Influence of Total Ginseng Saponin on Contractile Responses of Vasoconstrictors in the Isolated Rat Aorta

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Abstract

The effects of total ginseng saponin on contractile responses of isometric force transducer Physiograph in isolated rat aorta were evaluated. The aorta was precontracted with phenylephrine (10⁻⁵ - 10⁻⁶ M) and prostaglandin F₂α (5×10⁻⁶ M) or phenylephrine (3.5×10⁻³ M) or phenylephrine (5×10⁻² M) and calcium channel blocker nicardipine (10⁻⁶ M). Total ginseng saponin (600 μg/ml) inhibited the contractile responses of α₁-receptors in phenylephrine (10⁻⁵ - 10⁻⁶ M) and prostaglandin F₂α (5×10⁻⁶ M) or phenylephrine (3.5×10⁻³ M) or phenylephrine (5×10⁻² M) and calcium channel blocker nicardipine (10⁻⁶ M) in a dose-dependent manner. The inhibitory effect of total ginseng saponin on contractile responses of α₁-receptors was observed in phenylephrine (10⁻⁵ - 10⁻⁶ M) and prostaglandin F₂α (5×10⁻⁶ M) or phenylephrine (3.5×10⁻³ M) or phenylephrine (5×10⁻² M) and calcium channel blocker nicardipine (10⁻⁶ M) in a dose-dependent manner. (Korean Circulation J 1999;29(9):976-984)

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Introduction

It has been shown that total Ginseng saponin produces the pressor and depressor actions in the anesthetized normotensive rats. It has suggested that this depressor response is mediated in part through the blockade of adrenergic α-receptors as well as the stimulation of cholinergic muscarinic receptors, and that its pressor response is caused by stimulation of nicotinic cholinergic receptors at the sympathetic ganglia. Furthermore, Choi has reported that total Ginseng saponin can inhibit the releasing effect of catecholamines evoked by nicotinic receptor stimulation from the isolated perfused rat adrenal medulla, which seems to be associated to the direct inhibition of calcium influx into the rat adrenomedullary chromaffin cells. In the isolated perfused rat adrenal glands, total Ginseng saponin increases a calcium-dependent secretion of catecholamines via direct action on chromaffin cells with partly mediation of muscarinic action.

In previous studies, it has been known that Ginseng extract causes the hypotensive action while it rather produces the hypertensive action. Some studies have suggested that Ginseng extract causes a biphasic response on blood pressure, namely, transient fall followed by prolonged elevation. Furthermore, Ginseng, when given at small dose in spontaneously hypertensive rat (SHR), cause pressor response, but at relatively large dose rather produces dose-dependent hypotensive response with decreased plasma renin activity. Sokabe and his coworkers have shown that administration of Korean Red Ginseng powder for 11 weeks has no effect on blood pressure in normotensive Donryn rats, SHR and renal hypertensive rats, whereas it elevates slightly blood pressure in deoxycorticosterone salt hypertensive rats. Lim and his coworkers have also found that both of panaxadiol- and panaxatriol-type saponins cause the increased secretion of catecholamines (CA) in a Ca²⁺-dependent fashion from the isolated perfused rabbit adrenal glands through the activation of cholinergic (both nicotinic and muscarinic) receptors and partly the direct action on the rabbit adreno-medullary chromaffin cells. As mentioned so far, there are many controversial reports on vascular effects of Ginseng saponin.

Therefore, the present study was attempted to examine the effect of total Ginseng saponin on contractile responses evoked by stimulation of adrenergic α₁-receptors and membrane depolarization in the isolated rat aorta and to clarify the mechanism of its action.

Materials and Methods

Experimental procedure

Mature male Sprague-Dawley rats, weighing 150 to 350 grams, were used in the experiment. The animals were housed individually in separate cages, and food (Cheil Animal Chow) and tap water were allowed ad libitum for at least a week to adapt to experimental circumstances. On the day of experiment, a rat was anesthetized with thiopental sodium intraperitoneally, and tied in supine position on fixing panel. The thorax was opened by a midline incision, and the heart and surrounding area were exposed by placing three hook retractors. The heart and portion of the lung were not removed, but pushed over to the right side and covered by saline-soaked gauge pads in order to obtain enough working space for isolating aortic vessel. The aorta was isolated from the proximal part of the heart to the vicinity of liver and immediately immersed in cold Krebs solution. The blood within the aorta was rapidly removed. The aorta was cut into the ring of 4-5 mm length.

Recording of mechanical activity

The ring segment of aorta was mounted in a muscle bath by sliding the ring over two parallel stainless-steel hook (0.15 mm in diameter). The lower hook was fixed on bottom of the bath and the upper was connected to isometric transducer (Grass FT. 03). The signal from the transducer was displayed on a polygraph (Grass Instruments Model 79). The volume of bath was 25 ml and the bath solution was saturated with 95% O₂ and 5% CO₂ at 37°C.

The composition (mM) of Krebs was NaCl, 118. 4 KCl, 4.7 CaCl₂, 2.5 MgCl₂, 1.18 NaHCO₃, 25 KH₂PO₄, 1.2 glucose, 11.7. The final pH of the solut-
ion was maintained at 7.4–7.5. During equilibration period of 2 hours, the resting tension was adjust to 0.5 g. After the equilibration period, the ring was challenged with 35 mM KCl two times, and if it responded with contraction, the proper experiment were started. Vasocostricators were admin-istered into the bath in order to obtain dose-response curves. In the subsequent experiments, under the presence of total Ginseng saponin some vasoconstrictors were administered. The data were expressed as % of the control tension.

**Statistical analysis**

The statistical significance between groups was deter-

mined by the Student’ t-test. A P-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida and Murray.20)

**Drugs and their sources**

The following drugs were used[phenylephrine hydro-

chloride, prostaglandin F₃α tris salt, potassium chloride and nicardipine hydrochloride (Sigma Chemical Co., U. S. A.). Total Ginseng saponin was a gift from late Dr. Young-Ho Kim (Sejong University, Seoul, Korea). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required. Concentrations of all drugs used are expressed in terms of molar base except Total Ginseng saponin (g/ml).

**Results**

The effects of total Ginseng saponin on contractile responses induced by phenylephrine, high K⁺ and prostaglandin F₃α in the isolated rat aortic strips

The resting (basal) tension from the isolated rat aortic strips reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 90 min before the experimental protocol is initiated. The resting tension was adjusted to 0.5 g. The effects of total Ginseng saponin on phenylephrine- as well as potassium chloride-mediated contractile responses in the rat aorta were examined. In the present study, total Ginseng saponin itself did not produce any effect on the resting tension (data not shown).

When 10⁻⁶ M and 10⁻⁵ M of phenylephrine were ad-

ministered into the aortic bath, the active tensions of them were 0.75±0.05 g and 1.02±0.12 g from the resting tension level, respectively. However, under the preloading with total Ginseng saponin at a concentration of 600 µg/ml, phenylephrine-induced tensions were significantly potentiated to 1.14±0.11 g (p<0.01) and 1.52±0.13 g (p<0.01) from resting level from 7 rat aortic strips, which were 152.0% and 149.1% of the control contractile responses, respectively, as shown in Figs. 1 and 2.

High K⁺ exerts two distinct effects on cells[1] (1) depolarization of cell membrane, and (2) depolarization-induced influx of calcium via voltage-dependent calcium channels.21)

When added through the bath, high potassium at the concentration of 35 mM and 56 mM, which is mem-
brane depolarizing agent, caused an increase in aortic contraction, respectively. As shown in Fig. 3 and 4, high potassium-induced contractile responses before preloading with total Ginseng saponin were 1.13±0.19 g and 1.58±0.17 g, while after pretreatment with total Ginseng saponin at a concentration of 600 μg/ml they were greatly enhanced to 1.56±0.18 g (p<0.01) and 2.27±0.21 g (p<0.01), which were 138.1% and 143.7% of the corresponding control from 8 rat aortic strips, respectively.

Since it has been found that prostaglandin F\textsubscript{2α} (5×10\textsuperscript{-6} M) is a vasoconstrictor in dog cerebral arteries\textsuperscript{22}, it is likely interesting to examine the effect of total Ginseng saponin on prostaglandin F\textsubscript{2α}-induced contraction in the rat aortic strips. Prostaglandin F\textsubscript{2α} (5×10\textsuperscript{-6} M) produced prominent and steady-state contraction in the rat aortic strips as shown in Fig. 5. In 6 rat aortic strips, prostaglandin F\textsubscript{2α} (5×10\textsuperscript{-6} M) caused the contractile response of 1.32±0.18 g from the resting tension level but, even in the presence of total Ginseng saponin (600 μg/ml), it was not affected (98.5% of the control) as in Fig. 6.

The effect of nicardipine on total Ginseng saponin-induced potentiation of the contractile response evoked by high K\textsuperscript{+} in the isolated rat aorta

In order to investigate the effect of nicardipine, a dihydropyridine derivative and L-type Ca\textsuperscript{2+} channel blocker\textsuperscript{23} on total Ginseng saponin-induced potentiation of the contractile response evoked by high potassium in the rat aortic strips, nicardipine (10\textsuperscript{-6} M) was preloaded into the bath. In the presence of nicardipine effect, total Ginseng saponin-induced potentiation of the contractile response evoked by high potassium (35 mM) was inhibited.
mM were greatly depressed to 0.31 ± 0.02 g (19.4% of the control, p<0.01) from the resting tension level from 6 adrenal glands in comparison with their corresponding control values of 1.60 ± 0.15 g as depicted in Fig. 7 and 8.

Discussion

The present experimental results suggest that total Ginseng saponin potentiates the contractile responses induced by stimulation of adrenergic α₁-receptor and the membrane depolarization in the isolated rat aortic strips, which appears to be in relation to calcium influx.

Generally, extracellular Ca²⁺ has been unequivocally shown to play a critical role in excitation-contraction coupling in vascular smooth muscle. Most of cellular mechanisms accepted in accounting for the contraction of vascular smooth muscle by various agents are based on increased intracellular Ca²⁺ either through influx of extracellular Ca²⁺ or through release

**Fig. 4.** Influence of total Ginseng saponin (GTS) on high potassium-induced contractile responses in the isolated rat aortic strips. High potassium (35 and 56 mM, respectively) was added into the bath before and after pretreatment with GTS (600 μg/ml). Other legends are the same as in Fig. 2. *** p<0.01

**Fig. 5.** The typical tracing showing the effect of total Ginseng saponin (GTS) on prostaglandine F₂α (PGF₂α)-induced contractile responses in the rat aortic strip. Upper[] PGF₂α-induced contractile response. Lower[] PGF₂α-induced contractile response in the presence of total Ginseng saponin (600 μg/ml). At dots, the indicated dose (5×10⁻⁶M) of phenylephrine was added to the bath. The chart speed was 5 mm/min.

**Fig. 6.** Influence of total Ginseng (GTS) saponin on prostaglandin F₂α-induced contractile response in the isolated rat aortic strips. Prostaglandin F₂α (5 M) was added into the bath before and after pretreatment with GTS (600 μg/ml). Other legends are the same as in Fig. 2. ns nonsignificance.
of intracellularly stored Ca^{2+}. And it well known that potassium chloride (KCl) opens voltage-dependent calcium channels by depolarizing the cell membrane of vascular smooth muscle, resulting in increased influx of extracellular Ca^{2+}. Kim and his colleagues have shown that the contractile responses of vascular smooth muscle induced by CaCl_2 and KCl may result most likely from increased influx of extracellular Ca^{2+} through the voltage-dependent calcium channels. In terms of these results, the present findings that total Ginseng saponin enhances the contraction of rat aortic smooth muscle evoked by phenylephrine (α₁-adrenergic receptor agonist) and KCl (membrane depolarizer) suggest strongly that total Ginseng saponin can facilitate influx of extracellular Ca^{2+}.

In previous studies, three cellular mechanisms have been proposed to explain relaxant response of vascular smooth muscle: (i) blockade of extracellular Ca^{2+} entry into cells, (ii) increase in binding or sequestration of intracellular Ca^{2+}, and (iii) inhibiting the release of intracellular stored Ca^{2+}. In the light of these findings, the present result suggests that total Ginseng saponin can enhance the contractile responses of vascular smooth muscle evoked by phenylephrine and /or KCl through increased extracellular Ca^{2+} entry into the muscle cells. Because the pretreatment with 5 M nicardipine, an inhibitor of the dihydropyridine Ca^{2+} channel, abolished almost completely total Ginseng saponin-induced potentiation of contraction evoked by KCl in the present study.

Moreover, this effect of total Ginseng saponin seems
to contribute at least partly to the facts that Ginseng extract causes the hypertensive action\(^9\rightarrow10\), but not to the facts that it rather produces the hypotensive action.\(^4\rightarrow6\)

The contractions of vascular smooth muscles induced by neurohumoral agents have been composed of two components\(^3\) Phasic contraction induced by the Ca\(^{2+}\) released from inside the cell and tonic tension related to the Ca\(^{2+}\) influx,\(^3(38)\) both leading to increased intracellular calcium.

In the present study, prostaglandin F\(_2\alpha\), which is known as a vasoconstrictor in cerebral arteries of the dog\(^22\) and the pig\(^39\), caused a contractile response in the isolated rat aortic smooth muscles. However, prostaglandin F\(_2\alpha\)-induced contractile response was never influenced by the pretreatment with total Ginseng saponin. This finding indicates that total Ginseng saponin does not affect prostaglandin receptors. Responses to prostaglandin F\(_2\alpha\) vary with species and vascular bed. It is a potent constrictor of both pulmonary arteries and veins in human beings.\(^40\rightarrow41\)

Thus, the most plausible explanation accounting for the facilitatory action of total Ginseng saponin on the vascular contractions induced by stimulation of adrenergic \(\alpha\)-receptors and the membrane depolarization in the isolated rat aortic strips is the increased extracellular calcium into muscle cells likewise in neuronal tissues.

**Summary**

**Background :** It has been known that Ginseng extract causes the hypertensive action while it rather produces the hypertensive action. Some studies have suggested that Ginseng extract causes a biphasic response on blood pressure, namely, transient fall followed by prolonged elevation. It has been also shown that administration of Korean Red Ginseng powder has no effect on blood pressure in normotensive and hypertensive rats. The present study was designed to examine the effect of total Ginseng saponin on contractile responses of vasoconstrictors in the rat aorta and to establish the mechanism of its action.

**Methods :** The ring segment of aorta was mounted in a muscle bath filled with oxygenated Krebs solution for the measurement of isometric tension. After the equilibration period, under the presence of total Ginseng saponin, isometric tension induced by some vasoconstrictors were observed and compared to the control responses. The data were expressed as \% of the control tension.

**Results :** Phenylephrine (an adrenergic \(\alpha\)-receptor agonist) and high potassium (a membrane depolarizing agent) caused greatly contractile responses in the rat aorta, respectively. However, in the presence of total ginseng saponin (600 g/ml), the contractile responses of phenylephrine (\(10^{-6}\) and \(10^{-5}\) M) and high potassium (\(3.5 \times 10^{-2}\) and \(5.6 \times 10^{-2}\) M) were markedly potentiated whereas prostaglandin F\(_2\alpha\) (\(5 \times 10^{-6}\) M) -induced contractile responses was not affected. The contractile responses induced by phenylephrine (\(10^{-5}\) M) and high potassium (\(3.5 \times 10^{-2}\) M) even under the presence of total ginseng saponin (600 g/ml) were greatly inhibited by the pretreatment of nicardipine (\(10^{-6}\) M), a calcium channel blocker.

**Conclusion :** Taken together, these experimental results suggest that total ginseng saponin can enhance the contractile responses evoked by stimulation of adrenergic \(\alpha\)-receptor and the membrane depolarization in the isolated rat aortic strips, which seems to be associated to calcium influx.

**KEY WORDS** Isolated rat aortic smooth muscle· Total ginseng saponin· Vasoconstriction.

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**REFERENCES**

2) Choi H. Influence of total Ginseng saponin on nicotinic stimulation-induced catecholamine secretion from the perfused rat adrenal gland. MS Thesis, Chosun University Graduate School;1996.
3) Lim DY, Park KB, Kim KH, Moon JK, Kim YH. Influence of total Ginseng saponin on secretion of catecholamines in the isolated adrenal gland of rabbits. Korean Biochem
38) Bevan JA. Selective action of diltiazem on cerebral va-
