Pharmacological Effects of Ginseng Saponins on Receptor Stimulation-responses

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ABSTRACT

We investigated the influence of the root of *Panax ginseng* C. A. Meyer on the secretion of catecholamines from bovine adrenal chromaffin cells, which are used as a model of nervous systems. In two major parts extracted from the ginseng root, the crude saponin fraction, but not the non-saponin fraction, reduced the secretion from the cells stimulated by acetylcholine (ACh). Ginseng saponins (ginsenosides) are classified into three groups, the panaxadiol, the panaxatriol and the oleanolic acid groups, on the basis of the chemical structures of their sapogenins. Both the panaxadiol and the panaxatriol saponins, excluding only one oleanolic acid saponin ginsenoside-Ro, generally reduced the ACh-evoked secretion. The inhibitory effects of the panaxatriols were much stronger than those of the panaxadiols. However, ginsenoside-Rg_3 and -Rh_2 in the panaxadiol saponins were the potent inhibitors comparable to the panaxatriol saponins. Ginsenoside-Rg_2 in the panaxatriols was the most effective. It is probable that the ginsenoside inhibition of the catecholamine secretion is due to the suppression of the function of the nicotinic ACh receptor-cation channels. On the other hand, ginsenoside-Rg_2 did not affect the angiotensin Ⅱ-, the bradykinin-, the histamine- and the neurotensin-induced catecholamine secretions from the chromaffin cells and the muscarine- and the histamine-induced contraction of the ileum in guinea-pigs. Ginsenoside-Rb_1, a panaxadiol saponin, and ginsenoside-Ro had no or only a slight effect on them. On the contrary, ginsenoside-Rg_3 not only competitively inhibited the muscarine-induced ileum contraction but also reduced the angiotensin Ⅱ-, the bradykinin-, the histamine- and the neurotensin-induced catecholamine secretions. Thus, the ginseng root contains active ingredients, namely some ginsensides, which suppress the responses induced by receptor stimulations. The inhibitory effects of ginseng saponins may be one of the action mechanisms for the pharmacological effects of the *Panax ginseng* root.

Introduction

The root of *Panax ginseng* C. A. Meyer has been used as an important component of many

Chinese traditional prescriptions for more than two thousand years and is now well known as a natural medicine throughout the world. However, until quite recently, the pharmacological effects of the ginseng root have scarcely been scientifically demonstrated. The oldest Chinese traditional medical book, *Sheng-nong Ben-cao Jing,* stated that the root has many effects, e.g. replenishment of vital energy, tranquilization, elevation of mood and prevention of aging. Some of these pharmacological effects led us to consider that the ginseng root may affect the nervous systems. Actually, several investigators have reported that the ginseng saponins have several effects on the nervous systems[1]. However, the mechanisms underlying the ginseng effects are still obscure.

The adrenal chromaffin cells are well known as a useful model of nervous systems, especially the sympathetic nerves, because they are embryologically derived from neural crest tissue. The chromaffin cells secrete catecholamines via stimulation of the nicotinic ACh receptors by a physiological secretagogues, acetylcholine (ACh), which is released from the terminal of the splanchnic nerve. The process of catecholamine secretion is as follows:[1] ACh binds to the nicotinic receptors; 2) the influx of Na^+ occurs throught the receptor-operated cation channels; 3) the cell membrane is depolarized and the influx of Ca^{2+} occurs through the voltage-sensitive Ca^{2+} channels; and 4) the intracellular free Ca^{2+} concentration increases which results in exocytotic catecholamine secretion[2].

Therefore, we investigated the effect of the ginseng root extracts and the ingredients on the secretion of catecholamines from the bovine adrenal chromaffin cells. The results showed that the panaxatriol saponins strongly inhibited the secretion from the cells stimulated by ACh, due to suppressing the ability of the nicotinic ACh receptors. Furthermore, the effects of the ginseng saponins on the other receptor-stimulation responses were also studied.

Materials and Methods

Materials

Ginseng saponins were supplied by Korea Tobacco & Ginseng Corporation and Japan Korea Red Ginseng Co., Ltd. (Kobe, Japan). Purities of the ginseng saponins used in this study were checked by TLC and NMR according to the method of Kawashima and Samukawa[3], and they were more than 98% pure. Oxygenated Krebs-Ringer-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (KRH buffer) (pH 7.4) was used as the incubation medium and was composed of 125 mM NaCl, 4.8 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM HEPES, 5.6 mM glucose, and 0.5% bovine serum albumin. In 56 mM KCl-KRH buffer, the amount of NaCl was reduced to maintain the isotonicity of the medium. All other chemicals were of the highest grade available from commercial sources.

Isolation and Primary culture of bovine adrenal chromaffin cells

Bovine adrenal glands were kindly provided by the Center of Iwate Livertock Industry. Adrenal chromaffin cells were prepared by the method of collagenase digestion as previously described[4]. To remove the nonchromaffin cells including firbroblasts or epithelial cells, the differential plating procedure was used[5]. The chromaffin cells were maintained as a monolayer culture in 35-mm diameter dishes at a density of 2 x 10^6 cells. The cells were cultured at 37℃ in a CO_2 incubator (95% air/5% CO_2) for four days. The purity of the cultured cells was confirmed by the Grimelius method[6] and the final cell preparation contained at least 80-90% chromaffin cells.

Measurements of catecholamine secretion

After four days of culturing, the chromaffin cells were washed twice with KRH buffer and then preincubated with or without the ginseng extracts or the saponins in KRH buffer for 10 min at 37℃. The cells were incubated with or without secretagogues for 7 min in the presence or absence of the above agents except as otherwise described below. The reaction was terminated by transferring the incubation medium to tubes in an ice-cold bath. The catecholamines secreted into the medium were extracted with 0.4 M perchloric acid and adsorbed on aluminum hydroxide. Their amounts were estimated by the ethylenediamine condensation method, using a fluorescence spectrophotometer (650-10S; Hitachi, Tokyo, Japan) at an excitation wavelength of 420 nm and an emission wavelength of 540 nm. At these wavelengths, epinephrine and norepinephrine showed the same fluorescence intensity.

Measurement of intracellular free Ca^{2+} concentration($[Ca^{2+}]_i$)

Fura-2 was loaded into the chromaffin cells using a previously described method[2]. Briefly, the isolated cells were cultured for four days on coverslips cut to fit into the spectrofluorophotometer cuvette. The cultured cells on the coverslips were incubated with 5 μM fura-2-acetoxymethy ester in the culture medium at 37℃. After 40 min, the incubation medium was replaced with KRH buffer. The coverslip was washed three times with KRH buffer and placed in the cuvette. The cells in the cuvette were preincubated with KRH or Ca^{2+} -free KRH buffer for 10 min at 37℃ in the fluorescence meter, and then the test agents were added to the cuvette. Increases and decreases in the fluorescence induced by the fura-2-Ca^{2+} complex in the cells were simultaneously measured with a spectrofluorophotometer (CAF-100, Nikon Bunko, Tokyo, Japan) at an excitation wavelength of 340 nm and an emission wavelength of 500 nm and at an excitation wavelength of 380 nm and an emission wavelength of 500 nm, respectively. $[Ca^{2+}]i$ was calculated as described by Grynkiewicz *et al.*[7].

Measurement of intracellular free Na^{2+} concentration ($[Na^+]_i$)

The isolated cells were incubated with 5 μM sodium-binding benzoforan isophthalate (SBFI) tetraacetoxy methyl ester and 0.02% Pluronic F-127 in KRH buffer for two hrs at 37℃ and washed

three times with KRH buffer. The cells were preincubated with KRH buffer or Na^+-free sucrose buffer for 10 min at 37℃ in the fluorescence meter, and then the test agents were added. Increases and decreases in the fluorescence induced from the SBFI-Na^+ complex were simultaneously recorded at excitation wavelengths of 340 and 380 nm, respectively, and at an emission wavelength of 500 nm. The change in $[Na^+]_i$ was expressed as the ratio of the fluorescence at excitation wavelengths of 340 and 380 nm.

Isolation and contraction of guinea-pig ileum

The ileum of starved (48 h) male guinea-pigs (400-500 g) was isolated and washed with Tyrode solution. One end of the muscle with a length of 3 cm was fixed while the other end was connected to a light lever, it was suspended in the solution in a glass chamber of 10 ml capacity bubbled with oxygen for 30 min at 35℃. The ileum was preincubated with or without ginsenosides for 1 min and then incubated with or without various stimuli. The contractions of the muscle were recorded by an isotonic transducer. An initial load of 0.5 g was applied to the muscle.

Results and Discussion

Effects of crude extracts and ginseng saponins (ginsenosides) isolated from ginseng root on catecholamine secretion

First, we examined the effects (Fig 1) of two major parts, which are the non-saponin and crude saponin fractions extracted from the root of ginseng, on the secretion of catecholamines from bovine adrenal chromaffin cells evoked by ACh. The crude saponin fraction dose-dependently reduced the ACh-evoked secretion from the cells (40-800μg/ml), whereas the non-saponin fraction (20-800μg/ml) did not affect it (Fig. 1) even at a higher concentration (1 mg/ml) (data not shown). Next, the effects of various ginsenosides (14 kinds) on the secretion were examined. Ginsenosides are classified into three groups, which are the panaxadiol, the panaxatriol and the oleanolic acid groups. In the three groups, the panaxatriol saponins(ginsenoside-Re, -Rf, -Rg_1, -Rg_2 and -Rh_1) greatly suppressed the ACh-evoked secretion, and ginsenoside-Rg_2 in the panaxatriols was the most effective (Fig. 2) However, the panaxadiol (ginsenoside -Rb_1, -Rb_2, -Rb_3, -Rc, -Rd and -Rs_1) and the oleanolic acid (ginsenoside-Ro) saponins did not or only slightly inhibited the secretion excluding ginsenoside-Rg_3 and -Rh_2, which were the potent inhibitors comparable to the panaxatriol saponins (Fig. 2).

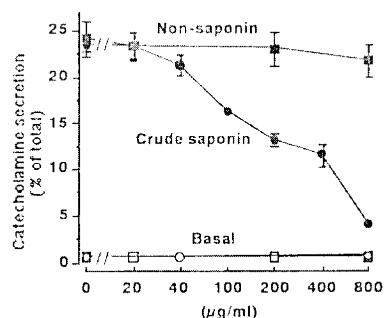

Fig. 1. Effects of crude saponin and non-saponin fractions on ACh-evoked CA secretion. Bovine adrenal chromaffin cells were preincubated with various concentrations of crude saponin or non-saponin for 10 min at 37°C, and then the cells were incubate or without various concentrations of crude saponin or non-saponi the presence (●, ■) or absence of ACh (50 μM) (○, □) for 7 min a

Properties of ginsenoside-Rg_2 inhibition of catecholamine secretion(Fig. 2)

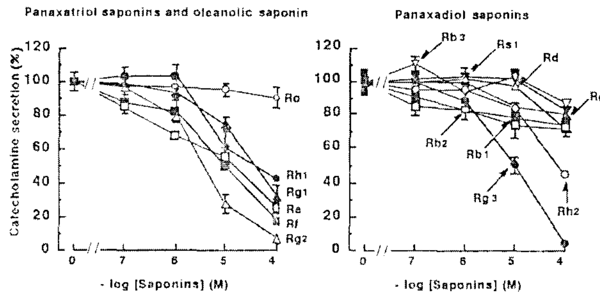

Fig. 2 Effects of 14 kinds of representative ginseng saponins (ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, Rh_2, Ro and Rs_1) on ACh-evoked CA secretion. The ACh-evoked secretion was assigned a value of 100%.

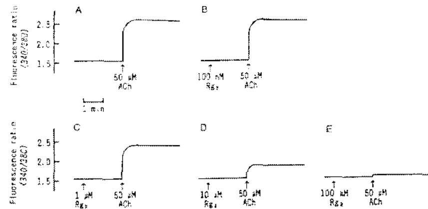

Fig. 3. Effect of ginsenoside-Rg_2 on $[Na^+]_i$.

We investigated the inhibitory mechanism of the ginseng saponins using ginsenoside-Rg_2. Ginsenoside-Rg_2 inhibited both the ACh-evoked Na^+ (Fig. 3) and Ca^{2+} influxes into the cells (data not shown) in a concentration-dependent manner similar to that of the inhibition of the ACh-evoked catecholamine secretion. However, it had no effect on the secretion of catecholamines from the cells induced by high K^+, which depolarizes the cell membranes and results in Ca^{2+} influx through the voltage-sensitive Ca^{2+} channels (data not shown). The inhibitory effect of ginsenoside-Rg_2 on the secretion was not affected by increasing the external Ca^{2+} (2.6-7.8 mM) or ACh concentrations (20-200 μM), while it was overcome by increasing the external Na^+ concentrations (125-250 mM) (data not shown).

Effects of various ginsenosides on angiotensin Ⅱ-, bradykinin- histamine- andneurotensin- induced secretion of catecholamines from the cells

The bovine adrenal chromaffin cells secrete catecholamines via stimulations of each receptor by angiotensin Ⅱ, bradykinin, histamine and neurotensin as well as ACh. The effects of the representative ginseng saponins in the three groups on the catecholamine secretion induced by the stimuli were studied (Table 1). A panaxadiol saponin, ginsenoside-Rg_2, (1-100 μM) reduced only the ACh-evoked secretion, but the saponin did not affect the angiotensin Ⅱ-, the bradykinin-, histamine- and neurotensin- induced secretions. On the other hand, a panaxadiol saponin, ginsenoside-Rg_3, (1-100 μM)suppressed not only the ACh-induced secretion but also, at higher concentrations (10-100 μM), the angiotensin Ⅱ-, the bradykinin-, the histamine- and the neurotensin- induced secretions. However, another panaxadiol saponin, ginsenoside-Rb_1, and an oleanolic acid saponin, ginsenoside-Ro, had no significant effect on their responses.

Effects of various ginsenosides on muscarine- and histamine-induced contraction of ileum

Ginsenoside-Ro, -Rb_1, and -Rg_2 at 100 μM scarcely affected the contraction of the guinea-pig

Table 1. Effects of various ginseng saponins on receptor stimulation-responses

		Ginsenosides			
		Oleanolic acid	Panaxadiols	Panaxatriols	
		Ro	Rb_1	Rg_2	Rg_3
Bovine					
Chromaffin cells	Nicotine	-	±	++	++
	Histamine	-	-	+	-
	Angiotensin II	-	-	+	-
	Bradykinin	-	-	+	-
	N eurotensin	-	-	+	-
Guinea-pig		-			
ileum	Muscarine	-	±	++	-
	Histamine	-	±	+	-

- : No effect; ± : Slight inhibition; + : Inhibition; ++ : Strong inhibition,
ND : Not determined

ileum induced by muscarine or histamine. On the other hand, ginsenoside-Rg_3 at 2.5 μM suppressed the muscarine (at 30-100 nM) -induced ileum contraction. However, the inhibitory were overcome by increasing the muscarine concentration (200-400 nM), indicating that the inhibitory effect of the saponin is competitive with muscarine. The histamine-induced contraction of the ileum are also strongly reduced by ginsenoside-Rg_3 at a higher concentration (100 μM) (Table 1).

This study demonstrated that the ginseng root contains some saponins (ginsenosides) having pharmacological effects on the receptor-stimulation responses. In bovine adrenal chromaffin cells, the panaxatriol saponins inhibited the secretion of catecholamines from the cells stimulated by ACh more strongly than the panaxadiol saponins except for ginsenoside-Rg_3 and -Rh_2. There was the relationship between the inhibitory effects and the structures of the ginsenosides[8]. The ginsenosides' inhibition of the ACh-evoked secretion is considered to be due to a suppression of the function of the nicotinic ACh receptors[9] (Fig. 4), because ginsenoside-Rg_2 inhibited the Ach- evoked Na^+ and Ca^{2+} influxes and catecholamine secretion. However, the saponin did not alter the high K^+ -induced secretion. Furthermore, the ginsenoside-Rg_2 inhibition was overcome by increasing the extermal Na^+ concentrations. It is likely that the ginsenoside-Rg_2 inhibition is specific to nicotinic ACh receptors in bovine adrenal chromaffin cells, while ginsenoside-Rg_3 may act on not only the nicotinic receptors but also the muscarinic ACh receptors in the ileum of guinea-pigs (Table 1). Although ginsenoside-Rg_3 inhibited the responses induced by other receptor stimuli, relatively higher concentrations (10-100 μM) were required for their inhibi-

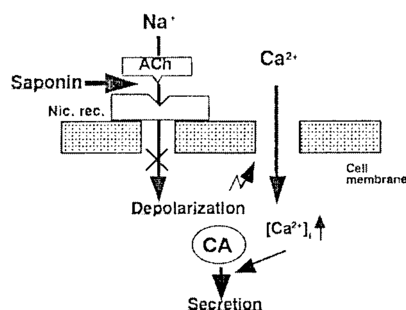

Fig. 4. Mechanism of ginsenoside inhibition of CA secretion

tions. Therefore, the inhibitory effect seem to be non-specific actions on the plasma membranes. Other representative ginsenosides had no effects on the receptor stimulation-responses and the cell membranes. These actions antagonistic to neurotransmitters may account for the pharmacological effects of the ginseng root on nervous systems.

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