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Effects of Ginsenosides on Vasodilator Nerve Actions in the Rat Perfused Mesentery are Mediated by Nitric Oxide

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Abstract

This study was designed to explore the effect of ginsenosides, saponins from *Panax ginseng*, on the vasodilator nerve actions in the rat perfused mesentery and the mechanism of this effect.

In the rat perfused mesentery, when adrenergic nerves were blocked by guanethidine (5×10^{-6} M) and vascular muscle tone was increased with methoxamine (5×10^{-6}–10^{-5} M), transmural field stimulation produced a frequency-dependent vasodilator response, which is due to the release of calcitonin gene-related peptide; ginsenosides significantly suppressed this vasodilator response in a concentration-dependent manner (3–30 $\mu g\,mL^{-1}$). After pretreatment with saponin (50 $\mu g\,mL^{-1}$, 3 min) to damage endothelial cells, this suppressing effect of ginsenosides was unaltered. However, the effect was abolished by N^{ω}-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M), an inhibitor of nitric oxide synthesis and addition of L-arginine (3×10^{-4} M) restored this suppressing effect. Methylene blue (10^{-5} M), an inhibitor of guanylate cyclase, also abolished the suppressing effect of ginsenosides. However, ginsenosides did not alter the relaxation responses caused by exogenous calcitonin gene-related peptide administration.

We conclude that ginsenosides can produce an inhibitory effect on the vasodilator response prejunctionally in the rat perfused mesentery and that this effect of ginsenosides may be mediated by nitric oxide released from non-adrenergic, non-cholinergic nerves.

Ginsenosides, saponins from *Panax ginseng*, have been documented to possess complex cardiovascular effects. Ginsenosides have been shown to produce different responses in different blood vessels (Chen et al 1984). Recently, it has been reported that vasodilator responses to ginsenosides are related to an L-arginine-nitric oxide-cyclic GMP (L-arg-NO-cGMP) pathway (Chen et al 1993; Kim et al 1993). Besides possessing cardiovascular effects, ginsenosides can modulate the release of neurotransmitter from sympathetic nerves in pithed rats (Zhang & Chen 1987).

It has been demonstrated that capsaicin-sensitive sensory nerves are present in the rat mesentery and that calcitonin gene-related peptide (CGRP), a principal transmitter in vascular sensory nerves, may play a role in the modulation of the total peripheral resistance of the systemic circulation through local reflex mechanisms (Kawasaki et al 1988; Li & Duckles 1992). Our previous study has shown that CGRP release caused by transmural field stimulation can be modulated, in an inhibitory fashion, by the endogenous nitric oxide, probably released by non-adrenergic, non-cholinergic (NANC) nerves (Li et al 1993).

Since mesenteric vasculature is innervated by both sympathetic and capsaicin-sensitive sensory nerves (Kawasaki et al 1988; Li & Duckles 1992), and ginsenosides can inhibit sympathetic neurotransmission (Zhang & Chen 1987), in the present study we examined whether ginsenosides can affect the actions of sensory nerves in the rat perfused mesentery. The modulation of capsaicin-sensitive sensory nerves involves nitric oxide, possibly from NANC nerves (Li et al 1993), and ginsenosides can relax vascular smooth muscle via an L-arg-NO-cGMP pathway (Chen et al 1993; Kim et al 1993); therefore, we further explored whether the effect of ginsenosides on sensory nerve action is correlated with an L-arg-NO-cGMP pathway.

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Materials and Methods

Tissue preparation and perfusion

Mesenteric vasculature of male Sprague-Dawley rats, 180–250 g, was isolated and prepared for perfusion as described previously (Li et al 1993). Rats were decapitated, and the mesenteric artery was quickly cannulated at its origin at the aorta with PE 50 tubing and perfused with Krebs solution, saturated with 95% O_2–5% CO_2. Preparations were then placed in a water-jacketed organ bath (volume 200 mL) maintained at 37°C. The system was perfused with Krebs solution with the help of a peristaltic pump at a rate of $5 \pm 0{\cdot}2\,mL\,min^{-1}$, and superfused by gravity feed at a rate of $1 \pm 0{\cdot}2\,mL\,min^{-1}$. The Krebs solution had the following composition (mM): NaCl 118, KCl 4·8, $CaCl_2$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 2·5, $MgSO_4$ 1·2, EDTA.Na 0·107, dextrose 11·5. The perfusion pressure was monitored and recorded by a pressure transducer and a LMS-2B two-channel recorder.

Two platinum electrodes, one placed around the superior mesentery artery and the other resting on the vasculature in a lower part of the preparation, were used to create transmural field stimulation. Transmural field stimulation (amplitude of 60 V, 80 pulses and 3 ms duration) was applied at various frequencies using a Nihonkohden S3201 stimulator. Sufficient time was allowed between each stimulation train for the perfusion pressure to return to a stable level, usually 10–20 min.

Experimental protocols

Tissues were equilibrated for 60 min before each experiment was begun. Responses to transmural field stimulation were stable in the absence of drug. All drugs were administered intraluminally by switching the perfusion solution to a solution containing the drug in the concentrations indicated. For measurement of vasodilator responses, tissues were pretreated with guanethidine (5×10^{-6} M) and then contracted with methoxamine (5×10^{-6}–10^{-5} M). For ginsenosides, preparations were exposed for 10 min and these remained in the perfusate for the remainder of the study. Transmural field stimulation was applied 10 min after drug administration. For measurement of vasodilator responses to ginsenosides, various concentrations of ginsenosides were tested in a cumulative fashion. In the case of studies of the effect of L-NAME, L-NAME in the presence of L-arginine, or methylene blue on responses to ginsenosides, exposure to L-NAME, L-NAME in the presence of L-arginine, or methylene blue was for 10 min and then responses to transmural field stimulation were tested. After measurement of control responses to transmural field stimulation, tissues were exposed to ginsenosides in the presence of L-NAME, L-arginine and L-NAME, or methylene blue before final stimulation was tested. For CGRP, various concentrations of CGRP were tested. To remove the endothelium, preparations were perfused with distilled water containing saponin ($50\,\mu g\,mL^{-1}$) for 3 min. Selective removal of the endothelium was confirmed by demonstrating the lack of a vasodilator response to acetylcholine. For all studies a paired design was used, that is, the same tissue was studied both before and after treatment with the particular test reagent.

Drugs

The following drugs were used: saponin, methoxamine, L-arginine, N^{ω}-nitro-L-arginine methyl ester (L-NAME) and guanethidine (Sigma, St Louis, MO); calcitonin gene-related peptide (Peninsula Laboratory, Belmont, CA); ginsenosides (extracted from *Panax ginseng* C. A. Meyer according to the Shibata methods) (Shibata et al 1965) and methylene blue (Medical and Pharmaceutical Co. of China, Beijing). All drugs were dissolved in Krebs solution.

Statistics

Results are expressed as means ± s.e.m. Student's *t*-test was used to determine statistical differences between two means. The level of significance was chosen as $P < 0.05$.

Table 1. Effect of various concentrations of ginsenosides on vasodilator response to transmural field stimulation (n = 5). Relaxation was calculated as percentage of contraction to methoxamine.

Ginsenosides ($\mu g\,mL^{-1}$)	Frequency (Hz)		
	1	2	4
0 (Control)	21·1 ± 7·9	35·7 ± 9·9	45·1 ± 5·9
3	18·8 ± 7·9	27·5 ± 7·1	38·6 ± 4·9
10	10·5 ± 3·7*	19·4 ± 7·8**	28·2 ± 8·2**
30	5·6 ± 3·1**	13·4 ± 6·1**	22·2 ± 4·5**

*$P < 0.05$, **$P < 0.01$ compared with control.

Table 2. Effect of ginsenosides ($10\,\mu g\,mL^{-1}$) on vasodilator responses to transmural field stimulation in the rat perfused mesentery with endothelium and without endothelium (n = 5). Relaxation was calculated as percentage of contraction to methoxamine.

	Frequency (Hz)		
	1	2	4
Control	27·1 ± 6·8	39·8 ± 5·4	46·7 ± 5·3
With endothelium	13·0 ± 3·2*	25·4 ± 4·7**	32·0 ± 7·1*
Control	24·8 ± 6·0	38·0 ± 3·4	48·8 ± 8·8
Without endothelium	17·0 ± 6·1*	30·0 ± 2·5**#	37·6 ± 6·8**#

*$P < 0.05$, **$P < 0.01$ compared with control, #$P > 0.20$ compared with corresponding experiment with endothelium.

Results

Guanethidine (5×10^{-6} M) was used to block sympathetic nerves and methoxamine (5×10^{-6}–10^{-5} M) was added to increase smooth muscle tone in the perfused rat mesenteric vascular bed, with a basal perfusion pressure of 22 ± 3 mmHg (n = 24). Transmural field stimulation caused a frequency-dependent vasodilator response, which is due to the release of CGRP (Kawasaki et al 1988). Ginsenosides significantly suppressed vasodilator responses to transmural field stimulation at all indicated stimulation frequencies (Table 1) in a concentration-dependent manner.

To further investigate whether endothelium is involved in the suppressing effect of ginsenosides, tissues were perfused with saponin to damage endothelial cells. As we have shown previously (Li et al 1993), after removal of endothelium by saponin, vasodilator responses to acetylcholine were abolished, and microscopy revealed that the great majority of endothelial cells were removed from the basal lamina. However, removal of endothelium did not alter the suppressing effect of ginsenosides on the vasodilator responses to transmural field stimulation. As shown in Table 2, there was no significant difference in relaxation when endothelium-intact and endothelium-denuded preparations were compared ($P > 0.20$).

The effects of ginsenosides at various concentrations were tested in the presence of guanethidine and methoxamine. At the concentrations of 3 and $10\,\mu g\,mL^{-1}$, ginsenoside treatment alone did not cause vascular relaxation, while at a higher concentration ($30\,\mu g\,mL^{-1}$), ginsenosides caused a vasodilator response (19·9 ± 4·8%, n = 5). However, after removal of endothelium, ginsenosides at various concentrations did not cause relaxation of methoxamine-contracted mesentery.

Table 3. Effect of ginsenosides on vasodilator responses to exogenous CGRP (n = 4). Relaxation was calculated as percentage of contraction to methoxamine.

	CGRP concn (M)		
	10^{-9}	3×10^{-9}	10^{-8}
CGRP	26·0 ± 1·1	42·8 ± 2·4	58·1 ± 3·8
CGRP + ginsenosides	26·5 ± 1·1	47·6 ± 2·7	60·8 ± 2·8

Table 4. Effect of L-NAME (10^{-4} M) or L-arginine (3×10^{-4} M) in the presence of L-NAME on inhibition by ginsenosides ($10\,\mu g\,mL^{-1}$) of vasodilator responses to transmural field stimulation (n = 5). Relaxation was calculated as percentage of contraction to methoxamine.

	Frequency (Hz)		
	1	2	4
Control	18·1 ± 2·8	32·4 ± 4·2	46·4 ± 6·2
L-NAME + ginsenosides	18·2 ± 2·0	28·8 ± 2·4	45·2 ± 2·1
Control	27·1 ± 9·0	40·6 ± 2·6	52·2 ± 2·4
L-NAME + ginsenosides + L-arginine	14·9 ± 3·7*	20·8 ± 2·5**	35·1 ± 4·6**

*$P < 0.05$, **$P < 0.01$ compared with control.

Table 5. Effect of methylene blue (10^{-5} M) on inhibition by ginsenosides ($10\,\mu g\,mL^{-1}$) of vasodilator responses to transmural field stimulation (n = 5). Relaxation was calculated as percentage of contraction to methoxamine.

	Frequency (Hz)		
	1	2	4
Control	25·6 ± 2·8	35·7 ± 3·1	49·6 ± 2·8
Ginsenosides	14·6 ± 3·8*	21·1 ± 2·8**	31·2 ± 1·7**
Ginsenosides + methylene blue	24·3 ± 4·2	34·8 ± 5·5	47·0 ± 5·8

*$P < 0.05$, **$P < 0.01$ compared with control.

In tissues pretreated with guanethidine and methoxamine vasodilator responses to exogenous CGRP were unaltered in the presence of $10\,\mu g\,mL^{-1}$ ginsenosides (Table 3).

The inhibitory action of ginsenoside ($10\,\mu g\,mL^{-1}$) was completely abolished in the presence of L-NAME (3×10^{-4} M). However, in the presence of L-arginine, a precursor of nitric oxide synthesis, subsequent reversal of the action of L-NAME was observed, as reappearance of inhibitory effects of vasodilator responses to transmural field stimulation (Table 4).

Inhibition of vasodilator responses to transmural field stimulation by ginsenosides was abolished in the presence of 10^{-5} M methylene blue (Table 5).

Discussion

Ginsenosides can inhibit sympathetic nervous activity by an action on presynaptic α_2-receptors to reduce transmitter release (Zhang & Chen 1987). In the present study, when sympathetic nerve action was blocked by guanethidine, and the perfusion pressure was increased by methoxamine, transmural nerve stimulation caused a frequency-dependent relaxation of the perfused rat mesentery, which is due to the release of CGRP (Kawasaki et al 1988) from the capsaicin-sensitive sensory nerves. Ginsenosides, however, significantly inhibited this frequency-dependent relaxation in a concentration-dependent manner (Table 1). This suggests that ginsenosides cannot only inhibit sympathetic nerve action, but also suppress capsaicin-sensitive sensory nerve action. Furthermore, ginsenosides had no effect on vasodilator responses to exogenous CGRP. This suggests that inhibition of neurogenic responses by ginsenosides must result from an action at prejunctional sites.

Endothelial factors such as endothelium-derived relaxing factor, besides exerting direct actions on vascular smooth muscle, regulate vascular tone through modulation of sympathetic nerve transmission (Li & Duckles 1992). In the present study, ginsenoside treatment ($30\,\mu g\,mL^{-1}$) alone caused an endothelium-dependent relaxation. Similar results have been seen in a variety of tissues (Chen et al 1993; Kim et al 1993). These results indicate that ginsenosides can facilitate release of nitric oxide from endothelial cells. However, as has been shown previously, endothelium, which can release nitric oxide, does not mediate the vasodilator effects of sensory nerves, and vasodilator responses to CGRP are endothelium-independent in the rat mesentery (Li & Duckles 1992). Then the inhibitory effect of ginsenosides on sensory nerves in the rat perfused mesentery is mediated by endogenous nitric oxide release from tissues other than the endothelium. In our experiment, in the rat perfused mesenteric vasculature without endothelium, ginsenosides still inhibited vasodilator responses to transmural field stimulation. These results suggest that the inhibitory effect of ginsenosides on vasodilator nerves is not related to endothelial function (Table 2).

Although the endothelium is not involved in modulation of sensory vasodilator nerves in the perfused mesentery of the rat, nitric oxide released from tissues other than the endothelium has been shown to regulate peptidergic neurotransmission in guinea-pig ileum and in the rat perfused mesentery (see Li et al 1993; Gustafsson et al 1990). To explore the possible contribution of endogenous nitric oxide, we used L-NAME, an inhibitor of nitric oxide synthesis. Treatment with L-NAME abolished the inhibition of vasodilator responses to transmural field stimulation by ginsenosides, while this effect of L-NAME was reversed completely by addition of L-arginine. These results suggest that ginsenosides inhibit the actions of sensory vasodilator nerves in the perfused mesentery via stimulation of endogenous nitric oxide release. Our previous investigation has shown that, in the preparations without endothelium, inhibition of synthesis or action of nitric oxide significantly enhances vasodilator responses to transmural field stimulation, and that stimulation of nitric oxide release suppresses vasodilator responses to transmural field stimulation (Li et al 1993).

There is an increasing amount of evidence to suggest that nitric oxide, besides being present in the central nervous system and endothelial cells, may be released from vascular smooth muscle cells as well as from NANC nerves (Garthwaite 1991; Yoshida et al 1993). Recently, it has been reported that the neurally-induced relaxation is associated with nitric oxide released from NANC nerves, that activate guanylate cyclase and increases the synthesis of cGMP in monkey mesenteric artery and in dog and monkey cerebral arteries (Toda & Okamura 1990, 1992). These results, together with the findings of our present study, suggest that it is possible that the inhibitory effect of ginsenosides on the actions of sensory nerves was mediated by the nitric oxide release from NANC nerves in the perfused mesentery of the rat.

Nitric oxide activates guanylate cyclase, with a subsequent elevation of tissue levels of cGMP, resulting in relaxation of vascular smooth muscle and inhibition of neurotransmission (Garthwaite 1991). Methylene blue, an inhibitor of guanylate cyclase, is widely used as a tool to evaluate the mechanism of action of vasodilators (Martin et al 1985; Watanabe et al 1988). We explored the effect of methylene blue on the inhibition of the actions of sensory nerves by ginsenosides. Our results showed that the inhibition of vasodilator responses to transmural field stimulation by ginsenosides was abolished by methylene blue. This suggests the effects of ginsenosides may be secondary to the elevation of cGMP via nitric oxide stimulation of guanylate cyclase.

In summary, the present results suggest that ginsenosides inhibit the actions of vasodilator nerves prejunctionally in the rat perfused mesentery, the inhibitory effect of ginsenosides is mediated by nitric oxide and the nitric oxide which participates in modulating neurotransmission may be from NANC nerves.

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