Ginsenosides Evoke Vasorelaxation in Rat Aortic Rings: Involvement of Ca^{2+}-dependent K^+ Channels

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ABSTRACT

Administration of ginsenosides, a mixture of saponin extracted from *Panax ginseng,* decreased blood pressure in rat. Previous studies have shown that ginsenosides caused endothelium-dependent relaxation which was associated with the formation of cyclic GMP, suggested that ginsenosides caused release of nitric oxide (NO) from the vascular endothelium. The aim of the present study was to characterize the endothelium-independent relaxation to ginsenosides in the isolated rat aorta. Ginsenosides caused a concentration-dependent relaxation of rat aortic rings without endothelium constricted with 25 mM KCl but affected only minimally those constricted with 60 mM KCl. Ginsenoside Rg_3 (Rg_3) was a more potent vasorelaxing agonist than total ginsenoside mixture and also the ginsenoside PPT and PPD groups. Relaxations to ginsenosides were markedly reduced by TEA, but not by glibenclamide. Rg_3 significantly inhibited Ca^{2+}-induced concentration-contraction curves and the $^{45}Ca^{2+}$ influx in aortic rings incubated in 25 mM KCl whereas those responses were not affected in 60 mM KCl. Rg3 caused efflux of ^{86}Rb in aortic rings that was inhibited by tetraethylammonium (TEA), an inhibitor of Ca^{2+}-dependent K^+ channels, but not by glibenclamide, an inhibitor of ATP-dependent K^+ channels. These findings indicate that ginsenosides may induce vasorelaxation via activation of Ca^{2+}-dependent K^+ channels resulting in hyperpolarization of the vascular smooth muscle with subsequent inhibition of the opening of voltage-dependent Ca^{2+} channels. These effects could contribute to explain the red ginseng-associated vasodilation and the beneficial effect on the cardiovascular system.

Introduction

Potassium channel openers elicit a vasorelaxing effect that is markedly blunted in blood vessels constricted by a high KCl solution (Hamilton *et al.,* 1986; Weir and Weston, 1986; Matsuda *et al.,* 1991) and also inhibit the Ca^{2+}-induced contraction in a low but not high KCl solutions and the $^{45}Ca^{2+}$ influx elicited by a low but not high KCl solutions. K^+ channel openers such as cromakalim, pinacidil and nicorandil increases the permeability of the vascular smooth muscle cell membrane to K^+ resulting in a hyperpolarization that relaxes indirectly the blood vessel by decreasing the opening of voltage-sensitive Ca^{2+} channels (Edwards and Weston, 1995; Lawson, 1996; Hamilton *et al.,*

1986). Consistent with such a concept, cromakalim hyperpolarized rat aortic rings, an effect which was mediated by the activation of an outward K^+ current (Bray *et al.*, 1988).

Ginsenosides are a mixture of saponin from *Panax ginseng*, the major form of glycosides belong either to the protopanaxadiol group (PPD) or to the protopanaxatriol group (PPT) (Ando *et al.*, 1971). Ginsenosides induced endothelium-dependent relaxation and increased tissue content of cGMP in isolated rat thoracic aorta, possibly due to the release of EDRF (Kim *et al.*, 1994). PPT and its purified ginsenoside Rg_1 (Rg_1) and Re caused endothelium-dependent relaxation which is associated with the formation of cyclic GMP. In contrast, PPD and its purified ginsenoside Rg_1 (Rg_1) and Rc did not affect vascular tone or production of cGMP in rat aorta (Kang *et al.*, 1995). However, Rg_1 and Re were less effective endothelium-dependent vasodilator than were ginsenosides(total saponin) and PPT (Kim et al., 1994; Kang *et al.*, 1995). Recently, we found that Rg_3 was the most potent vasidilator (Kim *et al.*, 1998).

Recently, we also found that in addition to the endothelium-dependent relaxation, ginsenosides inhibited effectively the tone of aortic rings without endothelium contracted with 25 mM KCl whereas only a small relaxatiom was found in those contracted with pheylephrine. The purpose of the present study was to characterize the mechanisms underlying the direct relaxing effect of Rg_3 on the blood vessel wall.

Materials and methods

Materials

Ginsenoside Rg_3 was isolated from ginsenosides, which was extracted from *Panax ginseng,* by the methods of Kitagawa (1983). Total ginsenosides, protopanaxatriol ginsenoside group (PPT) and protopaxadiol ginsenoside group (PPD) were provided by the Korean Ginseng and Tobacco Research Institute (Taejon, South Korea). Tetraethylammonium (TEA) and glibenclamide were purchased from Sigma Chemical Co. (St. Louis, MO.).

Organ chamber studies

Male Sprague-Dawley rats (270~330g) were sacrificed and thoracic aortas were removed and placed in a modified Krebs-Ringer-bicarbonate solution containing (in mM): NaCl, 118.3; KCl, 4.7; MgSO4, 1.2; KH_2PO_4, 1.2; CaCl2, 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). 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℃, 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

The rings were stretched progressively to the optimal tension (2g) before the addition of phenylephrine (PE, 10^{-6} M). Once the plateau of the contraction to PE was obtained, the aortic rings were rinsed three times with warm (37℃) control solution. After a resting period (30 min), the aortic rings were exposed again to PE (10^{-6} M). When the contraction had stabilized, ACh (10^{-6} M) was added to test the presence of endothelium. Cumulative concentration-relaxation curves to ginsenosides were obtained following the contraction of aortic rings by replacing the control solution with control solution containing 25 mM or 60 mM KCl. In some experiments, TEA and glibenclamide were added 30 min before the addition of the KCl-rich solution.

Calcium-induced contraction studies

Aortic rings were incubated in a Ca^{2+}-free control solution containing 2 mM EGTA. After a 20 min-incubation period during which the incubation medium was changed three times, a cumulative concentration-response curve to $CaCl_2$ (10^{-4}~5×10^{-3} M) was obtained in KCl depolarizing solution containing either 25 mM or 60 mM KCl. Cromakalim (10^{-6} M), nifedipine (10^{-6} M), and Rg_3 (5×10^{-5} g/ml) were added 5 min before the addition of $CaCl_2$. Contractions were expressed as a percentage of the maximum contraction evoked by 60 mM KCl.

$^{45}Ca^{2+}$ influx studies

$^{45}Ca^{2+}$ influx measurements were performed as previously described (Godfraind, 1976, 1983).

^{86}Rb efflux studies

^{86}Rb efflux measurements were performed as previously described (Lodge *et al.*, 1991).

Results

Organ chamber studies

Rg_3 (10^{-6}~10^{-4} g/ml) produced a concentration-dependent relaxation in rat aortic rings without endothelium contracted with 25 mM KCl (Fig.1). A relaxation was also found in response to the

Table 1. Ginsenoside Rg_3 relaxation of K^+ contraction in the presence of K^+ channel blockers in rat aorta

	% relaxation of KCl contraction	
	25 mM KCl	60 mM KCl
Rg_3	80 (5)	9(3)
Rg_3 + TEA (10^{-3} M)	20 (5) ***	-
Rg_3 + glibenclamide (10^{-5} M)	90 (5)	-

** Significantly different from ginsenoside Rg_3 relaxation ($P<0.001$). Parentheses indicate the number of animals used

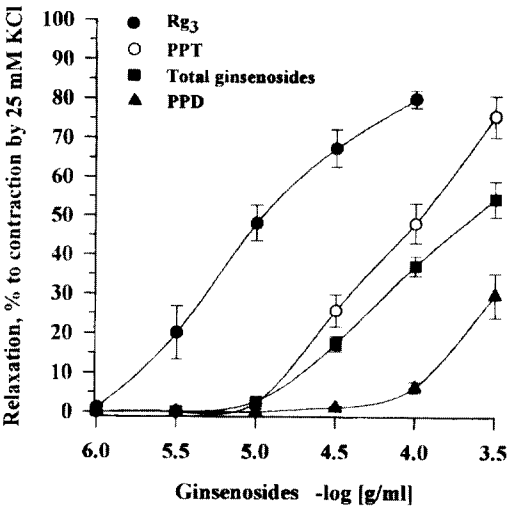

Fig 1. Concentration relaxation curves to a total mixture of ginsenosides extracted from red ginseng, the mixture of ginsenosides from the PPT group, the mixture of ginsenosides from PPD group and Rg_3 in endothelium-denued rat aortic rings contracted with 25 mM KCL. Results are shown as mean ± SEM of 4 to 8 experiments

total ginsenoside mixture, PPT and PPD, however, these agents were much less potent than Rg_3 (Fig.1). Although Rg_3 effectively relaxed 25 mM KCl-induced contraction, those induced by 60 mM KCl were affected only minimally (Table 1).

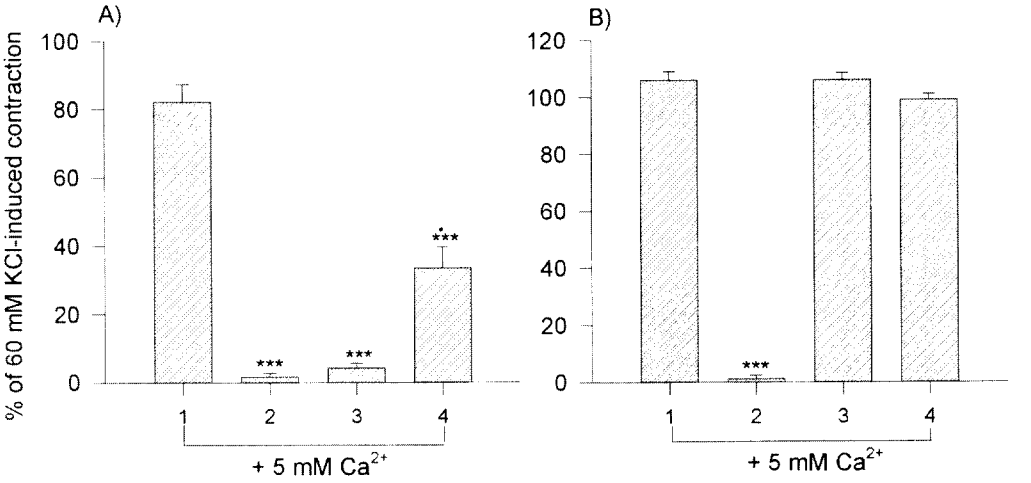

Fig 2. Ca^{2+} -induced contraction of rat aortic rings without endothelium stimulated with A) 25mM KCl and B) 60 mM KCl in the presence of 1) absence of inhibition; 2) nifedipine (10^{-6}M); 3) cromakalim (10^{-6}M) and 4) Rg_3 (5×10^{-5}g/ml) Contraction was expressed as a percentage of the maximal contraction to 60 mM KCl.
Results are shown as mean ± SEM of 4 to 6 experiments

Exposure of aortic rings to TEA (10^{-3} M) significantly reduced the maximal Rg_3-evoked relaxation by 75%, whereas glibenclamide (10^{-5} M) exerted no such effect (Table 1). Calcium induced a contraction of aortic rings incubated in either 25 mM KCl or 60 mM KCl(Fig. 2A and 2B). Calcium-induced contraction evoked in 25 mM KCl were abolished by nifedipine (10^{-6}M) and cromakalim (10^{-6}M) and significantly reduced by Rg_3 (5×10^{-5} g/ml) (Fig. 2A). Increasing KCl from 25 to 60 mM abolished the inhibitory effect of cromakalim and Rg_3 whereas that evoked by nifedipine was unaffected (Fig. 2B).

Effect of Rg_3 on $^{45}Ca^{2+}$ influx

Exposure of aortic rings to KCl for 2 min significantly increased $^{45}Ca^{2+}$ influx. Rg_3 (5×10^{-5} g/ml added 5 min prior to KCl) significantly inhibited $^{45}Ca^{2+}$ influx induced by 25 mM KCl and reduced that evoked by 60 mM KCl, this inhibition did not reach statistical significance (Table 2).

Table 2. Effect of ginsenoside Rg_3 on $^{45}Ca^{2+}$-influx in rat aortic rings bathed in KCl solution

		$^{45}Ca^{2+}$ influx (nmol/g wet wt.)	% Inhibition
25 mM KCl		818.7 ± 55.8	
-	(6)		
Rg_3 (5×10^{-5} g/ml)	(6)	613.9 ± 46.9 **	25
60mM KCl		1027.7 ± 93.8	
-	(6)		
Rg_3 (5×10^{-5} g/ml)	(6)	899.6 ± 44.9	12

*** Significant inhibition of 25 mM KCl stimulated $^{45}Ca^{2+}$ influx ($P<0.01$)
Parentheses indicate the number of animals used.

Effect of Rg_3 on ^{86}Rb efflux

Rg_3 (5×10^{-5} g/ml) transiently increased the basal ^{86}Rb efflux rate coefficient, this response was maximal after about 3 min and amounted to a 7-fold increase from 0.009 ± 0.001 min^{-1} to 0.058 ± 0.01 min^{-1}, n=12) (Fig. 2). The stimulatory effect of Rg_3 was concentration-dependent (Table 3).

Table 3. Effect of ginsenoside Rg_3 on $^{86}Rb^+$ efflux from rat aortic rings without endothelium

Ginsenoside Rg_3	Ratio fo $^{86}Rb^+$ efflux to basal rate	% Inhibition
Control (8)	1.7 ± 0.3	0
10^{-6} g/ml (6)	3.9 ± 1.4*	229
10^{-5} g/ml (6)	8.2 ± 2.9	482
10^{-4} g/ml (6)	9.0 ± 1.2**	529

* Significantly different from control ($P<0.05$) ** Significantly different from control ($P<0.01$) Parentheses indicate the number of animals used.

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Parentheses indicate the number of animals used.

Table 4. Effects of K^+ Channel blockers on Rg_3-stimulated $^{86}Rb^+$ efflux from rat aortic drings without endothelium

Ginsenoside Rg_3 (5×10^{-5} g/ml)		Ratio fo $^{86}Rb^+$ efflux to basal rate	% Inhibition
Rg_3	(6)	10.4 ± 1.5	0
Rg_3 with TEA (10^{-3} M)	(6)	4.4 ± 0.98	58
Rg_3 with glibenclamide (10^{-5} M)	(6)	6.9 ± 1.44	34

* Significant inhibition of Rg_3 (5×10^{-5} g/ml-evoked $^{86}Rb^+$ efflux ($P<0.05$)
Parentheses indicate the number of animals used.

Exposure of aortic rings to TEA (10^{-3} M) did not affect the basal rate of ^{86}Rb efflux (0.01 ± 0.002 min^{-1}) but significantly reduced the stimulatory effect of Rg_3 (5×10^{-5} g/ml), the stimulatory effect was reduced from 10.4 $\pm$ 1.5 to a 4.4 $\pm$ 1.0-fold increase (Table 4). Exposure of aortic rings to glibenclamide (10^{-5} M) did not alter the basal rate of ^{86}Rb efflux (0.01 ± 0.002 min^{-1}) and reduced slightly but not significantly the stimulatory effect of Rg_3 (to 6.9 ± 1.4-fold increase) (Table 4).

Table 4. Effects of K^+ Channel blockers on Rg_3-stimulated $^{86}Rb^+$ efflux from rat aortic drings without endothelium

Ginsenoside Rg_3 (5×10^{-5} g/ml)		Ratio fo $^{86}Rb^+$ efflux to basal rate	% Inhibition
Rg_3	(6)	10.4 $\pm$ 1.5	0
Rg_3 with TEA (10^{-3} M)	(6)	4.4 $\pm$ 0.98	58
Rg_3 with glibenclamide (10^{-5} M)	(6)	6.9 $\pm$ 1.44	34

* Significant inhibition of Rg_3 (5×10^{-5} g/ml-evoked $^{86}Rb^+$ efflux ($P<0.05$)
Parentheses indicate the number of animals used.

Summary

The present findings indicate that ginsenosides are able to directly inhibit the vascular smooth muscle tone. However, such endothelium-independent relaxations to ginsenosides are produced only in aortic rings constricted with a low (25 mM) but not a high (60 mM) concentration of KCl. The triterpene Rg_3 was about one order of magnitude more potent to relax the vascular smooth muscle than the total ginsenoside mixture and the PPT group of ginsenosides, and about 2-orders of magnitude more potent than the PPD group of ginsenosides. Rg_3 inhibited both the Ca^{2+}-induced contraction in aortic rings exposed to a 25 mM KCl solution and the 25 mM KCl -induced $^{45}Ca^{2+}$ influx, but not to a 60 mM KCl. The vasorelaxing effect of Rg_3 is associated with ^{86}Rb efflux. Moreover, since both the Rg_3-induced vasorelaxation and ^{86}Rb efflux are significantly prevented by TEA but not by glibenclamide, ginsenosides may induce vasorelaxation via activation of Ca^{2+}-dependent K^+ channels resulting in hyperpolarization of the vascular smooth muscle with subsequent inhibition of the opening of voltage-dependent Ca^{2+} channels.

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