Aqueous Extract of *Rosa rugosa* Radix Dilates Vascular Smooth Muscle Via a NO-cGMP Pathway

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While conducting an in vitro screening of various medicinal plant extracts, an aqueous extract of *Rosa rugosa* Radix (ARR) was found to exhibit a distinct vasorelaxant activity. ARR induced a concentration-dependent relaxation of the phenylephrine-precontracted aorta. This effect disappeared with the removal of functional endothelium. Pretreatment of the aortic tissues with NG-nitro-L-arginine methyl ester (L-NAME) or 1H-[1,2,4]-oxadiazole-[4,3-]-quinoxalin-1-one (ODQ) completely inhibited the relaxation induced by ARR. ARR-induced vascular relaxations were also markedly attenuated by addition of diltiazem or verapamil. However, the relaxant effect of ARR was not blocked by pretreatment with indomethacine, tetraethylammonium (TEA), glibenclamide, atropine, or propranolol. Taken together, the present study suggests that ARR dilates vascular smooth muscle via endothelium-dependent NO/cGMP signaling.

Key words : *Rosa rugosa* Radix (ARR), vasorelaxation, NO/cGMP

Introduction

Endothelial cells respond to various neurohumoral and physical stimuli by releasing endothelium-dependent vasodilators such as endothelium-derived relaxing factor (EDRF)[1], prostacyclin[2], and endothelium-derived hyperpolarizing factor (EDHF)[3]. EDRF has been identified as nitric oxide (NO), which is produced in a reaction catalyzed bynitric oxide synthase (NOS) using L-arginine as a substrate[4]. NO activates soluble guanylyl cyclase and thereby, indirectly increases the production of guanosine 3′, 5′-cyclic monophosphate (cGMP). This leads to protein kinase G (PKG) activation, which inhibits Ca^{2+} influx and decreases the sensitivity of contractile elements to Ca^{2+} [5]. Vascular tone plays an important role in the regulation of blood pressure. The development and maintenance of hypertension has been suggested to involve an inappropriately reduced endothelium-dependent vasodilator influence on the vascular tissue. Indeed, endothelium-dependent vascular relaxation is impaired in human and experimental hypertension[6,7], and the ability of nitric oxide (NO) to maintain vascular tone has been shown to be deficient in this condition[6,7]. Since NO is a potent vasodilator, a deficient production of endothelium-derived NO results in diminished vasodilator tone, allowing vascular resistance to increase, and this contributes to the elevated blood pressure[6]. Therefore, many studies have been performed to search plant resources in which dilate vascular smooth muscle via stimulation of NO release from vascular tissues[8-15].

While conducting an in vitro screen of various medicinal plant extracts, an aqueous extract of *Rosa rugosa* Radix (ARR) was found to exhibit a distinct vasorelaxant activity. Rosa rugosa roots has been reported to have an anti-diabetic activity, anti-hyperlipidemia, and diuretic activity[16-18]. Quercetin, β-sistosterol, and campesterol are well-known constituents of R. rugosa roots[19,20]. To our best knowledge, the effects of ARR on the vascular system have not been examined previously. The present study, therefore, was designed to examine the effects of ARR on the vascular tone and possible mechanisms responsible were investigated.

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Materials and Methods

1. Extraction of ARR

The *Rosa rugosa* THUNB (Rosaceae) rhizome was purchased from an herbal medicine co-operative association in Jeonbuk Province, Korea, in April 2004. Dried Rosa rugosa rhizome (600 g) was extracted with 2 L of boiled distilled water at 100℃ for 2 hr. The aqueous extract was centrifuged at 2500 g for 20 min at 4℃ and filtered with Whatman No. 3

filter paper. The resulting supernatant was lyophilized to produce a powder, which was then kept at 4℃.

2. Preparation of aortic rings

The animal procedures were in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and were approved by the Institutional Animal Care and Utilization Committee for Medical Science of Wonkwang University. Male Sprague-Dawley rats were purchased from Korean Experimental Animals Co. (Daejeon, Korea). The rats (weighing 250 - 300 g) were sacrificed by decapitation. The thoracic aortae were rapidly and carefully dissected and placed into ice-cold Krebs solution (pH 7.4) containing 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.1 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 1.5 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, and 10 mmol/L Glucose. The aortae were removed free of connective tissue and fat, and then cut into rings with a width of approximately 3 mm. All dissecting procedures were done with extreme care to protect the endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface of the aortic ring back and forth several times with plastic tubing. Endothelial integrity or functional removal was verified by the presence or absence, respectively, of the relaxant response to 3 x 10^{-6} M acetylcholine on phenylephrine (3 x 10^{-6} M) contracted vessels.

3. Recording of isometric vascular tone

The aortic rings were suspended, by means of two L-shaped stainless-steel wires inserted into the lumen, in a tissue bath containing Krebs solution (pH 7.4) at 37 C. 95% O_2-5% CO_2 was continuously bubbled through the bath. The baseline load placed on the aortic rings was 2.0 g. Changes in isometric tension were recorded using a force-displacement transducer (Grass FT 03, Quincy, MA, USA) connected to a Grass polygraph recording system (Model 7E). In the first set of experiments, the aortic rings were contacted with phenylephrine (3 x 10^{-6} M) to obtain a maximal response. The aortic rings were then washed every 20 min with Krebs solution until the tension returned to the basal level. A concentration-dependent response curve to acetylcholine (ACh) (10^{-9} - 10^{-5} M) was generated as a positive control for endothelium-intact aortic rings contracted by 3 x 10^{-6} M phenylephrine. The rings were then exposed to various drugs for 30 min and aortic relaxation was carried out by the cumulative addition of ARR. The effect of vehicle, < 0.2% dimethylsulfoxide (DMSO), was also tested. After each test, the aortic rings were washed three times with fresh Krebs solution and allowed to equilibrate for 30 min.

4. Reagents

Acetylcholine chloride (ACh), phenylephrine HCl, NG-nitroarginine methyl ester (L-NAME), methylene blue hydrate, 1H-[1,2,4]-oxadiazole-[4,3-]-quinoxalin-1-one (ODQ), indomethacin, glibenclamide, tetraethylammonium (TEA) chloride, 3-isobutyl-1-methylxanthine (IBMX), (±)-verapamil HCl, atropine, and (±)-propranolol HCl were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetylcholine (ACh), phenylephrine, L-NAME, methylene blue, IBMX, and atropine were dissolved in distilled water. Stock solutions of indomethacin, ODQ, TEA, propranolol, and verapamil were dissolved in DMSO; working solutions were made in Krebs solution. Control experiments demonstrated that the highest DMSO level (0.2%) had no effect on vascular smooth muscle tone.

5. Statistical analysis

Relaxant responses are expressed as percentage relaxation from phenylephrine precontraction levels unless otherwise described in the figure legends. Results were expressed as means ± SEM. The statistical significance of the difference between the group means was determined using the one-way ANOVA and Student's t-test.

Results

With endothelium-intact aortic preparations, ARR relaxed phenylephrine (3 x 10^{-6} M) precontracted aortic rings in a dose-dependent manner (Fig. 1A). The maximal relaxant effect of ARR was 92.55 ± 1.26% (vs. phenylephrine contraction) under the concentration of 1 x 10^{-4} g/ml. The relaxant effect of ARR in aortic tissue was completely abolished by denudation of the endothelial layer. As shown in Fig. 1B, Pretreatment of aortic tissue with L-NAME (1 x 10^{-6} M) completely inhibited the ARR-induced relaxation. ODQ (1 x 10^{-6} M), inhibitor of soluble guanylyl cyclase, also completely blocked the concentration-dependent relaxation induced by ARR. We examined the effect of indomethacin on ARR-induced vascular relaxation in order to determine whether prostacyclin is involved in ARR-induced vasorelaxation As shown in Figure 2A, ARR-induced endothelium-dependent vascular relaxation was not altered by addition of 1 x 10^{-5} M indomethacin. To assess whether vascular relaxation induced by ARR is associated with the activation of muscarinic or adrenergic receptors, the effects of atropine or propranolol on the endothelium-dependent relaxation to ARR were examined.

Preincubation of the aortic rings with either atropine (1×10^{-6} M) or propranolol did not affect the relaxations to ARR (Fig. 2B).

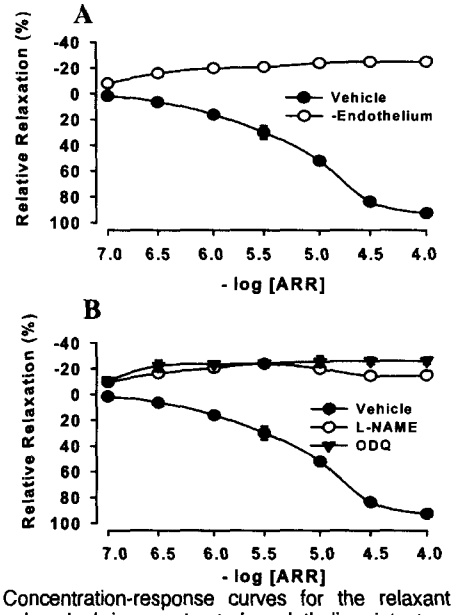

Fig. 1. Concentration-response curves for the relaxant effect of ARR in phenylephrine contracted endothelium-intact aortic rings or endothelium-denuded aortic ring (-endothelium) (A). Concentration-response curves for the relaxant effect of ARR in phenylephrine contracted endothelium-intact (vehicle) aortic rings in the absence (vehicle) or presence of 1×10^{-6} M L-NAME (L-NAME) or 1×10^{-6} M 1H-[1,2,4]-oxadiazole-[4,3-]-quinoxalin- 1-one (ODQ) (B). Each value shows mean ± SEM of six experiments.

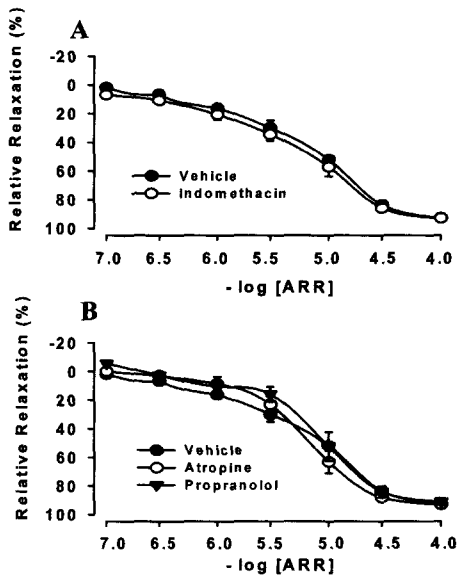

Fig. 2. Concentration-response curves for the relaxant effect of ARR in the endothelial intact aortic rings in the absence (vehicle) or presence of indomethacin (1×10^{-5} M) (A), atropine (1×10^{-6} M), or propranolol (1×10^{-6} M) (B). Each value shows mean ± SEM of six experiments.

To determine whether Ca^{2+} channel is involved in ARR-induced vasorelaxation, we also assayed the effects of Ca^{2+} channel blockers on ARR-induced relaxation. ARR-induced endothelium-dependent vascular relaxation was attenuated by addition of Ca^{2+} channel blockers such as verapamil (1×10^{-6} M) or diltiazem (1×10^{-5} M) (Fig. 3A). However, the relaxant effect of ARR was not altered by pretreatment with TEA (1×10^{-4} M), a nonselective K^+-channel blocker, or glibenclamide(1×10^{-5} M), an ATP-sensitive K^+-channel blocker (Fig. 3B).

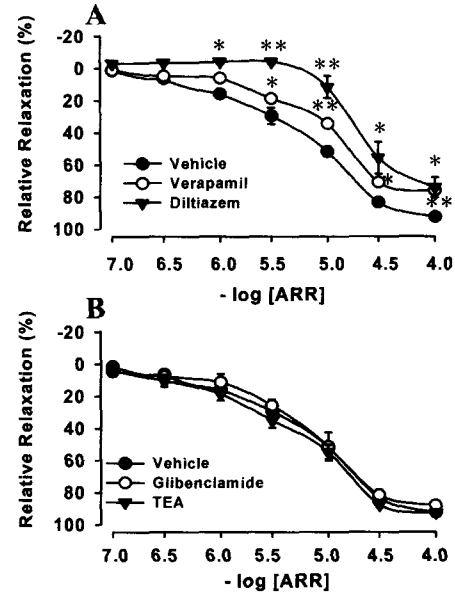

Fig. 3. Concentration-response curves for the relaxant effect of ARR in the endothelial intact aortic rings in the presence of diltiazem (1×10^{-5} M) or verapamil (1×10^{-6} M) (A), and glibenclamide (1×10^{-6} M) or tetraethylammonium (TEA) (1×10^{-4} M) (B). Each value shows mean ± SEM of six experiments. *p<0.05, **p<0.01 vs. vehicle.

Discussion

The present study showed that ARR exerts a vasorelaxant effect on phenylephrine-contracted aortic rings from Sprague-Dawley rats. Removing functional endothelium abolishes the relaxant response to ARR, suggesting that the vasorelaxation caused by ARR is endothelium-dependent. To verify the involvement of endothelium-derived vasodilators, the effects of various inhibitors on ARR-induced vascular relaxation were examined. Pretreatment of aortic tissues with L-NAME, a nitric oxide synthase inhibitor, abolished the ARR-induced vascular relaxation. The present study also showed that pretreatment aortic rings with ODQ, which are soluble guanylyl cyclase inhibitor, completely blocked the vascular relaxation induced by ARR. These results suggest that the ARR-induced vascular relaxation is closely related with the activation of a NO-cGMP pathway. To determine whether prostacyclin is involved in ARR-induced vasorelaxation, we also assayed the effects of indomethacin on ARR-induced relaxation. The endothelium-dependent relaxation caused by ARR in aortic rings was not affected by indomethacin, indicating that vasoactive prostacyclin (PGI2) may not contribute to the ARR-induced relaxation[21]. To assess whether

vascular relaxation induced by ARR is associated with the activation of muscarinic or adrenergic receptors, the effect of atropine on the endothelium-dependent relaxation to ARR was examined. Preincubation of the aortic rings with either atropine or propranolol did not affect the relaxant response to ARR. These findings indicate that ARR does not interact with muscarinic or adrenergic receptors in ARR-induced relaxation[22]. Since calcium and potassium channels play an essential role in NO synthesis and release in endothelial cells[23,24], we tested the effects of calcium and potassium channel blockers on ARR-induced vascular relaxation. Because ARR-induced relaxation was not inhibited by glibenclamide or tetraethylammonium (TEA), our results indicate that K^+ channels do not play a significant role. On the other hand, pretreatment with either verapamil or diltiazem, voltage-gated calcium channel blockers, markedly attenuated the relaxant response to ARR in aortic tissues. Therefore, ARR-induced relaxation may be closely related with L-type Ca^{2+} channel function.

Relaxation of vascular smooth muscle by NO-cGMP signaling involves a sequence of steps. Nitric oxide is formed in the endothelium by the activation of nitric oxide synthase using L-arginine as a substrate. Once formed, nitric oxide diffuses out of the endothelium with some entering the underlying vascular smooth muscle where it binds to and activates soluble guanylyl cyclase. This enzyme catalyzes the conversion of GTP to cGMP. cGMP-activated protein kinase G inhibits Ca^{2+} influx, augments Ca^{2+} sequestration and decreases the sensitivity of contractile elements to Ca^{2+} [5]. These findings are consistent with the hypothesis that ARR-induced vascular relaxation is due to the activation of a vascular NO/cGMP system, which may be causally related with L-type Ca^{2+} channels[23].

NO-cGMP pathway plays an important role in the not only relaxation of vascular smooth muscle but also inhibition of vascular smooth muscle cell (VSMC) proliferation, adhesion of platelets and leukocytes, endothelial permeability, and extracellular matrix collagen synthesis in the vascular system. A reduced production of NO by vascular endothelial cells is closely associated with the endothelial dysfunction or injury, which is proposed to be an important factor in severe pathologies such as atherosclerosis and hypertension[25]. Chronic inhibition of NO synthesis with the administration of inhibitor of NOS causes a vascular inflammation as well as hypertension in animal experiments. Therefore, the development of vasodilators acting by restoring the level of NO-cGMP in the vascular system can be of great value for the treatment of these cardiovascular diseases. Recently, many studies have been performed to find more suitable anti-hypertensive or anti-atherosclerotic from natural resources. Among them, extracts of hawthorn[10,11], Caesalpine sappan[12], Uncaria rhynchophylla[8,13], Cordyceps sinensis[9], Gynostemma pentaphyllum[15], Salviae miltiorrhizae[14], Cudrania tricuspidata[26], Fritillaria ussuriensis[27] exhibited an antihypertensive effect by endothelium-dependent vasorelaxation or direct stimulation of NO-cGMP release. In the present study, the endothelium-dependent vasorelaxant effect of MSC may be mediated via the endothelial NO signaling in aortic tissues. These results could be useful to further study to MSC on animal models with cardiovascular diseases.

Taken together, the present data suggest that aqueous extract of Rosa rugosa Radix (ARR) dilates vascular smooth muscle via activating NO/cGMP signaling.

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