Diphasic Effects of Astragalus membranaceus Bunge (Leguminosae) on Vascular Tone in Rat Thoracic Aorta

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This study was designed to investigate the effects of the aqueous ethanol extract of Astragalus membranaceus Bunge (Leguminosae) on rat thoracic aorta. Isometric tension was recorded in response to drugs in organ bath. In endothelium-intact aortic rings, A. membranaceus extract induced a significant dose-dependent relaxation of the rings precontracted by phenylephrine, which could be inhibited by preincubation with L-N(ω)-nitro-arginine methyl ester or methyloxanthinium chloride. In endothelium-denuded ones, the extract could dose-dependently relax the rings contracted by phenylephrine, not by KCl; and it could also attenuate contractile response to phenylephrine, not to caffeine or phorbol-12,13-diacetate in Ca2+-free medium; but it failed to affect the CaCl2-induced enhancement of contractile response to phenylephrine in Ca2+-free medium. These results indicate that nitric oxide signaling and Ca2+-handling pathway are involved in the A. membranaceus extract-induced vasodilatation.

Key words Astragalus membranaceus; nitric oxide; calcium ion; thoracic aorta; vasmotion

The roots of Astragalus membranaceus Bunge (Leguminosae) are amongst the most popular and important “Qi (pronounce chee) tonifying” adaptogenic herbs in China, their use dates back more than 2000 years, and are recorded in Shen Nong’s Materia Medica. According to ethnobotanical data collected in China, A. membranaceus has been prescribed for centuries for diarrhea, frequent colds, spontaneous sweats, fatigue, and edema. Furthermore, A. membranaceus is alleged to possess the effects of antioxidative damage, immune-stimulation, and antiviral infection. Recently, it has received more attention that the fact recorded in ancient Chinese texts that A. membranaceus possessed an antihypertensive effect by raising the yang qi of the spleen and kidney. Since deficiency qi formed by the disorder of spleen and kidney is considered to be the basic pathogenesis of hypertension. A. membranaceus was beneficial on the process of hypertension in animals and humans, and a cooperative mechanism of renin angiotensin aldosterone system, kallikrein bradykinin system, and central neuropeptide was used to explain this effect of A. membranaceus. We also found in the past experiments that the administration of A. membranaceus could attenuate the progression of blood pressure in spontaneously hypertensive rats concomitant with restoration of baroreflex sensitivity and reduction of Ca2+ concentration in lymphocyte and vascular smooth muscle cells (VSMC). Additionally, in vivo experiment had demonstrated that A. membranaceus could induce the vasodilatation. However, there is no experiment evidence available to show the exact mechanism of vaso-relaxation produced by A. membranaceus. The present study is conducted to elucidate various possible mechanisms in vasmotor response of A. membranaceus.

MATERIALS AND METHODS

Plant Material and Preparation of the Extracts A. membranaceus roots were collected in the northern region of Gansu Province, China, in the autumn (2001) and identified by the Quality Assessment of Di’ao Group, Sichuang, China. And a voucher specimen was deposited in the Academic Department of Di’ao Group (STQ-C-00203). A. membranaceus roots were air dried. A crude extract was prepared by decocction of 2.0×102 g in 500 ml water for 3 times (90 min per time). The obtained extract was combined, filtered and concentrated. Then all the aqueous extract was extracted with 400 ml ethanol (75% and 85%) twice. After the ethanol was retrieved, the active fractions were recovered, filtered and lyophilized (yield 0.28%). Consequently, the final extract was used in the study after dissolution in distilled water at 37 °C and stirred for 60 min.

Chemicals Phenylephrine (PE), acetylcholine (ACh), L-N(ω)-nitro-arginine methyl ester (L-NAME), methylthioninium chloride (MC), phosphoramidon, ethylene glycol-bis [β-aminoethyl ether]-N,N,N′,N′-tetraacetic acid (EGTA), caffeine and phorbol-12,13-diacetate (PD) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). They were dissolved in distilled water and diluted with Krebs–Henseleit (K–H) solution before use. All chemicals were of the highest purity available.

Experiment Animals The investigation conformed to the Chinese Regulation for Administration of Laboratory Animal (SSTC 1988/2). Health male Sprague–Dawley rats weighing 260—280 g (Laboratory Animal Center of Zhejiang College of Traditional Chinese Medicine, Hangzhou, China) were housed in conventional cages with free access to water and rodent chow at the controlled temperature and humidity with a 12-h light/dark cycle.

Preparation of Thoracic Arterial Rings from Sprague–Dawley Rats
Dawley Rats  Rats were euthanized by decapitation using small animal guillotine. The thoracic aorta was immediately isolated and immersed in oxygenated K–H solution 4°C of the following composition (in mM): NaCl 118.3; KCl 4.7; MgSO$_4$ 1.2; KH$_2$PO$_4$ 1.2; NaHCO$_3$ 25.0; CaCl$_2$ 2.5; Glucose 11.0. Then the adherent connective tissue was cleaned and blood vessel was cut into 3—4 mm rings, with the special care of avoiding damage of endothelium. In some preparations, endothelium was mechanically removed by gently rubbing the lumen. The rings were mounted horizontally between two stirrups in organ bath filled with 10 ml K–H solution at 37°C, ventilated continuously with 95% O$_2$ and 5% CO$_2$. The isometric tension was recorded with a force transducer (JZ101, metrical range: 0—5.0 g) and MedLab 5.0v recording system (Nanjing Medpace Science and Technology Co. Ltd).

Experimental Procedure of Isometric Tension  Rings were equilibrated for 60 min at 2.0 g resting tension, and then challenged with KCl (6.0×10$^{-2}$ M) at least 3 times until a reproducible maximal contractile response was obtained. After a further equilibration period of 30 min, the integrity of the endothelium was assessed in all preparations by determining the ability of ACh (1.0×10$^{-5}$ M) to induce more than 80% relaxation of rings pre-contracted with PE (1.0×10$^{-5}$ M). The endothelium was considered to be removed when there was less than 10% relaxation response to ACh.

Protocol 1: Effects of A. membranaceus on Vascular Tone: The first series of the experiments were conducted to assess endothelium-dependent or independent effects of A. membranaceus on isolated aortic rings. When the tension was at resting state or reached a plateau induced by PE (3.0×10$^{-7}$ M), A. membranaceus (2.0×10$^{-5}$—2.0×10$^{-1}$ g/l) was cumulatively added into the organ bath with 10 min interval. The rings with intact or denuded endothelium were always tested in parallel. The rings with intact or denuded endothelium were always run in parallel. Following the initial control response in Ca$^{2+}$-free medium and before the second test contraction (to PE or caffeine), a 6.0×10$^{-2}$ M KCl-induced contraction in K–H solution was obtained to refill the Ca$^{2+}$ stores in the vascular tissues.

Protocol 3: Endothelium-Dependent Vasomotor Response by A. membranaceus: In the third series of experiments, the potential influence of A. membranaceus on PE-induced vasomotor function in the intact rings was examined. For pretreatment, endothelium-intact rings were incubated with L-NAME (1.0×10$^{-4}$ M) (a specific inhibitor of nitric oxide synthase), MC (1.0×10$^{-3}$ M) (an inhibitor of guanylate cyclase) for 15 min$^{(15)}$ and phosphoramidon (5.0×10$^{-6}$ M) (an inhibitor of endothelin converting enzyme) for 20 min$^{(16)}$ before administration of PE. Then the response curves of A. membranaceus were recorded. The rings with or without the treatment of inhibitors were always tested in parallel.

Statistical Analysis  Relaxant responses were expressed as the percentage decreases of the magnitude of the contraction induced by PE or KCl (=100%) before the application of vasodilators, and the contractile responses as the ratio of the responses following and before the application of A. membranaceus (second response/first response). All results are expressed as mean±S.E.M. Statistical analysis was performed with t-test or ANOVA followed by Newman–Keuls test. Differences were accepted as statistically significant at p values<0.05 (GraphPad Prism).

RESULTS

PE (1.0×10$^{-6}$ M) induced a similar sustained contraction of aortic rings in each group with a peak tension of about 3.11±0.27 g in intact aortic rings and 3.02±0.19 g in denuded ones.

Effects of A. membranaceus on the Vasotension  All doses (2.0×10$^{-5}$—2.0×10$^{-1}$ g/l) of A. membranaceus exhibited negligible vasomotor actions on aortic rings with or without endothelium at resting tension (Fig. 1). A. membranaceus (2.0×10$^{-5}$—2.0×10$^{-1}$ g/l) produced dose-dependent relaxation in endothelium-denuded rings (Fig. 2), after the plateau tension induced by PE. However, in PE-precontracted endothelium-intact rings, low doses (2.0×10$^{-4}$—6.0×10$^{-3}$ g/l) of A. membranaceus showed a more significant relaxation than that in denuded ones (Fig. 2), while high dose (2.0×10$^{-2}$—2.0×10$^{-1}$ g/l) of the extract induced a transient contraction (Figs. 2, 3).

Endothelium-Independent Vasodilatation by A. membranaceus  In contrast to the effect on PE-contracted endothelium-denuded rings in K–H solution, A. membranaceus (2.0×10$^{-4}$—2.0×10$^{-1}$ g/l) had no dilatation-effect on rings stimulated by KCl (3.0×10$^{-2}$ M) (Fig. 4).

And in the experiment with Ca$^{2+}$-free medium, aortic contraction elicited by PE, caffeine or PD was decreased substantially, as these vascular contractions in Ca$^{2+}$-free medium are mediated only via the release of Ca$^{2+}$ from the intracellular stores or phosphorylation of myosin light chain. A. membranaceus (6.0×10$^{-3}$ g/l) could inhibit the contraction induced by PE (3.0×10$^{-7}$ M), but not block the extracellular Ca$^{2+}$ inflow, because 2.5 mM CaCl$_2$ induced a similar enhancement of contraction by PE, compared with the control (95.84±2.63% vs. 96.96±1.87%, p>0.05). Furthermore, A. membranaceus (6.0×10$^{-3}$ g/l) failed to induce an attenuation of contractile response by PD (1.0×10$^{-5}$ M) and caffeine (2.5×10$^{-3}$ M) (Fig. 5).

Endothelium-Dependent Vasomotor Effects by A. membranaceus  Pre-incubation of the intact rings with L-
NAME (1.0×10⁻⁴ M) markedly, incompletely inhibited A. membranaceus (6.0×10⁻³ g/l)-induced relaxation from 36.0 to 7.3% (Fig. 6). Similarly, MC (1.0×10⁻⁵ M) also showed an inhibition of endothelium-dependent vasodilatation from 36.0 to 8.0% (Fig. 6). Thus, NO signaling might play a major, but not full role in the vasodilatation effect of A. membranaceus.

When the dose of A. membranaceus in the organ bath was stepwise raised to 2.0×10⁻²—2.0×10⁻¹ g/l, a transient contraction could be observed in endothelium-intact rings. Pre-treatment of endothelium-intact rings with phosphoramidon (5.0×10⁻⁶ M) significantly attenuated the transient contraction response (Fig. 3).

DISCUSSION

The present study first and detailedly explored the in vitro vascular effects of A. membranaceus in aortic rings isolated from rats. In the course of the study, a number of novel observations had been made, which contribute to better scien-
Denuded Rings

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ephrine (3.0 g/l) on KCl (3.0 Fig. 5. Inhibitory Effect of

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vasorelaxant Effects of

Nitro-arginine Methyl Ester (L-NAME) (1.0 through receptor-gated Ca2+ channels; 2) mobilizes Ca2+
from intracellular stores via the inositol-1,4,5-trisphosphate (IP3) receptor or induces secondly myosin light chain phosphorylation via activating PKC.17,18) That is, the vasorelaxant action of A. membranaceus seems to occur in a receptor-de-
dependent manner in SMVC of rat aortas. However, the result that A. membranaceus failed to inhibit the contraction caused by addition of CaCl2 in Ca2+ free solution was probably only due to its effects on the intracellular pathways. This hypothe-
ysis was confirmed in the further experiments that the contrac-
tions induced by PD and caffeine in Ca2+-free medium were not affected by A. membranaceus equally. Thus, it appears that the IP3-induced Ca2+ release channels might be the site of action for A. membranaceus on endothelium-removed aor-
tas.

Third, NO is a potent vasodilator synthesized in the endo-
thelium19) by NO synthase, and causes VSMC relaxation
through the activation of soluble guanylate cyclase.20) The
present study demonstrated that the extract of A. membranaceus dose-dependently inhibited the contraction in-
duced by PE in intact aorta isolated from rats. This vasore-
lexant action was mostly inhibited by treatment with L-
NAME or MC. Endothelium-dependent relaxation of A. membranaceus seemed to be associated with NO signaling
via guanylate cyclase activation since both L-NAME and MC
seemed to be associated with NO signaling.

In the untreated group, A. membranaceus or vehicle group was not pretreated with L-NAME or MC. Each column with a bar represents mean±S.E.M. of 8 experiments. Contractile responses are expressed as the percentage from maximal contraction elicited by phenylephrine (3.0x10^-3 M). **p<0.01, compared with value of the corresponding vehicle group.
the NO-GC pathway, whilst the effect of transient contraction of *A. membranaceus* at a high dose was related with endothelin release.

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**REFERENCES**