ELSEVIER

European Journal of Pharmacology 369 (1999) 23–32

ejp

Effects of ginseng saponins on responses induced by various receptor stimuli

Eiichi Tachikawa [a,*], Kenzo Kudo [a], Kazuho Harada [a], Takeshi Kashimoto [a], Yoshikazu Miyate [b], Atsushi Kakizaki [b], Eiji Takahashi [b]

[a] *Department of Pharmacology, School of Medicine, Iwate Medical University, Uchimaru 19-1, Morioka 020-8505, Japan*
[b] *Department of Medicine, School of Dentistry, Iwate Medical University, Hontyodori, Morioka 020-8505, Japan*

Received 12 January 1999; accepted 19 January 1999

Abstract

We investigated the effects of four ginseng saponins, ginsenoside-Rb_1, -Rg_2, -Rg_3 and -Ro, on the responses induced by receptor stimulation of various stimuli. Ginsenoside-Rg_2 (1–100 μM) reduced the secretions of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine and γ-aminobutyric acid but not by angiotensin II, bradykinin, histamine and neurotensin. In guinea-pig, the ginsenoside also diminished the nicotine-induced secretion of catecholamines from the adrenal chromaffin cells, but it did not affect the muscarine- and the histamine-induced ileum contractions. On the other hand, ginsenoside-Rg_3 (1–100 μM) reduced not only the acetylcholine-, the γ-aminobutyric acid- and the neurotensin-induced secretions but also, at a higher concentration (100 μM), the angiotensin II-, the bradykinin- and the histamine-induced secretions from the bovine chromaffin cells. Furthermore, the saponin (3–100 μM) significantly inhibited the muscarine- and the histamine-induced ileum contractions of the guinea-pig. Ginsenoside-Rb_1 and -Ro had no marked effect on their responses. These results strongly suggest that ginsenoside-Rg_2 is a potent selective blocker of nicotinic acetylcholine and γ-aminobutyric acid receptors (ionotropic receptors) and ginsenoside-Rg_3 is not only a blocker of ionotropic receptors but also an antagonist of muscarinic or histamine receptors.

Keywords: Catecholamine secretion; Chromaffin cell; Ginseng saponin; Ginsenoside; Muscarinic receptor; Nicotinic receptor

1. Introduction

The root of *Panax ginseng* C.A. Meyer has been widely used as an important component of many Chinese prescriptions called 'Kan-Pou medicine' from ancient times and is now well-known as a natural medicine throughout the world. The drug alone has also been used in various diseases and for health. The oldest Chinese traditional medical book, *Sheng-nong Ben-cao Jing*, mentions that the ginseng root has many effects (e.g., replenishment of vital energy, tranquillization, mood elevation and prevention of aging). Thus, it seems that the ginseng root regulates biological functions and cures a variety of diseases. Among the many effects of the ginseng roots, we focused on the tranquillizing action. The pharmacological effect led us to consider that the ginseng root may affect the nervous systems. In fact, there are several reports showing that ginseng saponins, which are isolated from the ginseng root, have effects on the nervous systems (Yoshimura et al., 1988; Saito, 1990). However, the mechanism underlying the effects has not yet been well understood.

The adrenal medulla can secrete catecholamines mainly via stimulation of the nicotinic acetylcholine receptors by a physiological secretagogue, acetylcholine, which is released from the terminal of the splanchnic nerve, although it also has several other receptors for stimuli such as histamine, angiotensin II, bradykinin etc. which induce the secretion. Binding of acetylcholine to the nicotinic receptors leads to depolarization of the cell membrane by an influx of Na^+ through receptor-operated cation channels, causing an influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels and results in catecholamine secretion by exocytosis (Douglas and Poisner, 1961; Wilson and Kirshner, 1977; Holz et al., 1982). Therefore, adrenal chromaffin cells are widely used in studies of catecholamine secretion as a useful model of the sympathetic nervous systems.

* Corresponding author. Tel.: +81-19-651-5111; Fax: +81-19-651-1660; E-mail: etachika@iwate-med.ac.jp

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00043-6

We have demonstrated the effects of two major parts, crude saponin and non-saponin fractions extracted from the ginseng root, on the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine (Kudo et al., 1992). The saponin fraction, but not the non-saponin fraction, significantly inhibited the secretion. The ginseng saponins, which are called ginsenosides, are classified into three major groups, the panaxadiol, panaxatriol, and oleanolic acid groups, on the basis of the chemical structures of their sapogenins (aglycones). At present, more than 30 kinds of ginsenosides are found in the ginseng root. All ginseng saponins (1–100 μM) (panaxadiol saponins: ginsenoside-Rb_1, -Rb_2, -Rb_3, -Rc, -Rd, -Rg_3, -Rh_2, -Rs_1; panaxatriol saponins: ginsenoside-Re, -Rf, -Rg_1, -Rg_2, -Rh_1) except for ginsenoside-Ro (oleanolic acid saponin), which were used in the study, had a tendency to reduce the acetylcholine-evoked secretion from the cells. The inhibitory effects of the panaxatriol saponins were much greater than those of the panaxadiols. Other plant saponins, such as saikosaponin-a, glycyrrhizin and cardiac glycosides, digitoxin and digoxin, had no significant inhibitory effect on the secretion, suggesting that the inhibitory effects of ginsenosides on the acetylcholine-evoked secretion are a unique property of the ginseng (Tachikawa et al., 1995; Kudo et al., 1998).

Furthermore, we explored the mechanism of ginsenoside inhibition on the secretion using ginsenoside-Rg_2 that showed the greatest inhibition among the ginsenosides tested and suggested that ginsenoside-Rg_2 acts on the nicotinic acetylcholine receptor-operated cation channels, inhibits Na^+ influx through the channels and consequently reduces both Ca^{2+} influx and catecholamine secretion in the cells (Tachikawa et al., 1995).

In this study, therefore, to investigate the influences of ginseng saponins on other receptors besides the nicotinic acetylcholine receptors, we examined the effects of representative ginseng saponins of three groups (panaxadiols: ginsenoside-Rb_1 and -Rg_3; panaxatriols: ginsenoside-Rg_2; oleanolic acid: ginsenoside-Ro) on the secretion of catecholamines from bovine and guinea-pig adrenal chromaffin cells and on the contraction of the ileum in the guinea-pig induced by various receptor stimulants (γ-aminobutyric acid (GABA), histamine, bradykinin, angiotensin II, neurotensin and muscarine).

2. Materials and methods

2.1. Materials

Ginsenosides were kindly supplied by Korea Tobacco and Ginseng and Japan Korea Red Ginseng (Kobe, Japan). The purities of the ginsenosides were checked by thin

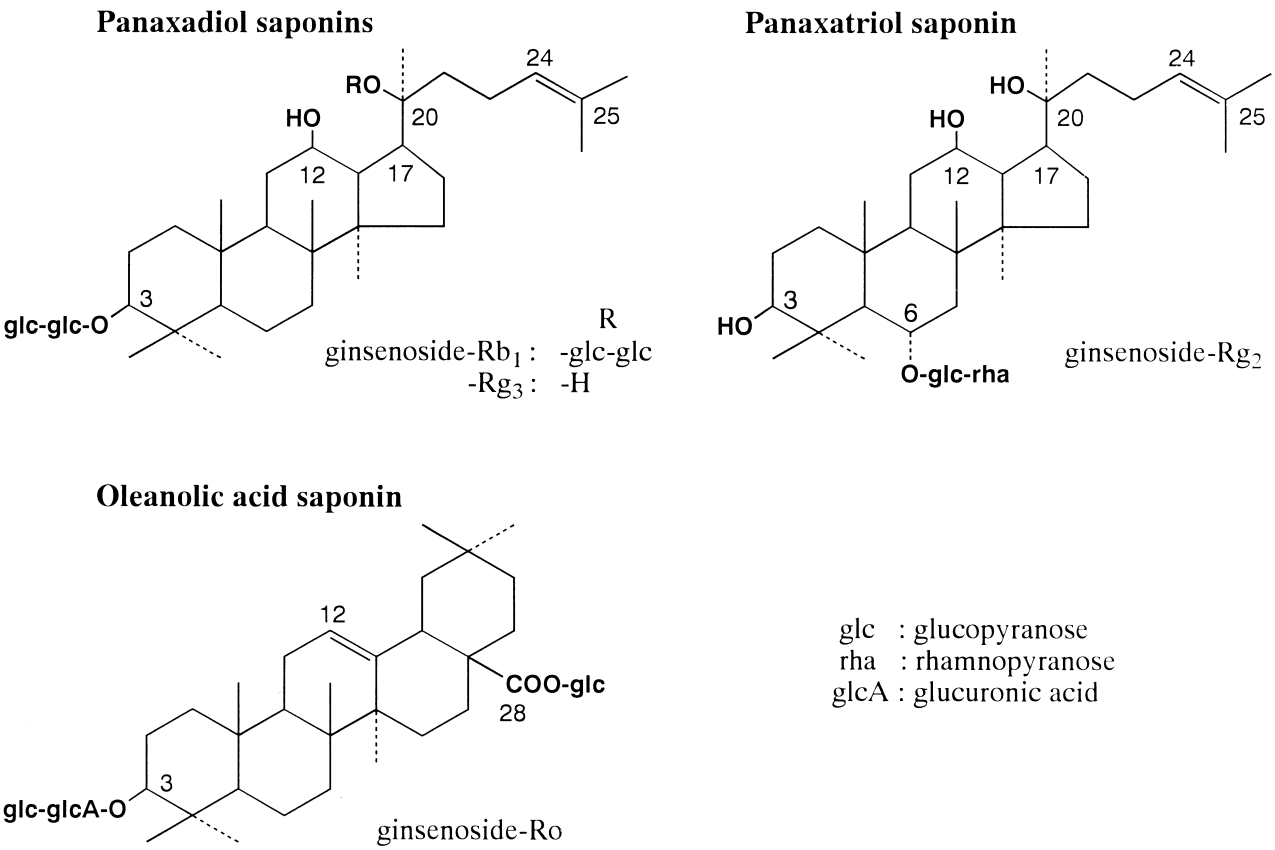

Fig. 1. Structures of ginsenosides.

layer chromatography and nuclear magnetic resonance according to the method of Kawashima and Samukawa (1986), and they were found to be more than 98% pure. The ginsenosides were dissolved in dimethyl sulfoxide. The concentration of dimethyl sulfoxide in the incubation medium was 1%, which had no effect on the secretion of catecholamines from bovine and guinea-pig adrenal chromaffin cells and on the ileum contraction in the guinea-pig under the conditions used in this study. Oxygenated Krebs–Ringer–HEPES buffer (KRH buffer) (pH 7.4) was used as an incubation medium for the chromaffin cells and was composed of (mM) 125 NaCl, 4.8 KCl, 2.6 $CaCl_2$, 1.2 $MgSO_4$, 25 HEPES, 5.6 glucose, and 0.5% bovine serum albumin. Oxygenated Tyrode solution was also used as an incubation medium for the ileum and was composed of (mM) 137 NaCl, 2.7 KCl, 1.8 $CaCl_2$, 1.1 $MgCl_2$, 0.4 NaH_2PO_4, 12 $NaHCO_3$ and 5.6 glucose and was adjusted to pH 7.8, when equilibrated with oxygen. Tissue culture instruments were obtained from the Falcon Plastics (Cockeysville, MD, USA). Angiotensin II, bradykinin and neurotensin were purchased from UCB-Bioproducts (Brussels, Belgium), and (±)-muscarine was from Sigma (St. Louis, MO, USA). γ-Aminobutyric acid was from Wako (Osaka, Japan). Eagle's minimum essential medium was obtained from Nissui Seiyaku (Tokyo, Japan). Calf serum and acetylcholine were obtained from Nacarai Tesque (Kyoto, Japan). All other chemicals were of the highest grade available from commercial sources.

2.2. Isolation and primary culture of bovine adrenal chromaffin cells

Bovine adrenal glands were kindly provided by the Center of Iwate Livestock Industry. Adrenal chromaffin

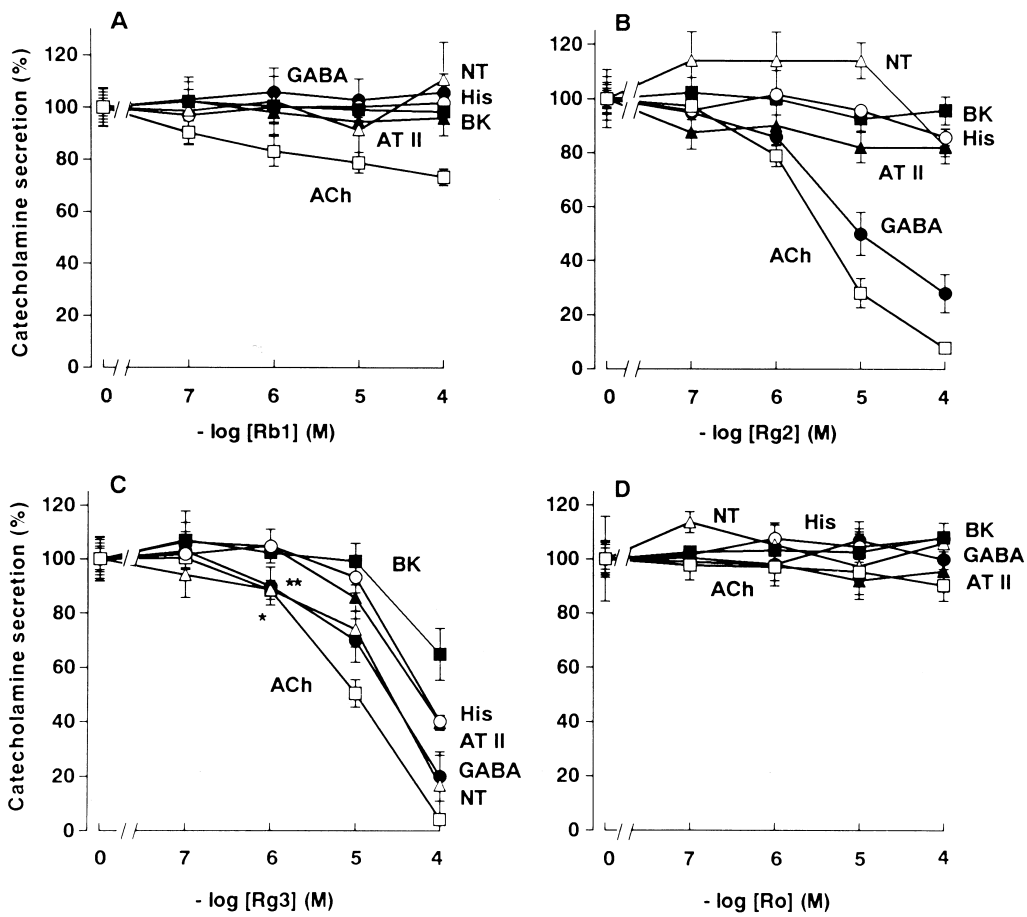

Fig. 2. Effects of different concentrations of ginsenoside-Rb_1, -Rg_2, -Rg_3 and -Ro on the secretion of catecholamines from bovine adrenal chromaffin cells induced by various stimuli. The isolated bovine adrenal chromaffin cells were cultured for four days and washed twice with prewarmed KRH buffer. The cells were preincubated with different concentrations of ginsenoside-Rb_1 (A), -Rg_2 (B), -Rg_3 (C), or -Ro (D) for 10 min at 37°C and then incubated with or without 100 μM acetylcholine (ACh; □) for 7 min, or 100 nM angiotensin II (AT II; ▲), 10 nM bradykinin (BK; ■), 40 μM γ-aminobutyric acid (GABA; ●), 10 μM histamine (His; ○) or 20 μM neurotensin (NT; △) for 30 min in the presence or absence of each ginsenoside used above. Catecholamines secreted from the cells into the medium were determined as described in Section 2. The values of the basal secretion were subtracted from the data, and the various stimuli-induced responses were assigned the value of 100%. The acetylcholine-, the angiotensin II-, the bradykinin-, the γ-aminobutyric acid-, the histamine- and the neurotensin-induced secretions were 29.5 ± 1.7, 1.9 ± 0.2, 3.5 ± 0.3, 1.6 ± 0.1, 6.5 ± 0.3 and 1.3 ± 0.1% of total cellular catecholamines, respectively. The basal secretion was 0.4 ± 0.1%. Values are means ± S.D. from at least four experiments. $^{*}P < 0.01$; significantly different from the neurotensin-induced secretion. $^{**}P < 0.01$; significantly different from the γ-aminoisobutyric acid-induced secretion.

cells were prepared by the method of collagenase digestion as previously described elsewhere (Tachikawa et al., 1991). The isolated cells were suspended in Eagle's minimum essential medium containing 10% calf serum and antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin, and 0.3 μg/ml amphotericin B) and were plated on 35-mm dishes at a density of 2×10^6 cells. The cells were cultured at 37°C in a CO_2 incubator (95% air and 5% CO_2) for 4 days. A total of 2×10^6 cells contained 34.7 ± 3.8 μg of catecholamines as epinephrine and norepinephrine.

2.3. Isolation of guinea-pig adrenal chromaffin cells

Guinea-pig adrenal chromaffin cells were prepared by the method of collagenase digestion of Hochman and Perlman (1976). Briefly, male guinea-pigs (Hartley strain) weighing 400–500 g were killed by being stunned and bled via the carotid arteries. In each experiment, adrenal glands from 10–12 guinea-pigs were used. After collagenase digestion of the adrenal glands, the isolated chromaffin cells were fully washed and suspended in KRH buffer at a density of $6–7 \times 10^5$ cells/ml. The viability of the cells, determined by the Trypan blue exclusion test, was more than 95%.

2.4. Measurements of catecholamine secretion

After 4 days of culturing, the bovine chromaffin cells were washed twice with KRH buffer and then preincubated with or without ginsenosides in KRH buffer for 10 min at 37°C. The cells were incubated with or without stimulus for 7 or 30 min in the presence or absence of each ginsenosides used above. The chromaffin cells of guinea-pigs were preincubated with or without ginsenosides for 10 min at 37°C and then incubated with or without stimuli for 20 min. 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 absorbed on aluminum hydroxide (Tachikawa et al., 1995). Their amounts were estimated by the ethylenediamine condensation method (Weil-Malherbe and Bone, 1952), using a fluorescence spectrophotometer (650-10S; Hitachi, Tokyo, Japan) at an excitation wavelength of 420 nm and an emission wave-

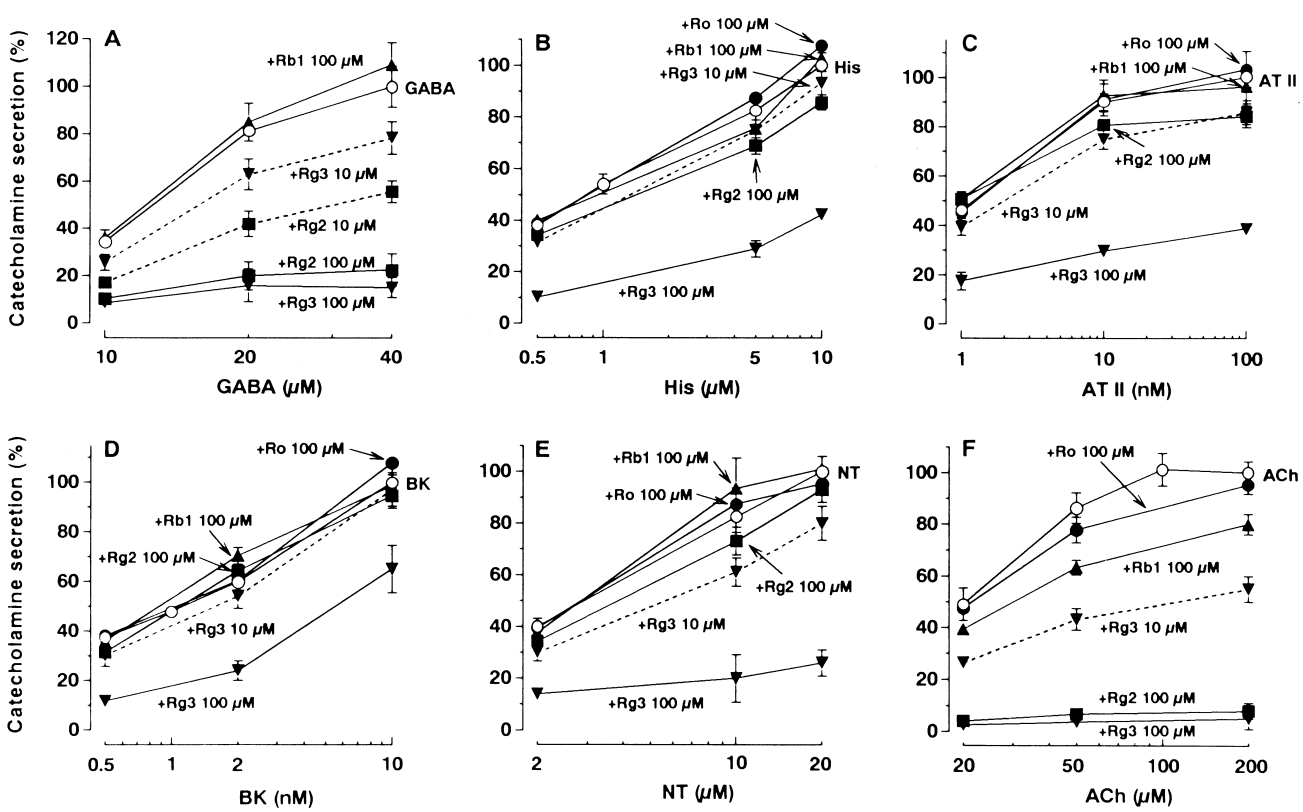

Fig. 3. Effects of ginsenoside-Rb_1, -Rg_2, -Rg_3 and -Ro on the secretion of catecholamines from bovine adrenal chromaffin cells induced by different concentrations of stimuli. The cultured cells were preincubated with ginsenoside-Rb_1 (100 μM), -Rg_2 (10 and 100 μM), -Rg_3 (10 and 100 μM) or -Ro (100 μM) for 10 min at 37°C and then incubated with or without different concentrations of γ-aminobutyric acid (GABA; A) (10–40 μM), histamine (His; B) (500 nM–10 μM), angiotensin II (AT II; C) (1–100 nM), bradykinin (BK; D) (500 pM–10 nM), neurotensin (NT; E) (2–20 μM) for 30 min or acetylcholine (ACh; F) (20–200 μM) for 7 min in the presence or absence of each ginsenoside used above. Catecholamines secreted from the cells into the medium were determined as described in Section 2. The values of the basal secretion were subtracted from the data, and the various stimuli-induced maximal responses were assigned the value of 100%. Values are means ± S.D. from at least four experiments.

length of 540 nm. At these wavelengths, epinephrine and nor-epinephrine showed the same fluorescence intensity.

2.5. 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°C. 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 (TD 112 S; Nihon Kohden Tokyo, Japan). An initial load of 0.5 g was applied to the muscle.

2.6. Statistics

Statistical calculations were made according to the methods of Snedecor and Cochran (1967). Differences were considered significant when P calculated by Student's t-test was < 0.05.

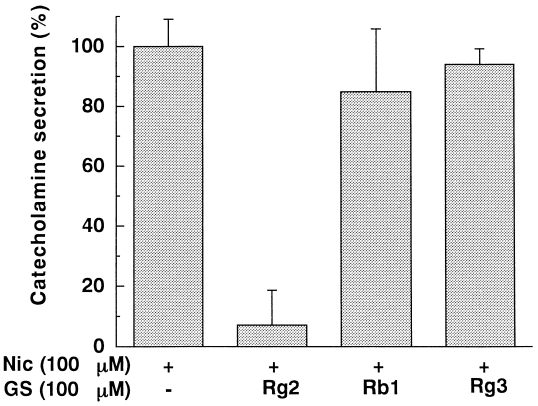

Fig. 4. Effects of ginsenoside-Rb_1, -Rg_2 and -Rg_3 on the secretion of catecholamines from guinea-pig adrenal chromaffin cells stimulated by nicotine. The isolated cells were preincubated with ginsenoside (GS) -Rb_1, -Rg_2 or -Rg_3 (100 μM) for 10 min at 37°C and then incubated with or without 100 μM nicotine (Nic) in the presence or absence of the ginsenosides used above for 20 min. Catecholamines secreted from the cells into the medium were determined as described in Section 2. The value of the basal secretion was subtracted from the data, and the nicotine-induced response was assigned the value of 100%. The nicotine-induced secretion was 10.0 ± 0.3% of total cellular catecholamines. The basal secretion was 6.3 ± 0.2%. Values are means ± S.D. from at least four experiments.

3. Results

3.1. Effects of ginsenosides on catecholamine secretion from bovine adrenal chromaffin cells stimulated by γ-aminobutyric acid, histamine, angiotensin II, bradykinin and neurotensin

First, we examined the effects of the panaxadiol saponins ginsenoside-Rb_1 and -Rg_3, the panaxatriol saponin ginsenoside-Rg_2 and the oleanolic acid saponin ginsenoside-Ro (Fig. 1) on the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by various stimuli. The bovine chromaffin cells secrete catecholamines via stimulation of each receptor by γ-aminobutyric acid (Kitayama et al., 1990), histamine (Schneider, 1969; Livett and Marley, 1986), angiotensin II (Noble et al., 1988; O'Sullivan and Burgoyne, 1989), bradykinin (O'Sullivan and Burgoyne, 1989; Bunn, 1990) or neurotensin (Noble et al., 1988; Bunn, 1990) (Figs. 2 and 3). As shown in Fig. 2C, ginsenoside-Rg_3 (1–100 μM) had a tendency to diminish all their stimuli-induced secretions. The saponin inhibited both the γ-aminobutyric acid (at 40 μM)-induced and the neurotensin (at 10 μM)-induced secretions more strongly than the others. The ginsenoside-Rg_3 inhibitions of the γ-aminobutyric acid-induced and the neurotensin-induced responses were detected at 1 μM, and at 10 and 100 μM, they were 25 and 26%, and 81 and 83%, respectively, while the inhibitions of other stimuli-induced secretions were not observed at 10 μM. The 50% inhibitory concentration (IC_{50}) values for ginsenoside-Rg_3 on various stimuli-induced catecholamine secretions were as follows (μM): 26 (γ-aminobutyric acid), 28 (neurotensin), 62 (histamine), 64 (angiotensin II) and > 100 (bradykinin) (Table 1). Ginsenoside-Rg_2 also strongly inhibited the γ-aminobutyric acid-induced secretion, but it did not alter the histamine-, the angiotensin II-, the bradykinin- and the neurotensin-induced secretions (Fig. 2B). The IC_{50} values for ginsenoside-Rg_2 on the γ-aminobutyric acid-induced secretion was 10 μM (Table 1). On the other hand, ginsenoside-Rb_1 and -Ro had no effect on their secretions (Fig. 2A,D). As previously reported (Tachikawa et al., 1995; Kudo et al., 1998), the acetylcholine-evoked secre-

Table 1
IC_{50} values of ginsenoside-Rg_2 and -Rg_3 on various stimuli-induced catecholamine secretions from bovine adrenal chromaffin cells

Stimuli	IC_{50} (μM)	
	Ginsenosides	
	Rg_2	Rg_3
ACh	4	10
GABA	10	26
His	−	62
AT II	−	64
BK	−	> 100
NT	−	28

The IC_{50} values were evaluated from the log dose–response curve of the ginsenosides on the acetylcholine (ACh) (at 100 μM)-, γ-aminobutyric acid (GABA) (at 40 μM)-, histamine (His) (at 10 μM)-, angiotensin II (AT II) (at 100 nM) -, bradykinin (BK) (at 10 nM)- or neurotensin (NT) (at 20 μM)-induced secretion of catecholamines.
−: No inhibition.

tion was strongly inhibited by ginsenoside-Rg_2 and -Rg_3 (IC_{50} = 4 and 10 μM) (Fig. 2B,C and Table 1). Although each stimulus was used at the concentrations which cause a maximal secretion (angiotensin II, 100 nM; bradykinin, 10 nM; γ-aminobutyric acid, 40 μM; histamine, 10 μM; neurotensin, 20 μM) (Fig. 2), even at the lower concentrations (angiotensin II, 1–10 nM; bradykinin, 500 pM–2 nM; γ-aminobutyric acid, 10–20 μM; histamine, 500 nM–5 μM; neurotensin, 2–10 μM), the inhibitory effects of the ginsenosides on the secretion were the same as those at the maximal concentrations (Fig. 3).

3.2. Effects of ginsenoside-Rb_1, -Rg_2 and -Rg_3 on catecholamine secretion from guinea-pig chromaffin cells stimulated by nicotine

To further confirm the actions of ginsenosides on nicotinic acetylcholine receptors, using guinea-pig adrenal chromaffin cells, we examined the effects of ginsenoside-Rb_1, -Rg_2 and -Rg_3 on the nicotine-induced catecholamine secretion. Ginsenoside-Rg_2 (100 μM) greatly reduced the nicotine-induced secretion from the chromaffin cells, whereas ginsenoside-Rb_1 and -Rg_3 did not affect it (Fig. 4).

3.3. Effects of ginsenosides on muscarine-induced contraction of ileum in guinea-pig

Guinea-pig ileum contracts via stimulation of a variety of receptors. Muscarine produced the contraction in a concentration-dependent manner (30 nM–3 μM) as shown in Fig. 5. Ginsenoside-Ro and -Rg_2 even at a higher concentration (100 μM) scarcely affected the muscarine-induced contraction (Fig. 5A,B). On the other hand, ginsenoside-Rg_3 at concentrations of 3–100 μM gave a parallel shift to the right of the concentration–response curve to muscarine (Fig. 5D). The Schild plot analysis showed that the slope of ginsenoside-Rg_3 was 0.70, suggesting that the mode of the ginsenoside-Rg_3 antagonism is unsurmountable (Table 2). The IC_{50} value for the saponin on the contraction induced by 300 nM muscarine whose concentration caused the submaximal response was 17 μM (Table 2). Ginsenoside-Rb_1, the same panaxadiol, at a higher concentration (100 μM) suppressed the contraction

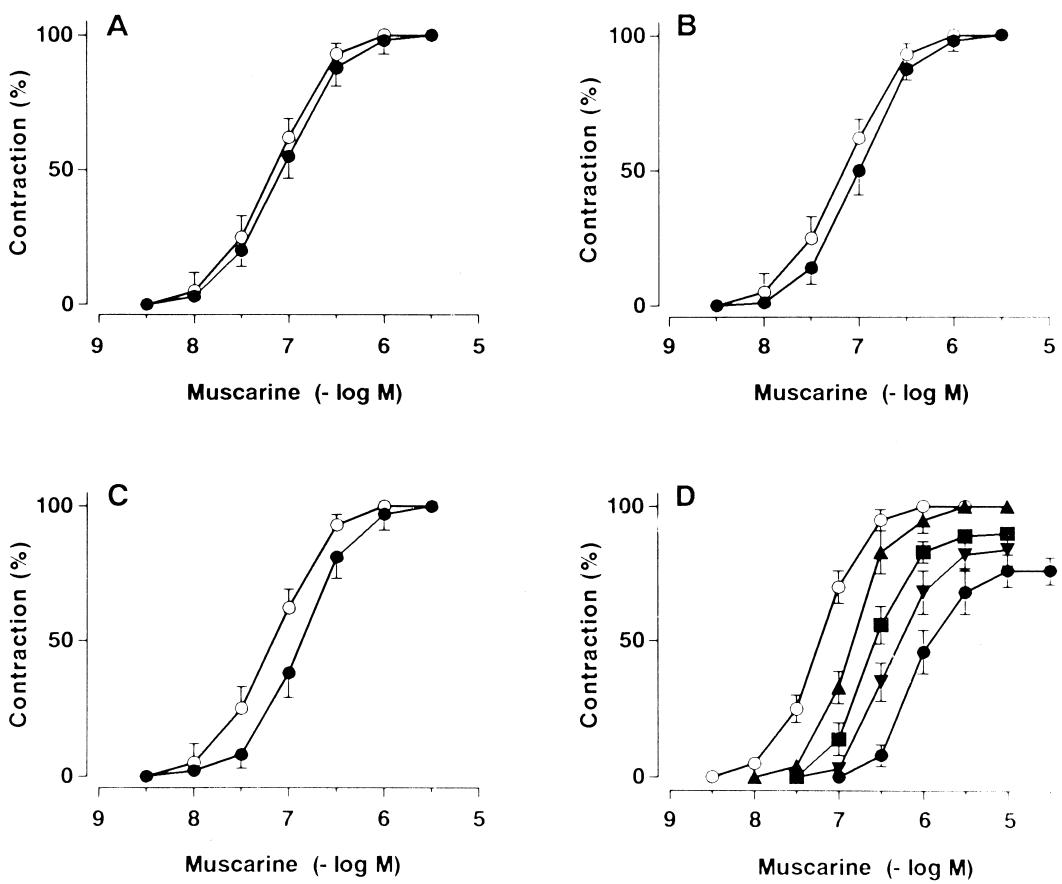

Fig. 5. Effects of ginsenoside-Rb_1, -Rg_2, -Rg_3 and -Ro on guinea-pig ileum contraction induced by muscarine. The isolated ileum was preincubated without (○) or with ginsenoside-Ro (100 μM; ●) (A), -Rg_2 (100 μM; ●) (B), -Rb_1 (100 μM; ●) (C) or -Rg_3 (3; ▲, 10; ■, 30; ▼ and 100 μM; ●) (D) in the medium for 1 min, and muscarine (3 nM–30 μM: final concentrations) was then added to the medium. The ileum contractions were determined as described in Section 2. The maximal contraction induced by 3 μM muscarine was expressed as 100%. Values are means ± S.D. from at least four experiments.

Table 2
Effects of ginsenoside-Rg_3 on muscarine- and histamine-induced ileum contractions in guinea-pig

Stimuli	Ginsenoside-Rg_3	
	IC_{50} (μM)	Slope
Mus	17	0.70
His	14	1.46

The IC_{50} values were evaluated from the log dose–response curve of ginsenoside-Rg_3 on the muscarine (Mus) (at 300 nM)- and the histamine (His) (at 3 μM)-induced ileum contractions in guinea-pig. The value of the slope was obtained by the Schild plot analysis.

induced by the lower concentrations of muscarine (30–300 nM; Fig. 5C).

3.4. Effects of ginsenosides on histamine-induced contraction of ileum

Histamine also contracted the ileum in a concentration-dependent manner (300 nM–10 μM) (Fig. 6). Ginsenoside-Rg_3 at concentrations of 3–100 μM gave a parallel shift to the right of the concentration–response curve to histamine (Fig. 6D). The Schild plot analysis showed that the slope of ginsenoside-Rg_3 was 1.46, suggesting that the mode of the ginsenoside-Rg_3 antagonism is also unsurmountable (Table 2). The IC_{50} value of the saponin on the contraction induced by 3 μM histamine whose concentration caused the submaximal responses was 14 μM (Table 2). On the other hand, neither ginsenoside-Ro, -Rg_2 nor -b_1 (100 μM) affected the contraction (Fig. 6A,B,C).

Muscarine and histamine receptors is considered to be also present in neural and mast cells in the isolated ileum as well as in the muscle. Therefore, we examined the effect of ginsenoside-Rg_3 on the muscarine-induced and the histamine-induced contractions of the ileum in the presence of hexamethonium (1 μM), atropine (100 nM) or diphenhydramine (200 nM) at the concentrations which did not affect other receptors. The muscarine (at 50 nM)-induced contraction was not altered by hexamethonium or diphenhydramine and also the histamine (200 nM)-induced contraction was not influenced by hexamethonium or atropine. Under these conditions, the ginsenoside-Rg_3 inhibition of the ileum contraction was still maintained (data not shown).

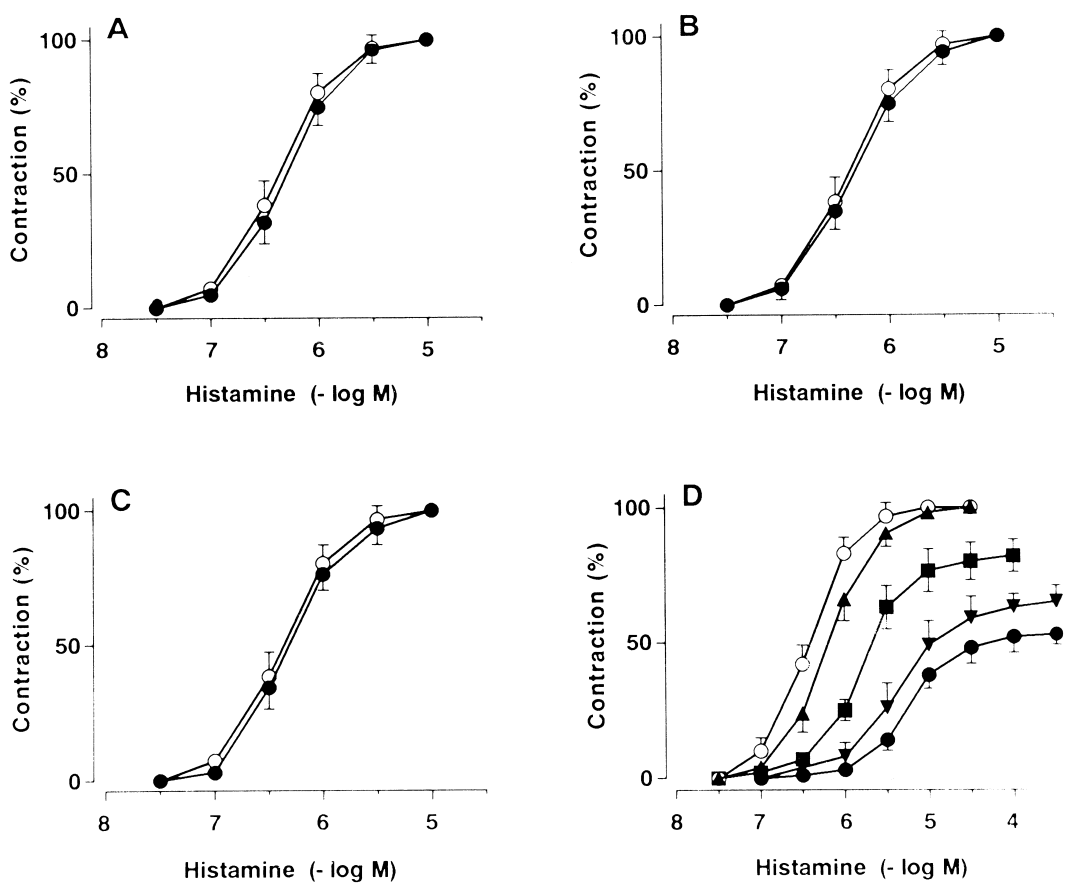

Fig. 6. Effects of ginsenoside-Rb_1, -Rg_2, -Rg_3 and -Ro on guinea-pig ileum contraction induced by histamine. The ileum was preincubated without (○) or with ginsenoside-Ro (100 μM; ●) (A), -Rg_2 (100 μM; ●) (B), -Rb_1 (100 μM; ●) (C) or -Rg_3 (3; ▲, 10; ■, 30; ▼, and 100 μM; ●) (D) in the medium for 1 min and histamine (His; 30 nM–300 μM) was added to the medium. The ileum contractions were determined as described in Section 2. The contraction induced by 10 μM histamine was expressed as 100%. Values are means ± S.D. from at least four experiments.

4. Discussion

In this study, we demonstrated the influences of representative ginseng saponins (ginsenosides) of each group (the panaxatriols, the panaxadiols and the oleanolic acid) on the responses to various receptor stimuli. The effects of ginsenosides on receptor stimulation-responses are summarized in Table 3. In bovine adrenal chromaffin cells, acetylcholine and γ-aminobutyric acid cause the secretion of catecholamines via stimulation of the nicotinic receptors and the $GABA_A$ receptors, respectively, which are ionotropic receptors (Kitayama et al., 1990; Tachikawa et al., 1995). On the other hand, other stimuli, angiotensin II, bradykinin, histamine and neurotensin, stimulate each GTP-binding protein coupled receptors and produce the secretion (Schneider, 1969; Livett and Marley, 1986; Noble et al., 1988; O'Sullivan and Burgoyne, 1989; Bunn, 1990).

We have already shown that ginsenoside-Rg_2, a panaxatriol saponin, inhibits Na^+ influx due to directly antagonizing nicotinic acetylcholine receptor-operated cation channels and consequently reduces the secretion of catecholamines in bovine adrenal chromaffin cells (Tachikawa et al., 1995). Also in the present study, ginsenoside-Rg_2 strongly inhibited the nicotine-induced secretion of catecholamines in the adrenal chromaffin cells of the guinea-pig (Fig. 4), confirming that ginsenoside-Rg_2 acts on the nicotinic receptors. The saponin also inhibited the catecholamine secretion from bovine adrenal chromaffin cells induced by γ-aminobutyric acid (Fig. 2B, Fig. 3A and Table 3). On the other hand, the muscarine-, the histamine-, the angiotensin II-, the bradykinin-, and the neurotensin-induced responses were not at all affected by ginsenoside-Rg_2 (Figs. 2, 3, 5 and 6 and Table 3). The $GABA_A$ receptors are ionotropic receptors selective in Cl^- and are pentametric oligomers, which consist of four kinds of subunits (Parramón et al., 1995), similar to the nicotinic acetylcholine receptors selective in cations. Thus, the antagonism of ginsenoside-Rg_2 seems to be selective for ionotropic receptors. Therefore, their receptors may have a common site to be sensitive to the saponin.

Panaxadiol saponins have shown very feeble inhibition of the acetylcholine-induced catecholamine secretion in bovine adrenal chromaffin cells (Tachikawa et al., 1995). However, contrary to our expectation, ginsenoside-Rg_3 in the panaxadiols produced a great inhibition of the secretion comparable to the ginsenoside-Rg_2 inhibition. The inhibitory effect of ginsenoside-Rg_3 was also caused by affecting nicotinic acetylcholine receptor-channels (Kudo et al., 1997). In this study, ginsenoside-Rg_3 (1–100 μM) strongly reduced the γ-aminobutyric acid-induced secretion from the bovine cells (Fig. 2C, Fig. 3A and Table 3). Furthermore, ginsenoside-Rg_3 strongly inhibited the muscarine- and the histamine-induced ileum contractions of the guinea-pig (Fig. 5D, Fig. 6D and Table 3). This evidence strongly indicates that ginsenoside-Rg_3 blocks not only ionotropic receptors but also muscarinic acetylcholine and histamine receptors. However, the inhibitory effect of ginsenoside-Rg_3 on the histamine-induced catecholamine secretion was not so strong (Fig. 2C, Fig. 3B and Table 3), suggesting that the subtypes of histamine receptors in the guinea-pig ileum may be distinct from those in the bovine cells. On the other hand, the ginsenoside (1–100 μM) greatly diminished the neurotensin-induced secretion in the bovine cells in a concentration-dependent manner (Fig. 2C, Fig. 3E and Table 3). The enhancement of the hydrolysis of phosphatidylinositides induced by neurotensin in bovine adrenal chromaffin cells appears to be mediated by neurotensin NT_2 receptors (Bunn, 1990). Ginsenoside-Rg_3, therefore, may also behave as a blocker to neurotensin NT_2 receptors. Further analysis of the ginsenoside-Rg_3 antagonism to the receptors is needed.

On the other hand, in the guinea-pig chromaffin cells, ginsenoside-Rg_3 did not suppress the nicotine-induced secretion (Fig. 4), but it inhibited the secretion in the bovine

Table 3
Effects of various ginseng saponins (ginsenosides) on receptor stimulation-responses

Stimuli		Ginsenosides			
		Oleanolic acid	Panaxadiols		Panaxatriols
		Ro	Rb_1	Rg_3	Rg_2
Bovine					
Chromaffin cells	Nicotine	−	±	++	++
	Histamine	−	−	+	−
	Angiotensin II	−	−	+	−
	Bradykinin	−	−	±	−
	Neurotensin	−	−	++	−
	γ-aminobutyric acid	−	−	++	++
Guinea-pig					
Chromaffin cells	Nicotine	ND	−	−	++
Ileum	Muscarine	−	±	++	−
	Histamine	−	−	++	−

−: No effect; ±: slight inhibition; +: inhibition; ++: strong inhibition; ND: not determined.

cells (Kudo et al., 1998). As mentioned above, neuronal nicotinic acetylcholine receptors are pentametric oligomers consisting of α (α2–α9)- and β (β2–β4)-subunits (Sala et al., 1996). A very recent report suggested that α3, β4 and α5 subunits in bovine chromaffin cells participate in the formation of the neuronal receptors involved in triggering the catecholamine secretion (Campos-Caro et al., 1997). Although the constituent subunits of the receptors of guinea-pig chromaffin cells are not yet characterized, they are estimated to be the neuronal type similar to those of the bovine cells. Therefore, there may be subtle differences in their subunits between the bovine and the guinea-pig receptors. Ginsenoside-Rg_3 may recognize such differences.

Ginsenoside-Rg_3 at the higher concentrations suppressed the histamine-, the angiotensin II- and the bradykinin-induced responses in the bovine chromaffin cells (Fig. 2C, Fig. 3B,C,D and Table 3), whereas other ginsenosides tested in this study did not affected them, except for ginsenoside-Rb_1 which somewhat suppressed the muscarine-induced contraction of the ileum (Fig. 5C and Table 3). Ginsenoside-Rg_3 is eluted into a more highly lipophilic fraction by high performance liquid chromatography (Kitagawa et al., 1983; Kitagawa et al., 1987). Hence, the saponin at the higher concentrations probably changes the fluidity of the membranes rather than directly acts on the receptors and may suppress the responses. This view is supported by the fact that ginsenoside-Rg_3 at 100 μM, but not at lower concentrations, showed the reversibility of the inhibitory effect on the catecholamine secretion less clearly than that of ginsenoside-Rg_2 in the bovine cells (data not shown) (Tachikawa et al., 1995).

Another panaxadiol saponin, ginsenoside-Rb_1, at the high concentration (100 μM) inhibited the ileum contraction induced by muscarine, whereas it had no effect on all other responses (Fig. 2A, Figs. 3–5C, Fig. 6C and Table 3). On the other hand, only one oleanolic acid saponin, ginsenoside-Ro, did not affect all receptor stimulation-responses (Fig. 2D, Figs. 3 and 5A, Fig. 6A and Table 3). Thus, the effects of ginsenosides on their responses seem to be related to the structures (type of aglycon, and type and number of sugar). The ginseng root has been reported to exhibit many pharmacological effects as a medicine (e.g., replenishment of vital energy, tranquilization, mood elevation and prevention of aging). Therefore, the diverse actions of ginsenosides to receptors may be one of the reasons why the ginseng root has so many effects. An investigation of other ginsenoside effects on the responses is now in progress.

Acknowledgements

We thank Ms. Yuko Takeda for her excellent technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research (C) from The Ministry of Education, Science, Sports and Culture.

References

Bunn, S.J., 1990. Receptor stimulated formation of inositol phosphates in cultures of bovine adrenal medullary cells: the effects of bradykinin, bombesin and neurotensin. Neuropeptides 15, 187–194.

Campos-Caro, A., Smillie, F.I., Demínguez del Toro, E., Rovira, J.C., Vicente-Agulló, F., Chapuli, J., Juíz, J.M., Sala, S., Sala, F., Ballesta, J.J., Criado, M., 1997. Neuronal nicotinic acetylcholine receptors on bovine chromaffin cells: cloning, expression and genomic organization of receptor subunits. J. Neurochem. 68, 488–497.

Douglas, W.W., Poisner, A.M., 1961. Stimulation of uptake of ^{45}calcium in the adrenal gland by acetylcholine. Nature 192, 1229.

Hochman, J., Perlman, R.L., 1976. Catecholamine secretion by isolated adrenal cells. Biochim. Biophys. Acta 421, 168–175.

Holz, R.H., Senter, R.A., Fry, R.A., 1982. Relationship between Ca^{2+} uptake and catecholamine secretion in primary dissociated cultures of adrenal medulla. J. Neurochem. 39, 635–646.

Kawashima, Y., Samukawa, K., 1986. Studies of ginseng. J. Med. Pharm. Soc. WAKAN-YAKU 3, 236–237.

Kitagawa, I., Yoshikawa, M., Yoshihara, M., Hayashi, T., Taniyama, T., 1983. Chemical studies on crude drug precession: I. On the constituents of Ginseng Radix Rubra (1). Yakugaku Zasshi 103, 612–622.

Kitagawa, I., Taniyama, T., Shibuya, H., Noda, T., Yoshikawa, M., 1987. Chemical studies on crude drug procession. V. On the constituents of Ginseng Radix Rubra (2): comparison of the constituents of white ginseng and red ginseng prepared from the same *Panax ginseng* root. Yakugaku Zasshi 107, 495–505.

Kitayama, S., Morita, K., Dohi, T., Tsujimoto, A., 1990. GABAergic modulation of catecholamine release from cultured bovine adrenal chromaffin cells. Evidence for the involvement of Cl^--dependent Ca^{2+} entry. Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 419–424.

Kudo, K., Akasaka, Y., Miyate, Y., Takahashi, E., Tachikawa, E., Kashimoto, T., 1992. Effects of red ginseng fractions on catecholamine secretion from bovine adrenal medullary cells. J. Med. Pharm. Soc. WAKAN-YAKU 9, 236–239.

Kudo, K., Tachikawa, E., Kashimoto, T., Takahashi, E., 1997. Comparison between ginsenoside-Rg_2 and -Rg_3 effects on catecholamine secretion from adrenal chromaffin cells. Jpn. J. Pharmacol. 73, 79P.

Kudo, K., Tachikawa, E., Kashimoto, T., Takahashi, E., 1998. Properties of ginseng saponins of catecholamine secretion in bovine adrenal chromaffin cells. Eur. J. Pharmacol. 341, 139–144.

Livett, B.G., Marley, P.D., 1986. Effects of opioid peptides and morphine on histamine-induced catecholamine secretion from cultured, bovine adrenal cells. Br. J. Pharmacol. 89, 327–334.

Noble, E.P., Bommer, M., Liebisch, D., Herz, A., 1988. H_1-histaminergic activation of catecholamine release by chromaffin cells. Biochem. Pharmacol. 37, 221–228.

O'Sullivan, A.J., Burgoyne, R.D., 1989. A comparison of bradykinin, angiotensin II and muscarinic stimulation of cultured bovine adrenal chromaffin cells. Biosci. Rep. 9, 243–252.

Parramón, M., González, M.P., Oset-Gasque, M.J., 1995. Pharmacological modulation of adrenal medullary $GABA_A$ receptor: consistent with its subunit composition. Br. J. Pharmacol. 116, 1875–1881.

Saito, H., 1990. Effects of ginsenoside Rb_1 and ginsenoside Rg_1 on learning and memory. In: Shibata, S., Ohtsuka, Y., Saito, H. (Eds.), Recent Advances in Ginseng Studies. Hirokawa Publishing, Tokyo, pp. 99–112.

Sala, C., Kimura, I., Santoro, G., Kimura, M., Fumagalli, G., 1996. Expression of two neuronal nicotinic receptor subunits in innervated and denervated adult rat muscle. Neurosci. Lett. 215, 71–74.

Schneider, F.H., 1969. Secretion from the cortex-free bovine adrenal medulla. Br. J. Pharmacol. 37, 371–379.

Snedecor, G.W., Cochran, W.G., 1967. Statistical Methods. Iowa State Univ. Press, Ames.

Tachikawa, E., Takahashi, S., Furumachi, K., Kashimoto, T., IIda, A., Nagaoka, Y., Fujita, T., Takaishi, Y., 1991. Trichosporin-B-III, an α-aminoisobutyric acid-containing peptide, causes Ca^{2+}-dependent catecholamine secretion from adrenal medullary chromaffin cells. Mol. Pharmacol. 40, 790–797.

Tachikawa, E., Kudo, K., Kashimoto, T., Takahashi, E., 1995. Ginseng saponins reduce acetylcholine-evoked Na^{+} influx and catecholamine secretion in bovine adrenal chromaffin cells. J. Pharmacol. Exp. Ther. 273, 629–636.

Weil-Malherbe, H., Bone, A.D., 1952. The chemical estimation of adrenalin-like substances in blood. Biochem. J. 51, 311–318.

Wilson, W.W., Kirshner, N., 1977. The acetylcholine receptor of the adrenal medulla. J. Neurochem. 28, 687–695.

Yoshimura, H., Watanabe, K., Ogawa, N., 1988. Acute and chronic effects of ginseng saponins on maternal aggression in mice. Eur. J. Pharmacol. 150, 319–324.