to morphine but not to the inhibition of the development of morphine tolerance (Table I). On the other hand, these compounds failed to reverse the tolerance development in MVD preparations.

Since electrically evoked contractions of GPI and MVD are mediated by acetylcholine and norepinephrine, respectively, and presumably these releases are regulated presynaptically by opioid receptors, it is conceivable that the tolerance development in these preparations may be due to changes in the sensitivity of opioid receptors or to changes in the release of neurotransmitters and in their receptors. In this experiment, ginseng saponins, GTS and PT, were effective to suppress the development of morphine tolerance in GPI preparations, but failed to affect the tolerance development in MVD preparations. Accordingly, the difference in the suppressive effect of ginseng saponins on the development of morphine tolerance between GPI and MVD may be attributable to the distinctive effect on the neurotransmitter systems, namely, cholinergic and adrenergic systems, rather than direct actions of the compounds on the opioid receptors. Actually, this speculation may be supported by our earlier findings that ginseng saponins inhibited contractions of GPI but facilitated contractions of MVD, and these effects were not mediated through opioid receptors.[9]

Thus, ginseng saponins can affect the cholinergic system to inhibit the development of morphine tolerance without direct opioid receptor mechanisms. In fact, it was suggested that drugs which affect the cholinergic mechanism must modify the acute and chronic actions of morphine.[10,11] Acetylcholine supersensitivity has been implicated in the development of morphine tolerance.[12,13]

Although the participation of the catecholaminergic system in morphine action cannot be neglected,[14,15] the cholinergic mechanism has much relevance to the inhibition by ginseng saponins of the development of morphine tolerance, as shown in this experiment.

The possibility that ginseng saponins inhibit morphine tolerance through the cholinergic system in GPI preparations may also be applicable to the brain, or *in vivo*. Kim *et al.* have found that GTS and PT prevented the development of tolerance to morphine analgesia, whereas PD had no such effect in mice.[1] In this experiment, GTS and PT but not PD inhibited morphine tolerance in GPI prearations. These similarities suggest an important role of the cholinergic system in the action of ginseng saponins, and also suggest that these compounds inhibit the development of morphine tolerance through their effect on the cholinergic system *in*

vivo and *in vitro*.

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