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The ginsenoside Rg_3 evokes endothelium-independent relaxation in rat aortic rings: role of K^+ channels

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Abstract

The purpose of the present study was to characterize the mechanism underlying the direct relaxing activity of ginsenosides on vascular smooth muscle. The total ginsenoside mixture, ginsenosides from either the protopanaxadiol group or the protopanaxatriol group, and the ginsenoside Rg_3 from the protopanaxatriol group caused a concentration-dependent relaxation of rat aortic rings without endothelium contracted with 25×10^{-3} M KCl but affected only minimally those contracted with 60×10^{-3} M KCl. Ginsenoside Rg_3 was the most potent relaxing agonist. Relaxations elicited by ginsenoside Rg_3 were markedly reduced by tetraethylammonium, a blocker of non-selective K^+ channels, but not by glibenclamide, a blocker of ATP-sensitive K^+ channels. Ginsenoside Rg_3 significantly inhibited Ca^{2+}-induced concentration–contraction curves and the $^{45}Ca^{2+}$ influx in aortic rings incubated with 25×10^{-3} M KCl whereas these responses were not affected in rings incubated with 60×10^{-3} M KCl. Ginsenoside Rg_3 caused a time- and concentration-dependent efflux of ^{86}Rb from aortic rings that was inhibited by tetraethylammonium but not by glibenclamide. These findings indicate that ginsenoside Rg_3 is a potent inhibitor of vascular smooth muscle tone and that this effect seems to be due to an inhibition of Ca^{2+} influx and stimulation of K^+ efflux, possibly via activation of tetraethylammonium-sensitive K^+ channels.

Keywords: Ginsenoside Rg_3; Vascular relaxation; Aorta; K^+ channel; (Rat)

1. Introduction

Administration of ginsenosides, a mixture of saponin extracted from *Panax ginseng*, decreases blood pressure in both hypertensive patients and experimental animals (Sohn et al., 1980; Kim et al., 1994). The antihypertensive effect of ginsenosides may be due, at least in part, to their ability to inhibit vascular tone. Indeed, ginsenosides concentration dependently relax isolated rabbit pulmonary arteries contracted with prostaglandin F_{2a} (Chen et al., 1984) and isolated rabbit and rat aorta contracted with phenylephrine (Kim et al., 1994). The inhibitory effect of ginsenosides requires the presence of a functional endothelium and is mediated by an increased formation of endothelium-derived nitric oxide (Kim et al., 1994). Studies examining the effect of various purified ginsenosides on vascular tone identified ginsenoside Rg_3, a triterpene glycoside which chemically belongs to the protopanaxadiol ginsenoside group, as the most potent vasodilator (Kim et al., 1998). Since the endothelium-dependent nitric oxide-mediated relaxation in response to ginsenoside Rg_3 in rat aorta was prevented by tetraethylammonium, a blocker of non-selective K^+ channels, but not by glibenclamide, an ATP-sensitive K^+ channel blocker, activation of tetraethylammonium-sensitive K^+ channels seems to be implicated in the formation of nitric oxide in endothelial cells. Recently, we found that, in addition to the endothelium-dependent relaxation, ginsenoside Rg_3 also inhibited the tone of aortic rings without endothelium contracted with 25×10^{-3} M KCl whereas only a small relaxation was found in rings contracted with phenylephrine (Kim et al., 1998). The purpose of the present study was to characterize the mechanisms underlying the direct relaxing effect of ginsenoside Rg_3 on the blood vessel wall.

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2. Materials and methods

2.1. Materials

Ginsenoside Rg_3 was isolated from an extract of ginsenosides, prepared from *P. ginseng*, by the methods of Kitagawa et al. (1983). Total ginsenosides, protopanaxatriol group ginsenosides and protopanaxadiol group ginsenosides were provided by the Korean Ginseng and Tobacco Research Institute (Daejun, South Korea). Tetraethylammonium and glibenclamide were purchased from Sigma (St. Louis, MO).

2.2. Organ chamber studies

Male Sprague–Dawley rats (270–330 g) were killed and their thoracic aortas were removed and placed in 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 eight 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°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 the other was connected to a transducer coupler (Narco bio-system) for the recording of isometric tension. The aortic 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 30-min resting period, 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. A cumulative concentration–relaxation curve for ginsenoside Rg_3, ginsenosides from either the protopanaxatriol or protopanaxadiol group and ginsenosides (total saponin) (10^{-6}–10^{-4} g/ml) was obtained following the contraction of aortic rings with solution containing 25 or 60×10^{-3} M KCl. The concentration of NaCl in the solution was lowered to adjust osmolarity. In some experiments, tetraethylammonium (10^{-3} M) or glibenclamide (10^{-5} M) was added 30 min before the addition of the high KCl solution.

2.3. Ca^{2+}-induced contraction studies

Aortic rings were incubated in a Ca^{2+}-free control solution containing 2×10^{-3} M ethylene glycol bis (β-aminoethyl ether) *N,N,N′N′*-tetraacetic acid (EGTA). After a 20-min incubation during which the incubation medium was changed three times, a cumulative concentration–response curve for $CaCl_2$ (10^{-4}–5×10^{-3} M) was obtained in KCl depolarizing solution containing either 25×10^{-3} M or 60×10^{-3} M KCl. Cromakalim (10^{-6} M), nifedipine (10^{-6} M), and ginsenoside Rg_3 (5×10^{-5} g/ml) were added 5 min before the addition of $CaCl_2$. Contractions are expressed as a percentage of the maximum contraction evoked by 60×10^{-3} M KCl.

2.4. $^{45}Ca^{2+}$ influx studies

$^{45}Ca^{2+}$ influx measurements were performed as previously described (Godfraind, 1976, 1983). Thoracic aortas were cut into rings (1 cm wide) and the endothelium was removed mechanically. Ca^{2+} influx was estimated by measuring changes in the specific activity of the Ca^{2+} fraction resistant to displacement by La^{3+} (Godfraind, 1976, 1983). Aortic rings were incubated for 60 min in control solution (in mM: NaCl 122, $NaHCO_3$ 25, KCl 5.0, $CaCl_2$ 1.25, $MgSO_4$ 1.2 and glucose 11, pH 7.4; this solution did not contain phosphates to minimize Ca^{2+} precipitation (Godfraind, 1976)) and then for an additional 30 min in the same solution with or without ginsenoside Rg_3 (5×10^{-5} g/ml). The aortic rings were incubated for 5 min in $^{45}Ca^{2+}$(1 μCi/ml)-containing control solution with or without ginsenoside Rg_3 and then for 2 min in the absence and presence of KCl (25×10^{-3} M or 60×10^{-3} M KCl). Thereafter, they were soaked for 5 min in ice-cold La^{3+} solution (in mM: NaCl, 122; KCl, 5.9; $MgCl_2$, 1.25; glucose, 11.0; $LaCl_3$, 50; and tris-maleate, 15, pH 6.8), to remove extracellular Ca^{2+} and prevent Ca^{2+} efflux and influx (Godfraind, 1976). The aortas were then placed

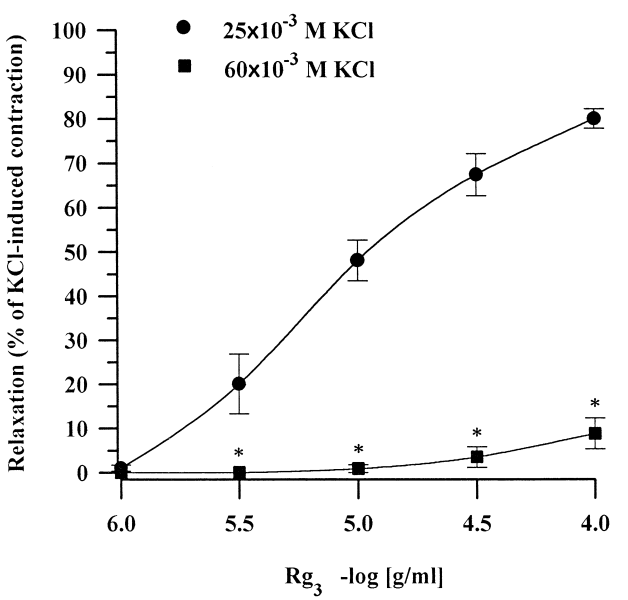

Fig. 1. Concentration–relaxation curves for ginsenoside Rg_3 in endothelium-denuded rat aortic rings constricted with either 25×10^{-3} M KCl or 60×10^{-3} M KCl. Tension induced by 25×10^{-3} M KCl and 60×10^{-3} M KCl was 2.88 ± 0.10 g and 3.61 ± 0.14 g, respectively. Results are shown as means ± S.E.M. of 3 to 5 experiments. $* P < 0.05$ versus aortic rings constricted with 25×10^{-3} M KCl.

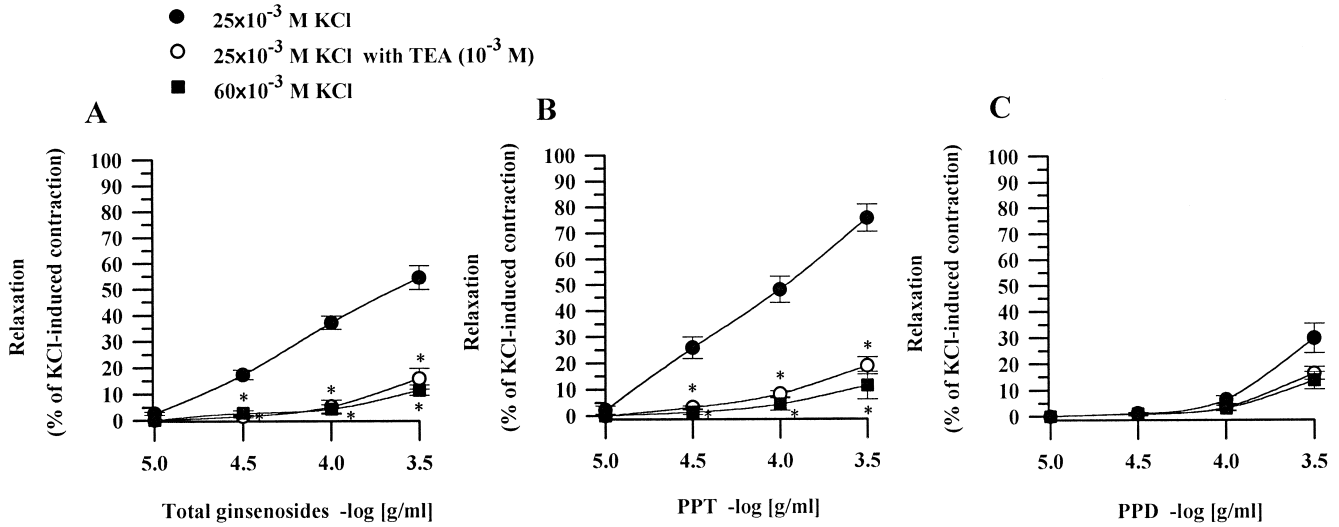

Fig. 2. Concentration–relaxation curves for A) a total mixture of ginsenosides extracted from *P. ginseng*, B) ginsenosides from the protopanaxatriol group (PPT) and C) ginsenosides from protopanaxadiol group (PPD) in endothelium-denuded rat aortic rings constricted with either 25×10^{-3} M KCl or 60×10^{-3} M KCl. The effect of tetraethylammonium (10^{-3} M) on the ginsenoside-induced relaxation is also shown. Results are shown as means ± S.E.M. of 3 to 6 experiments. $* P < 0.05$ versus aortic rings constricted with 25×10^{-3} M KCl.

between two sheets of filter paper and pressed three times with a roller weighing 350 g. Each aortic ring was weighed and then dissolved in 600 μl of soluene 350 (Packard Instrument) at 40°C overnight. The radioactivity of the sample was counted in a liquid scintillation counter (Wallac, 1409). The result of each determination was converted to the apparent tissue content of

$$^{45}Ca^{2+}\ (\text{nmol/g of wet tissue wt}) = \frac{\text{dpm in muscle}}{\text{wet tissue wt (g)}} \times \frac{\text{nmole of } Ca^{2+}/\text{l of medium}}{\text{dpm/l of medium}}$$

2.5. ^{86}Rb efflux studies

^{86}Rb efflux was measured as previously described (Lodge et al., 1991). Thoracic aortas were cut into eight rings (2–3 mm wide) and the endothelium was removed mechanically. Each ring was mounted on a stainless-steel wire without tension. After an equilibration period of 60 min, the aortic rings were immersed in control solution (same composition as for the organ bath study) containing trace amounts of ^{86}Rb (1 μCi/ml) and allowed to equilibrate for 3 h. Thereafter, the tissue was rinsed four times with fresh control solution and then transferred through a series of vials (3 min/vial) containing 3 ml of control solution bubbled with a mixture of 95% O_2 and 5% CO_2. The control solution was maintained at 37°C and stirred thoroughly by constant agitation in a shaker water bath. Ginsenoside Rg_3 (5×10^{-5} g/ml) was present from 24 to 48 min of the 60-min efflux observation period. In some experiments, tetraethylammonium (10^{-3} M) and glibenclamide (10^{-5} M) was added 20 min before the addition of ginsenoside Rg_3 (5×10^{-5} g/ml). At the end of the experiment, the tissues were dissolved in tissue solubilizer (soluene 350) overnight at 40°C. The radioactivity present in each single effluent (3 min) and in the tissues was measured using a liquid scintillation counter (Wallac, 1409). Results are expressed as the rate coefficients, which were calculated from the radioactivity of a single effluent (3 min) divided by the total tissue radioactivity, obtained by addition of the effluent radioactivities to the tissue residual radioactivity (Tamura et al., 1994). The average efflux rate obtained between 15 and 24 min of the efflux

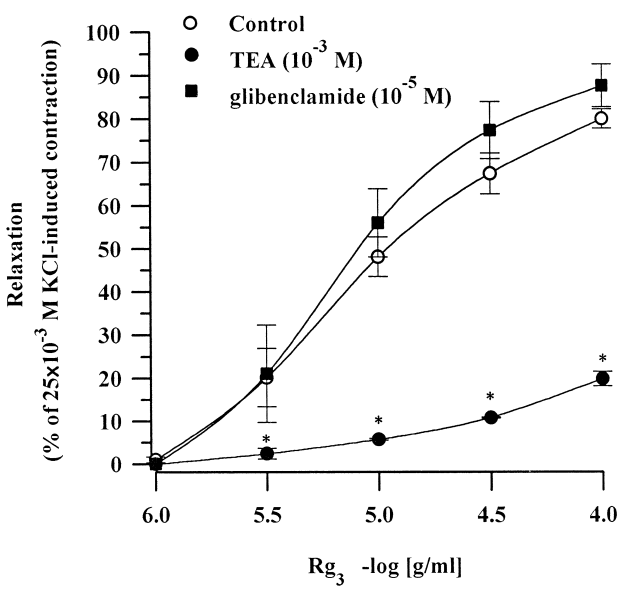

Fig. 3. Effect of tetraethylammonium (TEA, 10^{-3} M) and glibenclamide (10^{-5} M) on ginsenoside Rg_3-evoked relaxation in endothelium-denuded rat aortic rings constricted with 25×10^{-3} M KCl. Results are shown as means ± S.E.M. of 3 to 6 experiments. $* P < 0.05$ versus control.

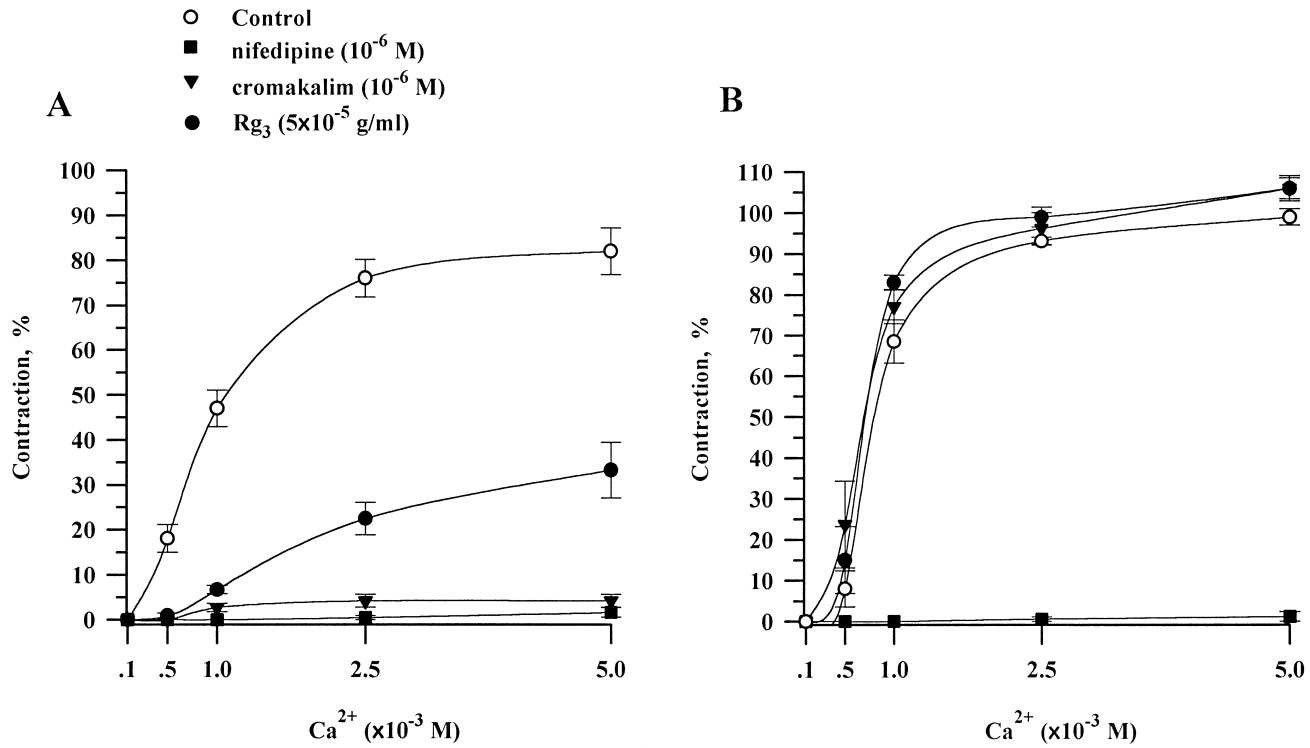

Fig. 4. Effect of nifedipine (10^{-6} M), cromakalim (10^{-6} M) and ginsenoside Rg_3 (5×10^{-5} g/ml) on the Ca^{2+}-induced contraction of rat aortic rings without endothelium stimulated with A) 25×10^{-3} M KCl and B) 60×10^{-3} M KCl. Contraction is expressed as a percentage of the maximal contraction in response to 60×10^{-3} M KCl. Results are shown as means ± S.E.M. of 4 to 6 experiments.

period was taken as the basal rate. The efflux rate stimulated by ginsenoside Rg_3 was calculated as the maximum efflux rate observed between 24 and 48 min of the efflux period divided by basal rate and is expressed as a percentage.

2.6. Statistical analysis

The data are expressed as means ± S.E.M. The number of rings obtained from different rats is represented by *n*. The relaxation response is expressed as percent decrease of the maximal contraction developed in response to 25×10^{-3} M or 60×10^{-3} M KCl. The statistical significance was analyzed by Student's *t*-test and *P* values of less than 0.05 were considered significant.

3. Results

3.1. Organ chamber studies

Ginsenoside Rg_3 (10^{-6}–10^{-4} g/ml) produced a concentration-dependent relaxation of rat aortic rings without endothelium contracted with 25×10^{-3} M KCl (Fig. 1). A relaxation was also found in response to the total ginsenoside mixture, and ginsenosides from either the protopanaxatriol or protopanaxadiol group of ginsenosides; however, these agents were much less potent than ginsenoside Rg_3 (Fig. 2). Although ginsenoside Rg_3 effectively inhibited the 25×10^{-3} M KCl-induced contraction, it affected the contraction induced by 60×10^{-3} M KCl only minimally

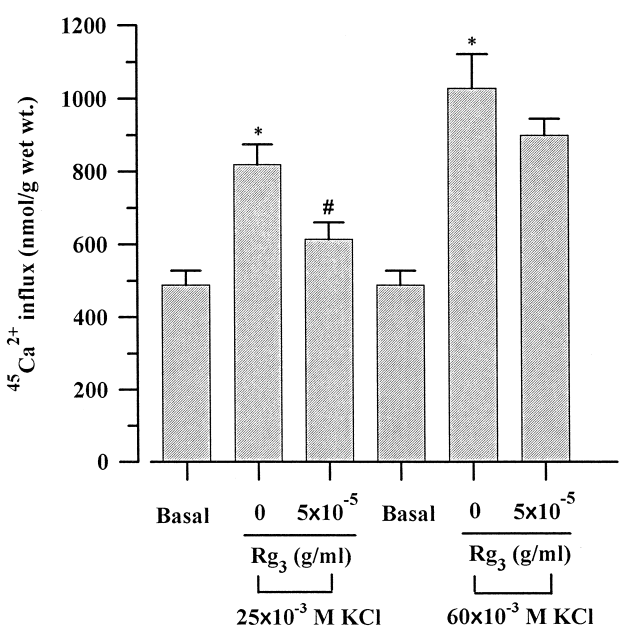

Fig. 5. Effect of ginsenoside Rg_3 (5×10^{-5} g/ml) on $^{45}Ca^{2+}$ influx into rat aortic rings without endothelium stimulated with either 25×10^{-3} M KCl or 60×10^{-3} M KCl. Results are shown as means ± S.E.M. of 15 experiments. $* P < 0.05$ versus basal, $\# P < 0.05$ versus rings stimulated with 25×10^{-3} M KCl in the absence of ginsenoside Rg_3.

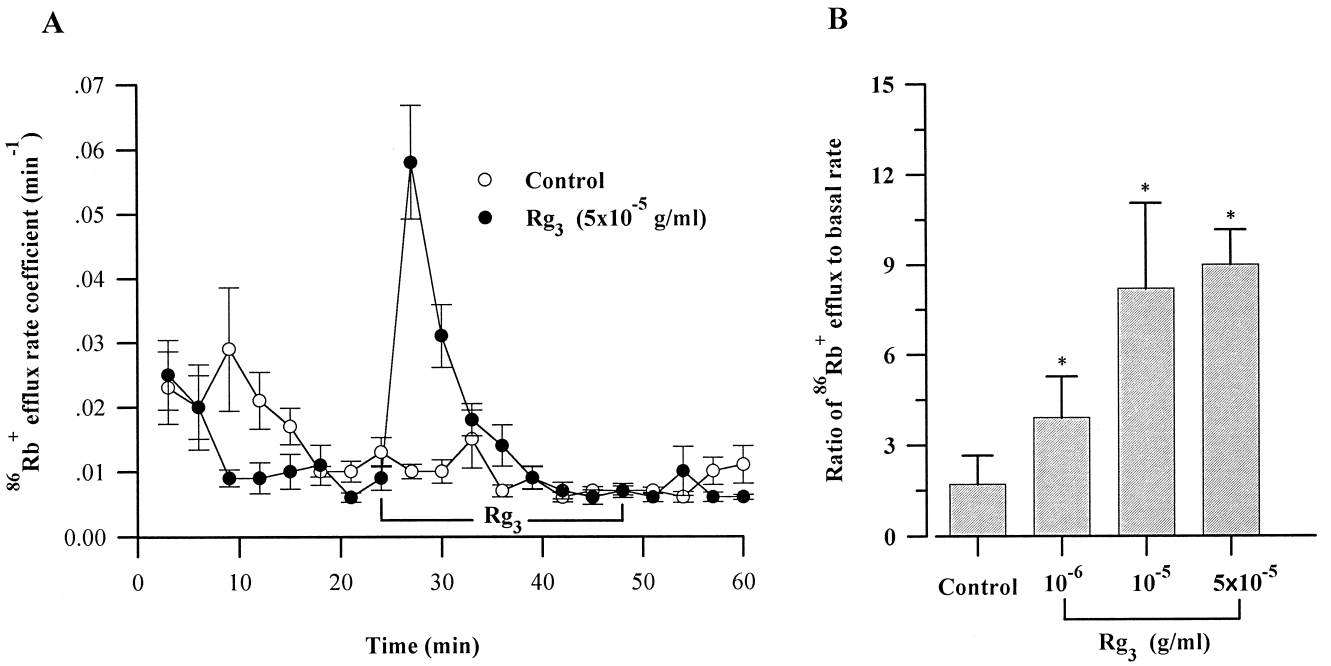

Fig. 6. A) Time- and B) concentration-dependent effect of ginsenoside Rg_3 on ^{86}Rb efflux from rat aortic rings without endothelium. Results are shown as means ± S.E.M. of 12 to 13 experiments. $* P < 0.05$ versus control.

(Fig. 1). Similarly, the total ginsenoside mixture and ginsenosides of either the protopanaxatriol or protopanaxadiol group had only minor effects on the contraction induced by 60×10^{-3} M KCl (Fig. 2). Exposure of aortic rings to tetraethylammonium (10^{-3} M) significantly shifted the concentration–relaxation curves for ginsenoside Rg_3 to the right and reduced the maximal relaxation by 75%, whereas glibenclamide (10^{-5} M) exerted no such effect (Fig. 3). Tetraethylammonium (10^{-3} M) also significantly inhibited the relaxation in response to the total ginsenoside mixture and ginsenosides of either the protopanaxatriol or protopanaxadiol group (Fig. 2).

Ca^{2+} induced a concentration-dependent contraction of aortic rings incubated with either 25×10^{-3} M KCl or 60×10^{-3} M KCl (Fig. 4). Ca^{2+}-induced contractions evoked by 25×10^{-3} M KCl were abolished by nifedipine (10^{-6} M) and cromakalim (10^{-6} M) and significantly reduced by ginsenoside Rg_3 (5×10^{-5} g/ml; Fig. 4A). Increasing KCl from 25 to 60×10^{-3} M abolished the inhibitory effect of cromakalim and ginsenoside Rg_3 but did not affect the inhibitory effect of nifedipine (Fig. 4B).

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

The basal $^{45}Ca^{2+}$ influx for 5 min was 480.6 ± 35 nmol/g wet weight ($n = 15$, Fig. 5). Exposure of aortic rings to KCl for 2 min significantly increased $^{45}Ca^{2+}$ influx (to 818 ± 56 nmol/g wet weight at 25×10^{-3} M KCl and to 1027.7 ± 93.8 nmol/g wet weight at 60×10^{-3} M KCl, Fig. 5). Ginsenoside Rg_3 (5×10^{-5} g/ml added 5 min prior to KCl) significantly inhibited the $^{45}Ca^{2+}$ influx induced by 25×10^{-3} M KCl (to 614 ± 47 nmol/g wet weight) and reduced that evoked by 60×10^{-3} M KCl (to 899.6 ± 44.9 nmol/g wet weight, this inhibition did not reach statistical significance; Fig. 5).

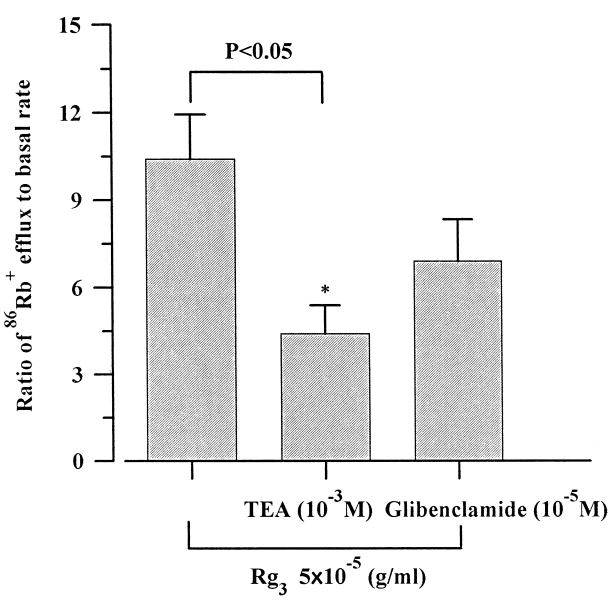

Fig. 7. Effects of tetraethylammonium (TEA, 10^{-3} M) and glibenclamide (10^{-5} M) on ginsenoside Rg_3 (5×10^{-5} g/ml)-evoked ^{86}Rb efflux from rat aortic rings without endothelium. The results are expressed as the ratio of maximal to basal ^{86}Rb efflux rate coefficient. Results are shown as means ± S.E.M. of 8 experiments. $* P < 0.05$ versus aortic rings in the absence of K^+ channel blocker.

3.3. Effect of Rg_3 on ^{86}Rb efflux

Ginsenoside Rg_3 (5×10^{-5} g/ml) transiently increased the basal ^{86}Rb efflux rate coefficient. This response was maximal 3 min after exposure to ginsenoside Rg_3 and amounted to a 7-fold increase (from 0.009 ± 0.001 min^{-1} to 0.058 ± 0.01 min^{-1}, $n = 12$, Fig. 6A). The stimulatory effect of ginsenoside Rg_3 was concentration dependent (Fig. 6B). Exposure of aortic rings to tetraethylammonium (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 ginsenoside Rg_3 (5×10^{-5} g/ml, the stimulatory effect was reduced from a 10.4 ± 1.5 to a 4.4 ± 1.0-fold increase, Fig. 7). 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 ginsenoside Rg_3 (to 6.9 ± 1.4-fold increase, Fig. 7).

4. Discussion

The present findings indicate that, in addition to the endothelium-dependent nitric oxide-mediated relaxation (Kim et al., 1998), ginsenosides are also able to inhibit directly vascular smooth muscle tone. However, this endothelium-independent relaxations in response to ginsenosides was pronounced only in aortic rings constricted with a low (25×10^{-3} M) but not a high (60×10^{-3} M) concentration of KCl. As for the endothelium-dependent nitric oxide-mediated relaxation, the triterpene Rg_3 extracted from the protopanaxatriol group of ginsenosides was about one order of magnitude more potent in relaxing vascular smooth muscle than the total ginsenoside mixture and the protopanaxatriol group of ginsenosides, and about 2-orders of magnitude more potent than the protopanaxadiol group of ginsenosides. Previous in vitro studies with rat sensory neurons (Nah et al., 1995) and cultured rat ventricular myocytes (Wang et al., 1994) have suggested that ginsenosides have Ca^{2+} channel-blocking effects. Thus inhibition of voltage-dependent Ca^{2+} channels might explain the vasorelaxant effects of ginsenosides. Consistent with such an idea, ginsenoside Rg_3, like nifedipine, inhibited significantly both the Ca^{2+}-induced contraction in aortic rings exposed to a 25×10^{-3} M KCl solution and the 25×10^{-3} M KCl-induced $^{45}Ca^{2+}$ influx. However since nifedipine abolished both responses elicited by a high (60×10^{-3} M) KCl solution whereas ginsenoside Rg_3 was ineffective, ginsenosides do not belong to the typical class of Ca^{2+}-channel blockers. Similarly to ginsenosides, K^+ 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 low but not high KCl solutions and the $^{45}Ca^{2+}$ influx elicited by low but not high KCl solutions (present findings). K^+ channel openers such as cromakalim, pinacidil and nicorandil increase 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 (Hamilton et al., 1986; Edwards and Weston, 1995; Lawson, 1996). Consistent with such a concept, cromakalim hyperpolarizes rat aortic rings, an effect which is mediated by the activation of an outward K^+ current (Bray et al., 1991). Since ginsenoside Rg_3, at concentrations evoking vasorelaxation, significantly increased ^{86}Rb efflux from rat aortic rings without endothelium, activation of K^+ channels seems to contribute to the vasorelaxing activity of ginsenosides. Moreover, tetraethylammonium significantly prevented the ginsenoside Rg_3-induced ^{86}Rb efflux and inhibited the 25×10^{-3} M KCl-induced contraction of aortic rings without endothelium whereas glibenclamide had no such effect. These findings suggest that ginsenosides relax vascular smooth muscle via activation of tetraethylammonium-sensitive K^+ channels rather than ATP-sensitive K^+ channels.

In conclusion, ginsenosides concentration dependently inhibit the contraction of rat aortic rings without endothelium elicited by a low but not a high KCl solution. The triterpene Rg_3 is a more potent vasorelaxing agonist than the total ginsenoside mixture and the ginsenosides of the protopanaxatriol and protopanaxadiol groups. The vasorelaxing effect of ginsenoside Rg_3 is associated with ^{86}Rb efflux. Moreover, since both the ginsenoside Rg_3-induced vasorelaxation and ^{86}Rb efflux were significantly prevented by tetraethylammonium but not by glibenclamide, ginsenoside Rg_3 may induce vasorelaxation via activation of tetraethylammonium-sensitive 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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