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Ginsenoside Rg_3 mediates endothelium-dependent relaxation in response to ginsenosides in rat aorta: role of K^+ channels

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Abstract

The aim of the present study was to characterize the endothelium-dependent relaxation elicited by ginsenosides, a mixture of saponin extracted from *Panax ginseng*, in isolated rat aorta. Relaxations elicited by ginsenosides were mimicked by ginsenoside Rg_3 and ginsenoside Rg_1, two major ginsenosides of the protopanaxatriol group. Ginsenoside Rg_3 was about 100-fold more potent than ginsenoside Rg_1. The endothelium-dependent relaxation in response to ginsenoside Rg_3 was associated with the formation of cyclc GMP. These effects were abolished by N^G-nitro-L-arginine and methylene blue. Relaxations in response to ginsenoside Rg_3 were unaffected by atropine, diphenhydramine, [D-Pro^2, D-Trp^{7,9}]substance P, propranolol, nifedipine, verapamil and glibenclamide but were markedly reduced by tetraethylammonium. Tetraethylammonium modestly reduced the relaxation induced by sodium nitroprusside. These findings indicate that ginsenoside Rg_3 is a major mediator of the endothelium-dependent nitric oxide-mediated relaxation in response to ginsenosides in isolated rat aorta, possibly via activation of tetraethylammonium-sensitive K^+ channels.

Keywords: Ginsenoside Rg_3; Nitric oxide (NO); cGMP; Vascular relaxation; K^+ channel; Aorta, rat

1. Introduction

The endothelium plays an important role in regulating vascular tone by releasing several vasoactive autacoids including prostacyclin (Vane et al., 1990), endothelium-derived relaxing factor (EDRF; Furchgott and Zawadzki, 1980) and endothelium-derived hyperpolarizing factor (EDHF; Feletou and Vanhoutte, 1988). EDRF has been identified as nitric oxide (NO), which is produced from L-arginine by the binding of Ca^{2+}-calmodulin to nitric oxide synthase (Ignarro et al., 1987; Palmer et al., 1987, 1988a,b; Furchgott, 1988). NO relaxes blood vessels mostly by stimulating soluble guanylyl cyclase, which leads to an increased production of cGMP in vascular smooth muscle (Rapoport and Murad, 1983). N^G-nitro-L-arginine (NLA), an inhibitor of nitric oxide synthase (Rees et al., 1990), and methylene blue, an inhibitor of soluble guanylyl cyclase (Katsuki and Murad, 1977), inhibit the endothelium-dependent relaxation and accumulation of cGMP induced by agonists in isolated blood vessels (Miller et al., 1984; Wood et al., 1990; Moncada et al., 1991; Gray and Marshall, 1992). EDRF is released under basal conditions and its release is further stimulated by various agonists, such as acetylcholine, histamine, and substance P (Furchgott, 1983; Gray and Marshall, 1992).

Receptor-binding agonists cause an increase in $[Ca^{2+}]_i$ by mobilization of Ca^{2+} from intracellular stores, which subsequently leads to the opening of Ca^{2+}-dependent K^+ channels in endothelial cells. The opening of the Ca^{2+}-dependent K^+ channel increases K^+ efflux, hyperpolarizing the endothelial cells. This hyperpolarization provides the driving force for transmembrane Ca^{2+} influx into endothelial cells and thus causes the synthesis and release of EDRF (Adams et al., 1989; Lückhoff and Busse, 1990; Mehrke and Daut, 1990). Tetraethylammonium inhibits the EDRF release and $[Ca^{2+}]_i$ elevation induced by acetylcholine whereas glibenclamide has no effect in rabbit aorta (Demirel et al., 1994).

Ginsenosides (a mixture of saponin from *Panax ginseng*) induce endothelium-dependent relaxation and increase the tissue content of cGMP in isolated rat thoracic

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aorta, possibly due to the release of EDRF (Kim et al., 1994). Ginsenosides are a mixture of triterpene glycosides. The major forms of these glycosides belong either to the protopanaxadiol group or to the protopanaxatriol group (Ando et al., 1971). The protopanaxatriol group of ginsenosides and the purified ginsenosides Rg_1 and Re cause endothelium-dependent relaxation that is associated with the formation of cGMP. In contrast, the protopanaxadiol group of ginsenosides and the purified ginsenosides Rb_1 and Rc do not affect vascular tone or the production of cGMP in rat aorta (Kang et al., 1995b). However, ginsenosides Rg_1 and Re are less effective as endothelium-dependent vasodilators than are the ginsenosides (total saponin) and the protopanxatriol group of ginsenosides (Kim et al., 1994; Kang et al., 1995b).

Recently, we detected ginsenoside Rg_3 in the protopanaxatriol group of ginsenosides, as determined by high-performance liquid chromatography (HPLC) (Park et al., 1996). The primary aim of this study was to determine whether ginsenoside Rg_3 causes endothelium-dependent relaxation by enhancing the release of nitric oxide from endothelial cells in the rat aorta. In addition, we were interested in the effect of K^+ channel blockers on the ginsenoside Rg_3-induced vasodilatation in rat aortic rings. We also determined the effect of tetraethylammonium and glibenclamide, which block different types of K^+ channels.

2. Materials and methods

2.1. Materials

N^G-nitro-L-arginine (NLA) was purchased from Aldrich Chemical (Milwaukee, WI). Ginsenoside Rg_3 was isolated

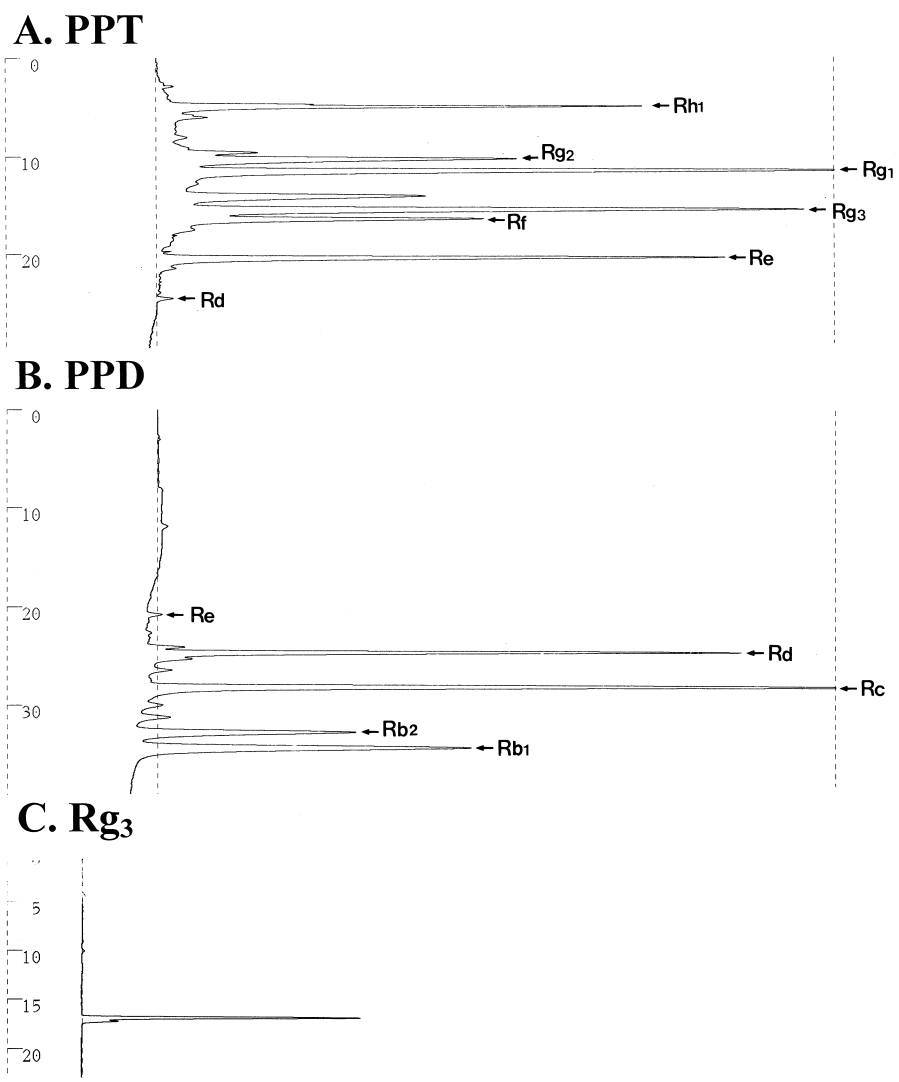

Fig. 1. Representative chromatogram of the (A) protopanaxatriol group of ginsenosides (PPT) and (B) protopanaxadiol group of ginsenosides (PPD) and (C) ginsenoside Rg_3. All ginsenosides were extracted from red ginseng (steamed ginseng).

from the protopanaxatriol group of ginsenosides, which were extracted from red ginseng, by the methods of Kitagawa et al. (1983) (Fig. 2). Protopanaxatriol and protopanaxadiol groups of ginsenosides, ginsenoside Rg_1 and ginsenosides were provided by the Korea Ginseng and Tobacco Research Institute (Daejun, South Korea).

2.2. HPLC analysis of protopanaxatriol and protopanaxadiol group of ginsenosides

Ginsenosides of the protopanaxatriol and protopanaxadiol groups were dissolved in methanol and were subjected to HPLC analysis. The HPLC system consisted of two Model SLC-100 pumps (Samsung, Suwon, South Korea), a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model ELSD IIA detector (Varex, Burtonsville, MD, USA) and a Model C-R4A chromatopac integrator (Schimadzu, Kyoto, Japan). The columns used for the separation of ginsenosides were LiChrosorb NH_2 (250 mm × 4 mm i.d., 5 μm, Merck). The separation of the ginsenosides was effected by gradient elution with (A) CH_3CN-Water-2-PrOH (80:5:15) and (B) CH_3CN-Water-2-PrOH (80:20:15). The solvent flow rate was held constant at 1.0 ml/min at ambient temperature throughout the analysis (Park et al., 1996).

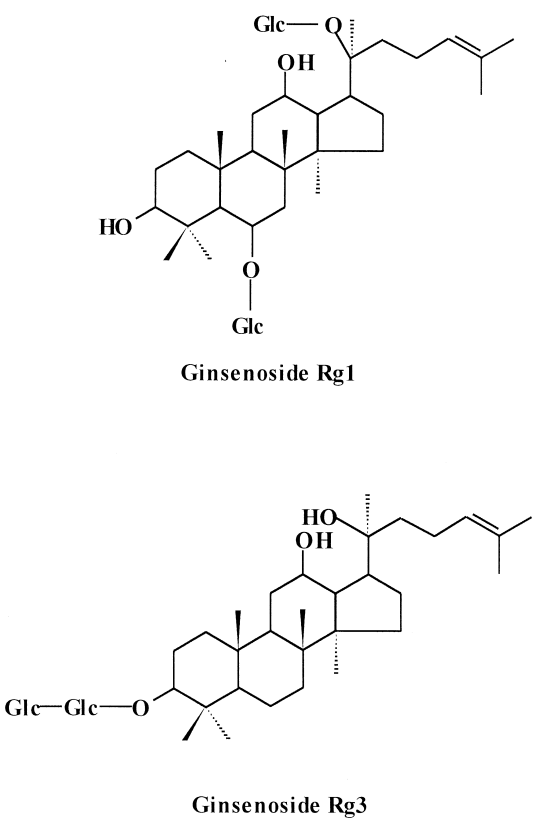

Fig. 2. Chemical structure of the ginsenoside Rg_1 and ginsenoside Rg_3 purified from the protopanaxatriol group of ginsenosides (PPT).

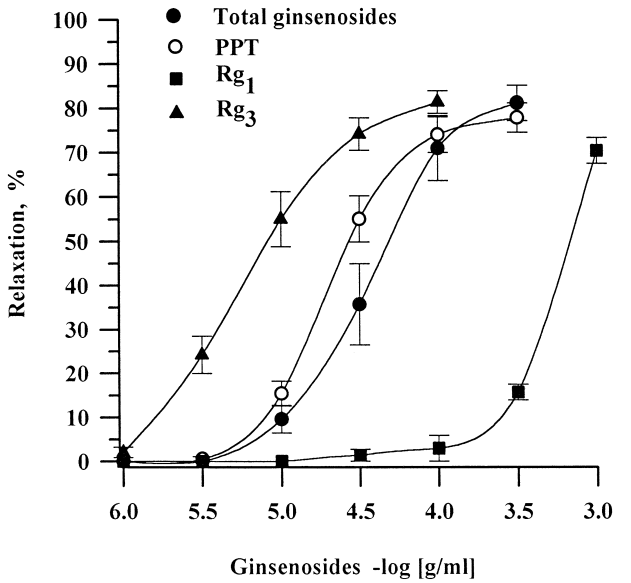

Fig. 3. Concentration–relaxation curves for a total mixture of ginsenosides extracted from red ginseng, ginsenosides from the protopanaxatriol group (PPT), and the purified ginsenosides from the protopanaxatriol group of ginsenosides (PPT), Rg_1 and Rg_3, in endothelium-intact rat aortic rings constricted with phenylephrine (10^{-6} M). The constriction induced by pheylephrine was 2.46 ± 0.18 g, 2.48 ± 0.19 g, 2.53 ± 0.12 g and 2.51 ± 0.11 g for total ginsenosides-, PPT-, Rg_1- and Rg_3-treated aortic rings, respectively. All experiments were performed in the presence of indomethacin (10^{-5} M). Results are shown as means ± S.E.M. of 4 to 8 experiments.

2.3. Organ chamber studies

Male Sprague–Dawley rats (270–330 g) were killed and thoracic aortas were removed and placed in a modified Krebs–Ringer–bicarbonate solution containing (in mM) NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; CaEDTA, 0.016; and glucose, 11.1 (control solution). The aortas were cleaned of loose connective tissue and then cut into rings (2–3 mm wide). In some rings, the endothelium was removed mechanically. The aortic rings were suspended horizontally between two stainless steel stirrups in organ chambers filled with 10 ml of control solution (37°C, pH 7.4) and bubbled with 95% O_2 and 5% CO_2. One of the stirrups was anchored to the organ chamber and one was connected to a transducer coupler (Narco bio-system) for the recording of isometric tension. The rings were stretched progressively to the optimal tension (2 g) before the addition of phenylephrine (10^{-6} M). Once the plateau of the contraction elicited by phenylephrine was obtained, the aortic rings were rinsed three times with warm (37°C) control solution. After a resting period (30 min), the aortic rings were exposed again to phenylephrine (10^{-6} M). When the contraction had stabilized, acetylcholine (10^{-6} M) was added to test for the presence of the endothelium. The organ chambers

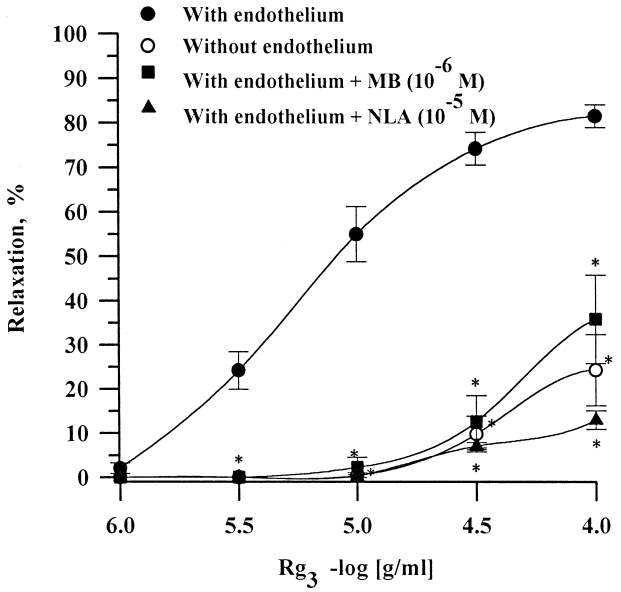

Fig. 4. Effect of methylene blue (MB, 10^{-6} M) and N^G-nitro-L-arginine (NLA, 10^{-5} M) on ginsenoside Rg_3-evoked endothelium-dependent relaxation in aortic rings constricted with phenylephrine (10^{-6} M). The small concentration-dependent relaxation evoked by ginsenoside Rg_3 in endothelium-denuded aortic rings is also shown. The constriction induced by phenylephrine was 2.53 ± 0.11 g, 2.80 ± 0.10 g, 2.82 ± 0.33 g and 3.30 ± 0.30 g in Rg_3-treated aortic rings with endothelium, with endothelium and NLA, with endothelium and methylene blue and without endothelium, respectively. All experiments were performed in the presence of indomethacin (10^{-5} M). Results are shown as means $\pm$ S.E.M. of 4 to 8 experiments. $*$ significant inhibitory effect of methylene blue (10^{-6} M) and NLA (10^{-5} M) ($P < 0.05$).

were rinsed three times with warm (37°C) control solution before the addition of indomethacin (10^{-5} M) to prevent the production of endogenous vasoactive prostanoids. In some experiments, rings were incubated with either N^G-nitro-L-arginine (10^{-5} M), an inhibitor of nitric oxide synthase; methylene blue (10^{-6} M), an inhibitor of soluble guanylyl cyclase; atropine (10^{-6} M), a muscarinic receptor antagonist; diphenhydramine (10^{-5} M), a histamine receptor antagonist; [D-Pro^2, D-Trp^{7,9}] substance P (5×10^{-6} M), a substance P receptor antagonist; propranolol (10^{-6} M), a β-adrenoceptor antagonist; tetraethylammonium (1–5 mM), a non-selective K^+ channel blocker; glibenclamide (10^{-5} M), an ATP-sensitive K^+ channel blocker; verapamil (10^{-6} M) or nifedipine (3×10^{-6} M), Ca^{2+} channel blockers, for 30 min before the addition of phenylephrine (10^{-6} M). Once the plateau of the contraction elicited by phenylephrine was achieved, a cumulative concentration–relaxation curve for ginsenoside Rg_3 (10^{-6}–10^{-4} g/ml) was made. To determine the effect of tetraethylammonium on the relaxation in response to sodium nitroprusside in endothelium-denuded aortic rings tetraethylammonium (5×10^{-3} M) was added before the addition of phenylephrine (10^{-6} M). A cumulative concentration–relaxation curve for sodium nitroprusside (10^{-9}–10^{-5} M) was then made.

2.4. Measurement of cGMP levels

The cGMP level of aortic rings was measured in rings that were not under tension (Vidal et al., 1991). Aortic rings with and without endothelium were incubated in flasks containing 10 ml of control solution for 30 min at 37°C and gassed with 95% O_2–5% CO_2. After the equilibration period, the incubation medium was removed and replaced with warmed (37°C), oxygenated control solution

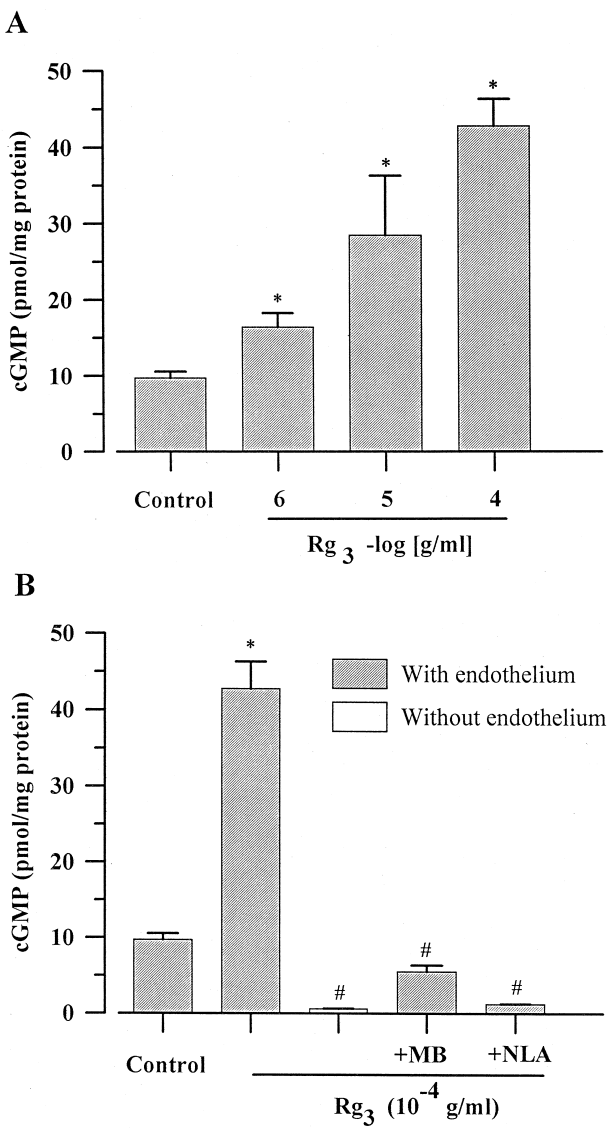

Fig. 5. (A) Ginsenoside Rg_3 caused a concentration-dependent accumulation of cGMP in aortic rings with endothelium. (B) Effect of methylene blue (MB, 10^{-6} M) and N^G-nitro-L-arginine (NLA, 10^{-5} M) on the ginsenoside Rg_3-induced accumulation of cGMP in aortic rings with endothelium. The effect of ginsenoside Rg_3 on aortic rings without endothelium is also shown. Aortic rings were treated with ginsenoside Rg_3 for 2 min in the presence of indomethacin (10^{-5} M) and IBMX (10^{-4} M). Results are shown as means $\pm$ S.E.M. from 5 to 10 experiments. $* P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. ginsenoside Rg_3.

containing indomethacin (10^{-5} M) and 3-isobutyl-1-methylxanthine (IBMX; a non-selective phosphodiesterase inhibitor; 10^{-4} M). In some experiments, methylene blue (10^{-6} M) or N^G-nitro-L-arginine (10^{-5} M) was added to the incubation medium. The vessels were then allowed to equilibrate for an additional 30 min before the addition of phenylephrine (10^{-6} M). After 7 min, ginsenoside Rg_3 (10^{-6}–10^{-4} g/ml, 2 min) was added. The tissues were frozen rapidly with an aluminium clamp cooled in liquid nitrogen. The frozen tissues were pulverized, homogenized in 1 ml of 6% trichloroacetic acid with a glass–glass potter and centrifuged at 13,600 *g* for 16 min. The supernatant was extracted four times with 4 vol of water-saturated ether, lyophilized and stored at −70°C. On the day of assay, each sample was resuspended in 0.5 ml of sodium acetate buffer (0.05 M, pH 6.2). The content of cGMP in each sample was determined by using a radioimmunoassay kit (Steiner et al., 1972). The amount of protein was determined by the method of Lowry et al. (1951).

2.5. Statistical analysis

The data are expressed as means ± S.E.M. The number of rings obtained from different rats is represented by *n*. The relaxing response is expressed in terms of the percent decrease of the maximal contraction developed in response to phenylephrine (10^{-6} M). Statistical significance was analyzed by Student's *t*-test or when more than two were treated compared by analysis of variances, and *P* values of less than 0.05 were considered as significant.

3. Results

3.1. HPLC chromatograms of ginsenosides of the protopanaxatriol and protopanaxadiol groups

Fig. 1A,B show typical chromatograms of the protopanaxatriol and protopanaxadiol ginsenosides extracted from red ginseng (Steamed ginseng). The elution profile for the protopanaxatriol group showed that ginsenoside Rh_1 was eluted first, followed by ginsenosides Rg_2, Rg_1, Rg_3, Rf, Re and Rd. The sequence of ginsenoside elution for the protopanaxadiol group was ginsenosides Re, Rd, Rc, Rb_2 and Rb_1. The major ginsenoside present in the protopanaxatriol group, ginsenoside Rg_3, was purified (Fig. 1C), and Fig. 2 shows the chemical structures of ginsenoside Rg_1 and Rg_3.

3.2. Effects of ginsenoside Rg_3 on vascular tone

The mixture of ginsenosides extracted from red ginseng caused a concentration-dependent relaxation of endothelium-intact aortic rings contracted with phenylephrine (Fig. 3). Although ginsenosides of the protopanaxadiol group did not affect the phenylephrine-induced tension in aortic rings with endothelium, those of the protopanaxatriol group induced a concentration-dependent relaxation (Fig. 3; Kang et al., 1995b). Ginsenosides of the protopanaxatriol group were slightly but significantly more potent than the total mixture of ginsenosides. In order to identify the ginsenosides mediating this relaxation, the effect of two major ginsenosides of the protopanaxatriol group, ginsenoside Rg_1 and Rg_3, was examined. Significant relaxations were induced by ginsenoside Rg_1 at concentrations greater than 10^{-4} g/ml and by ginsenoside Rg_3 at concentrations greater than 10^{-6} g/ml (Fig. 3). Since ginsenoside Rg_3 was the most potent relaxing ginsenoside of the protopanaxatriol group, experiments were planned to characterize the mechanisms underlying the relaxation. Although ginsenoside Rg_3 induced a concentration-dependent relaxation in aortic rings with endothelium, only a slight relaxation was observed in rings without endothelium (Fig. 4). The endothelium-dependent relaxation in response to ginsenoside Rg_3 was markedly inhibited by N^G-nitro-L-arginine (10^{-5} M) and by methylene blue (10^{-6} M; Fig. 4).

Table 1
The effects of the receptor antagonists, Ca^{2+}- and K^+-channel blockers on the Rg_3-induced concentration-dependent relaxation of aortic rings with endothelium constricted with phenylephrine (10^{-6} M)

		Phenylephrine-induced contraction after antagonists (g)	% Relaxation to Rg_3				
			6	5.5	5 (-log g/ml)	4.5	4
Control E(+)	(8)	2.38 ± 0.1	1.84 ± 0.9	18.9 ± 3.9	44.1 ± 6.6	72.3 ± 4.1	83.1 ± 2.6
with atropine (10^{-6} M)	(4)	2.01 ± 0.2	4.90 ± 2.3	18.4 ± 5.1	37.1 ± 6.1	78.4 ± 4.2	89.8 ± 0.8
with diphenhydramine (10^{-5} M)	(4)	2.17 ± 0.2	5.60 ± 1.4	23.8 ± 4.9	44.1 ± 7.1	79.7 ± 4.2	88.3 ± 1.4
with propranolol (10^{-6} M)	(6)	2.64 ± 0.1	3.75 ± 1.5	11.4 ± 4.2	37.6 ± 5.4	63.9 ± 4.8	78.4 ± 1.3
with [D-Pro,D-Trp]substance P (5×10^{-6} M)	(4)	2.14 ± 0.1	1.70 ± 1.5	29.9 ± 7.0	52.0 ± 3.0	70.0 ± 3.2	76.2 ± 5.5
with glibenclamide (10^{-5} M)	(5)	2.10 ± 0.3	2.48 ± 1.5	11.6 ± 2.0	36.4 ± 4.9	66.0 ± 3.1	84.3 ± 4.7
with verapamil (10^{-6} M)	(5)	1.68 ± 0.1[a]	4.80 ± 1.6	29.3 ± 6.7	58.0 ± 8.9	71.6 ± 8.1	82.4 ± 6.1
with nifedipine (3×10^{-6} M)	(5)	1.20 ± 0.1[a]	1.50 ± 1.5	23.2 ± 11.6	58.4 ± 9.7	80.1 ± 4.5	86.9 ± 5.4

The numbers in parenthesis indicate the number of experiments (n) with different animals. Results are shown as means ± S.E.M. of n experiments.
[a]significantly different from control.

3.3. Effects of ginsenoside Rg_3 on the cGMP content

Ginsenoside Rg_3 increased in a concentration-dependent manner the tissue content of cGMP in aortic rings with but not in those without endothelium (Fig. 5A,B). Both N^G-nitro-L-arginine (10^{-5} M) and methylene blue (10^{-6} M) abolished the stimulatory effect of ginsenoside Rg_3 (Fig. 5B).

3.4. Effects of receptor antagonists on the relaxation in response to ginsenoside Rg_3

Endothelium-dependent relaxations evoked by ginsenoside Rg_3 were affected by neither atropine (10^{-6} M), [D-Pro^2, D-Trp^{7,9}] substance P (5×10^{-6} M), propranolol (10^{-6} M) nor diphenhydramine (10^{-5} M; Table 1).

3.5. Effects of Ca^{2+} channel blockers and K^+ channel blockers on ginsenoside Rg_3-induced relaxation

Ginsenoside Rg_3-induced endothelium-dependent relaxation was affected neither by nifedipine (3×10^{-6} M) nor by verapamil (10^{-6} M; Table 1). Exposure of aortic rings to tetraethylammonium significantly shifted to the right the concentration-dependent relaxation curve for ginsenoside Rg_3 and reduced the maximal relaxation in rings with endothelium (Fig. 6) whereas glibenclamide had no such effect (Table 1). Tetraethylammonium (10^{-3} M and 5×10^{-3} M) did not affect basal tension.

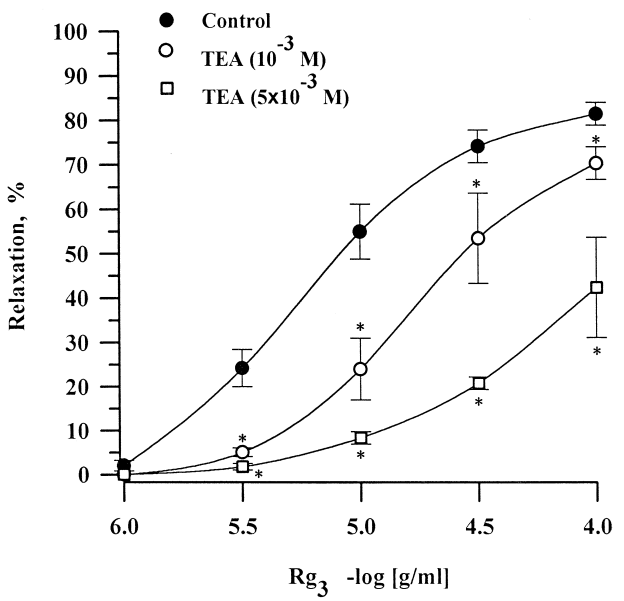

Fig. 6. Effect of tetraethylammonium (TEA) on the ginsenoside Rg_3-induced concentration-dependent relaxation of aortic rings with endothelium constricted with phenylephrine. The constriction induced by phenylephrine was 2.51 ± 0.11 g, 2.45 ± 0.06 g and 2.68 ± 0.14 g in control and tetraethylammonium (10^{-3} M and 5×10^{-3} M)-treated rings, respectively. All experiments were performed in the presence of indomethacin (10^{-5} M). Results are shown as means $\pm$ S.E.M. of 4 to 8 experiments. $*$ significant inhibitory effect of tetraethylammonium (10^{-3} M, $P < 0.05$; 5×10^{-3} M, $P < 0.01$).

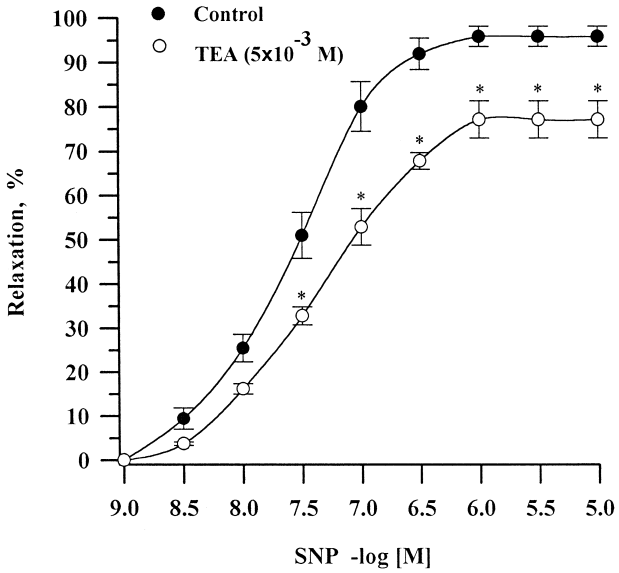

Fig. 7. Effect of tetraethylammonium (TEA) on the sodium nitroprusside (SNP)-induced concentration-dependent relaxation of aortic rings without endothelium constricted with phenylephrine. The constriction induced by phenylephrine was 3.38 ± 0.54 g and 3.10 ± 0.11 g in control and tetraethylammonium-treated rings, respectively. Results are shown as means $\pm$ S.E.M. of 4 experiments. $*$ significant inhibitory effect of tetraethylammonium (5×10^{-3} M, $P < 0.05$).

3.6. Effect of tetraethylammonium on sodium nitroprusside-induced relaxation

Exposure of aortic rings to tetraethylammonium slightly but significantly shifted to the right the relaxation curve for sodium nitroprusside and reduced the maximal relaxation in rings without endothelium (Fig. 7).

4. Discussion

The present study demonstrates that ginsenoside Rg_3 enhances the release of nitric oxide from endothelial cells in rat aorta. Recent studies from our laboratory have shown that ginsenosides cause endothelium-dependent relaxation of isolated rat and rabbit thoracic aortas (Kim et al., 1994). Ginsenosides extracted from *Panax ginseng* are a mixture of over 10 ginsenosides. Ginsenosides can be fractionated into two groups based on the type of aglycone, namely the protopanaxadiol ginsenoside group and the protopanaxatriol ginsenoside group. It was demonstrated that the protopanaxatriol group of ginsenosides and the purified ginsenosides Rg_1 and Re relaxed rings of rat aorta with endothelium whereas the protopanaxadiol group of ginsenosides and purified Rb_1 and Rc did not. However, ginsenoside Rg_1 and Re from the protopanaxatriol group

of ginsenosides turned out to be much less effective as endothelium-dependent vasodilators than unpurified ginsenosides (Kim et al., 1994) and the protopanaxatriol group of ginsenosides (Kang et al., 1995b), implying that ginsenosides Rg_1 and Re cannot fully account for the endothelium-dependent relaxation in response to the protopanaxatriol group of ginsenosides and unpurified ginsenosides. To identify a more effective endothelium-dependent vasodilator from the protopanaxatriol group of ginsenosides, protopanaxatriol ginsenosides were further purified by HPLC (Park et al., 1996). HPLC chromatograms showed that ginsenoside Rg_3 was present in addition to the ginsenosides Rh_1, Rg_2, Rg_1 Rf and Re, which are present in white ginseng (Park et al., 1996). It has been reported that ginsenoside Rg_3 is present in red ginseng (steamed ginseng) but not in white ginseng (dried ginseng) (Kitagawa et al., 1983; Park et al., 1996). Ginsenoside Rg_3 chemically belongs to the protopanaxadiol group of ginsenosides but is found in the protopanaxatriol group fraction because of its non-polar property. The protopanaxadiol ginsenosides are generally more polar than the protopanaxatriol ginsenosides. The potency of ginsenoside Rg_3 to induce endothelium-dependent relaxation was 3.4-fold that of protopanaxatriol ginsenosides, 6.2-fold that of unpurified ginsenosides and 79-fold that of ginsenoside Rg_1. Thus, ginsenoside Rg_3 was the most potent vasodilator among the examined ginsenosides (Kim et al., 1994; Kang et al., 1995b). The endothelium-dependent relaxation evoked by ginsenoside Rg_3 was reduced by methylene blue and N^G-nitro-L-arginine, an inhibitor of soluble guanylyl cyclase and an inhibitor of nitric oxide synthase, respectively.

The present findings indicate that ginsenoside Rg_3 enhances the production of NO from L-arginine in the vascular endothelium. This conclusion is also supported by the observation that, like the endothelium-dependent relaxing agent, acetylcholine, ginsenoside Rg_3 caused concentration-dependent accumulation of cGMP in rat aortic rings with endothelium but not in those without (Rapoport and Murad, 1983; Ignarro et al., 1987). The production of cGMP evoked by ginsenoside Rg_3 in rings with endothelium was abolished by methylene blue and N^G-nitro-L-arginine.

It has been demonstrated that administration of ginsenosides to anesthetized rats reduces the mean arterial blood pressure in a dose-dependent manner (Kim et al., 1994), and that addition of ginsenosides to the diet of rabbits on a high cholesterol diet restores the ability of contracted aortic rings to relax in response to acetylcholine (Kang et al., 1995a). Ginsenosides cause vasorelaxation, prevent manifestations of oxygen free radical injury due to electric stimulation of the perfused pulmonary bed of the rabbit, and cause the formation of L-citrulline from L-arginine in cultured endothelial cells, possibly by promoting NO release (Kim et al., 1992). Single injection of ginsenoside extract (200 mg/kg) increases nitrite and nitrate levels in rat serum and urine (Han and Kim, 1996). These effects are reversed by inhibition of NO synthase and restored by L-arginine. Intravenous administration of ginsenoside Rg_3 (10 mg/kg) to anesthetized rats increases nitrite levels (data not shown). Moreover, ginsenosides relax the corpus cavenosum in a concentration-dependent manner in in vitro superfusion experiments (Chen and Lee, 1995).

Taken together, the present findings indicate that ginsenoside Rg_3 effectively stimulates the formation of NO in endothelial cells, which accounts for the endothelium-dependent relaxation and production of cGMP in rat aorta. Endothelium-derived NO is released from endothelial cells under basal conditions, and its release is further stimulated by various agonists such as acetylcholine, histamine, substance P and isoproterenol via activation of muscarinic receptors, histamine receptors, substance P receptors and β-adrenoceptors, respectively (Furchgott, 1983; Hirano et al., 1991; Gray and Marshall, 1992).

To assess whether the enhanced release of NO by ginsenoside Rg_3 is linked to the activation of receptors, the effects of atropine, a muscarinic receptor antagonist, [D-Pro^2, D-$Trp^{7,9}$] substance P, a substance P receptor antagonist, and propranolol, a β-adrenoceptor antagonist, on the endothelium-dependent relaxation in response to ginsenoside Rg_3 were examined. Preincubation of aortic rings with the antagonists did not affect the relaxations elicited by ginsenoside Rg_3. Propranolol treatment inhibited the relaxations in response to ginsenoside Rg_3 only at a high concentration (10^{-5} M, Rapoport et al., 1985). These findings indicate that ginsenoside Rg_3 may not interact with muscarinic receptors, histamine receptors, substance P receptors or β-adrenoceptors to evoke endothelium-dependent relaxation. However, these observations do not rule out the possibility that other types of receptors may mediate the ginsenoside Rg_3-induced relaxation.

Calcium plays an essential role in NO synthesis/release in endothelial cells (Singer and Peach, 1982; Laskey et al., 1991). It has been suggested that endothelium-dependent vasodilators cause an increase in inositol triphosphate-mediated Ca^{2+} release from intracellular stores (Derian and Moskowitz, 1986; Pirotton et al., 1987), which subsequently leads to the opening of Ca^{2+}-dependent K^+ channels in endothelial cells. The opening of Ca^{2+}-dependent K^+ channels increases K^+ efflux, hyperpolarizing the endothelial cells, thus providing the driving force for Ca^{2+} influx into endothelial cells. (Busse et al., 1988; Adams et al., 1989; Lückhoff and Busse, 1990; Mehrke and Daut, 1990; Sakai, 1990; Chen and Cheung, 1992). The extracellular Ca^{2+} influx required for NO production is not mediated by voltage-dependent Ca^{2+} channels (VOC) (Jayakody et al., 1987) but probably involves other Ca^{2+} channels which remain to be identified (Lückhoff and Busse, 1986; Colden-Stanfield et al., 1987). The ginsenoside Rg_3-induced vasodilatation in rat aortic rings precontracted with prostaglandin $F_{2\alpha}$ was completely abolished in Ca^{2+}-free

solution (data not shown) but not, as expected, by nifedipine or verapamil, two Ca^{2+} channel blockers. The ginsenoside Rg_3-induced endothelium-dependent relaxation was markedly inhibited by tetraethylammonium, a non-selective K^+ channel blocker, but not by glibenclamide, an ATP-sensitive K^+ channel blocker. These findings suggest the ginsenoside Rg_3 activates tetraethylammonium-sensitive K^+ channels in endothelial cells, which presumably leads to an influx of Ca^{2+} and the subsequent activation of the endothelial nitric oxide synthase. However, since tetraethylammonium modestly but significantly impaired the endothelium-independent relaxation in response to sodium nitroprusside, an alteration of the cGMP relaxing pathway in smooth muscle may also contribute to the inhibitory effect of tetraethylammonium on ginsenoside Rg_3-induced relaxation. Consistent with this idea, activation of Ca^{2+}-dependent K^+ channels is involved in the vasodilator effect of cGMP on mesenteric microvessels (Carrier et al., 1997), and that of ATP-dependent K^+ channels on pial arteries (Armstead, 1996).

In conclusion, ginsenoside Rg_3 is a major mediator of the endothelium-dependent relaxation response to ginsenosides of rat aortic rings. The endothelium-dependent relaxation elicited by ginsenoside Rg_3 is due to an increased formation of NO by endothelial cells and seems to involve the activation of tetraethylammonium-sensitive K^+ channels. The increased formation of endothelium-derived NO could explain the red ginseng-associated vasodilatation and its beneficial effect on the cardiovascular system.

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