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Original Articles

The Inhibition Effect of Water Extract of *Acanthopanax senticosus* Harms Roots on the 5-HT Induced Vasocontraction in Rat

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Objective : To examine the inhibition of 5-Hydroxytryptamine (5-HT) induced vasocontraction of the water extract of *Acanthopanax senticosus* Harms roots (ASR) on rat thoracic aorta and mesenteric artery with and without endothelium.

Methods : Segments of thoracic aorta and mesenteric artery obtained from rats immediately after delivery were mounted in organ baths superfused on a polygraph.

Results : We found that the thoracic aorta segments responded to the water extract of ASR with a dose-dependent and concentration-dependent vasorelaxation. 5-HT produced a concentration-dependent contraction of the thoracic aorta and mesenteric artery. At high concentrations of ASR, the inhibition responses were 93.7% (Jang-su), 93.5% (Heok-ryong-kang-sung), 92.8% (Mt. Back-doo), and 83.5% (Yeon-byun) of the maximum 5-HT induced contraction. At high concentrations of ASR, the relaxational response at thoracic aorta and mesenteric artery with endothelium were 95.2% and 94.6%; without endothelium were 93.5% and 92.5% of the maximum 5-HT induced contraction.

Conclusions : In conclusion, the effect of water extract of ASR had potent inhibition at 5-HT and the effect of ASR in isolated thoracic aorta and mesenteric artery showed dose-dependent inhibition but endothelium-independent response.
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Key Words: *Acanthopanax senticosus* harms, 5-Hydroxytryptamine, thoracic aorta, mesenteric artery

Introduction

The herbal plant *Acanthopanax senticosus* Harms (Araliaceae) is widely found in Russia, Northeast China, Korea and Japan[1,4,5,7,9,15]. It is a typical oriental herb and its pharmacological effects are very similar to that of *Panax ginseng* CA Mayer.The A. senticosus exhibits wide pharmacological actions, like *P. ginseng*, called adaptogen. Unlike *P. ginseng, A. senticosus* is widely used but it is a poorly understood tonic. Plant medicines have a variety of pharmacological activities. The roots of A. senticosus have been used as a tonic, anti-rheumatic and prophylactic for chronic bronchitis, hypertension, tumor, and ischemic diseases[1-4].

It is reported that 5-HT induced vasocontraction inhibited by water extract of *Ginkgo biloba* Linne is endothelium dependent vasodilation. In this study, we examined the effect of ASR on 5-HT induced vaso-contraction and whether it is dependent on endothelium or not. ASR has a relaxation effect on rat isolated

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thoracic aorta and mesenteric artery with and without endothelium.

Materials and Methods

1. Plant Material

1) Plant Origin

The dried roots of *A. senticosus* were collected in June 2002, at Jang-su Agriculture Development & Technology Center, Korea, in August 2002, at Mt. Back-doo, Ryeon-byun, China, received as a gift from Pajin-bio company (Korea), and collected at Heok-ryong-kang-sung, China(Table 1).

2) Preparation of ASR

A water extract of ASR was prepared from dried roots native to Korea and China. They were cut into small pieces and mashed with a mortar and pestle. The roots (100 g) were extracted with boiling water (50 g/L), total volume 2 L. This mixture was stored in a refrigerator until the analyses. Before the analytical procedures the water extract was filtered through a syringe filter (0.45 μm pore size).

3) Handling of ASR

The extract of ASR dissolved in 1 ml of distilled water was given to the organ chamber (20 ml bath).

2. Animals

Male Sprague-Dawley rats weighing 300~350 g were used for all experiments.

They were purchased from Semtaco Animal Care. They were subjected to preliminary group-housing (3-4 per cage) under controlled conditions with food and water *ad libitum*. The animals were kept at 22±2 ℃ and controlled humidity (50-60%) under a 12 h light/dark cycle.

3. Materials

NaCl, KCl, NaH_2PO_4, $MgSO_4$, $CaCl_2$, $NaHCO_3$, glucose, 5-HT and acetylcholine were purchased from Sigma (Sigma, USA).

4. Preparation of isolated aortic rings

Male Sprague-Dawley rats (300-350 g) were killed with an overdose of chloral hydrate (400 mg/kg, ip) and the thoracic aorta and mesenteric artery were removed and cleaned of adherent tissue. The aorta and mesenteric artery were mounted on a length of scoured polythene tubing and placed in a petri dish containing modified physiological salt solution (PSS) of the following (mM) composition: NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.0, $CaCl_2$ 0.25. The aorta was cleared of surrounding adipose tissue and the endothelium was removed by gentle rubbing of the intimal surface with the polythene tube. Six to nine ring segments (2-3 mm length) were prepared from each aorta and were mounted between two stainless-steel wires in 5 ml organ baths, thermostatistically controlled at 37 ℃, containing modified PSS. The solution was bubbled with a gas mixture consisting of 95% O_2 and 5% CO_2 in order to keep a pH in the bath of around 7.35-7.38. Experiments were carried out after the vessel had equilibrated, usually

Table 1. Plant Materials used for Pharmacological Experiment. (Vouchers are in College of Oriental Medicine in KyungHee University)

Species	Vouchers	Origin	Locality	Date
Dried root of A. senticosus	KHU0101	Cultivated	China: Heok-ryong-kang-sung	Jul 2001
	KHU0202	Cultivated	China: Ryeon-byun	Jul 2002
	KHU0203	Wild	China: Mt. Back-doo	Jul 2002
	KHU0204	Cultivated	Korea: Jun-book: Jang-su	Jun 2002

within 1-2 h of mounting. The tension was recorded isometrically with a Grass FT03C force-displacement transducer and registered on a Grass model 7 polygraph. The vessels were given an initial passive load of about 50 mN and allowed to equilibrate for at least 30 min prior to the experiments. After the equilibrating period, vessels were stimulated with KCl (100 mM) in order to obtain a reference contraction. This contraction was defined as the maximal contraction to KCl.

Vessels that did not respond or responded abnormally were not tested further. ASR and other substances were dissolved in 0.9% NaCl and given to the baths in volumes of 5 ml.

The response to the added substances (contraction, relaxation) was expressed as a percentage of the maximal KCl-induced contraction exhibited by each ring.

5. Removal of endothelium

To preclude the possible role of endothelium in the vasodilation of ASR, the tests were conducted in endothelium-denuded preparation. The endothelium was removed by gently rubbing against the teeth of a pair of forceps. Success of the removal of the endothelium was characterized using the failure of 10^{-6} M acetylcholine to relax the rings precontracted with 10^{-4} M 5-HT

6. Vasodilation of ASR

After the resting tension became stabilized, 10^{-4} M 5-HT were administrated into bathing buffer to induce a rapid increase of vascular tone followed by the stable vasoconstriction. Treatments groups were administered ASR from concentrations of $10^{-4}$to 1 mg/ml to observe vasodilation (the decrease of tonic contraction). Concentration-relaxation curves were generated in a cumulative fashion.

7. Statistical analysis

Analysis of data from two groups was performed using Student' s *t*-test. Data from several groups were examined using analysis of variance (ANOVA), using the computer program GraphPad Prism (GraphPad Software, San Diego, CA). Significance levels were set as follows: $p = 0.05$(*), $p = 0.01$(**), $p = 0.001$(***).

Results

1. 5-HT induced vasocontraction on thoracic aorta

5-HT (10^{-9} M~10^{-4} M) produced contraction of the thoracic aorta. Thoracic aorta segments responded to 5-HT with a concentration-dependent and dose-dependent vasocontraction. At 10^{-9} M~10^{-5} M 5-HT, the contractile responses were 0.0%, 0.0%, 0.0%, 27.6%, 89.9% and 103.7%; the maximal contractile response was 103.7% of the maximum KCl-response. EC_{50} (-logEC_{50}) was 5.7 (Fig. 1).

2. Effect of regional ASR on 5-HT-induced contraction of thoracic aorta

Thoracic aorta segments responded to 5-HT with a dose-dependent vasocontraction. At maximum 5-HT (10^{-4} M), the maximum contractile responses of the

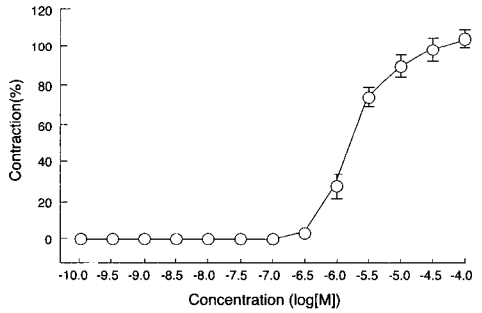

Fig. 1. Contractile response of 5-HT (○) at concentrations ranging from 10^{-10} M to 10^{-4} M. Results represent mean ± SEM on the maximal contractional response. On the X-axis: log[M] ; on the Y-axis: contraction (%). n=5-6.

egional ASR (10 mg/ml) were 93.7% (JS), 93.5% (HR), 92.8% (BD) and 83.5% (RB) of the maximum 5-HT induced contraction(Fig. 2).

3. The inhibition effect of ASR on 5-HT-induced vasocontraction in thoracic aorta and mesenteric arteries

Thoracic aorta and mesenteric artery segments responded to 5-HT with a dose-dependent vasoconstriction. At 10 mg/ml ASR (HR), the inhibition effects of 5-HT induced contractions at thoracic aorta and mesenteric artery with endothelium were 95.2% and 94.6%. EC_{50} were 3.8 mg/ml and 3.1 mg/ml; without endothelium were 93.5% and 92.5% of the maximum 5-HT induced contraction. EC_{50} were 4.6 mg/ml and 4.4 mg/ml(Fig. 3).

Discussion

The roots of *A. senticosus* were sold on the market as Siberian ginseng. The Koreans call it *ga-si-o-ga-pi* and the Chinese called it North *wujiapia*. It has been frequently confused with several similarly named herbs (*o-ga-pi*) in Korea. It has been used as a tonic, antirheumatic and prophylactic for chronic bronchitis, hypertension and ischemic disease1-4). The properties and taste are pungent, bitter and warm. It enters the liver and kidney meridians. The functions are to dispel wind and dampness, to strengthen the tendons and bones, to treat deficient liver and kidney manifesting as soreness, weakness and pain in the lumbar region and the knee, and to benefit urination[5]. The herb exhibits pharmacological actions; its effects are very similar to those of ginseng[6]. The drug has also been shown to have significant sedative action in mice[7].

5-HT produced a concentration-dependent contraction of the thoracic aorta and mesenteric artery. We found that the thoracic aorta and mesenteric artery vessel segments with ASR responded dose-dependent inhibition on 5-HT induced vasocontraction.

At high concentrations of ASR, the inhibition of 5-HT induced contraction was potent, but it wasn' t endothelium-dependent. It was reported that the inhibition of water extract of G. biloba on 5-HT induced vasocontraction was endothelium-dependent. ASR such as *P. ginseng*, *Rheum palmatum* Linne and *R. undu-*

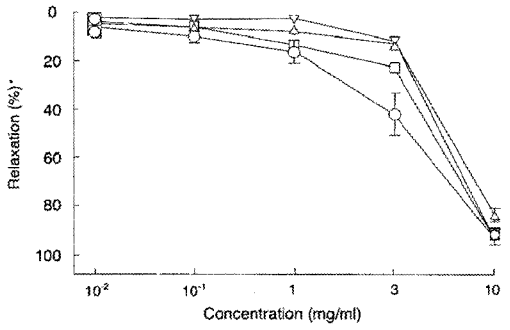

Fig. 2. The dried roots extract of ASH caused concentration-dependent relaxation in the 10^{-4} M 5-HT-induced contraction of rat thoracic aorta without endothelium. Heok-ryong-kang-sung (○); Yeon-byun (△); Mt. Back-doo (▽); Jang-su (□). Data represent mean ± SEM. n=4.

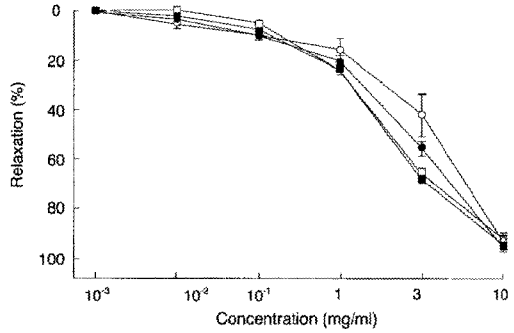

Fig. 3. ASH-caused concentration-dependent relaxation in the 5-HT-induced contraction of rat thoracic aorta (TA) and mesenteric artery (MA) with (TA, ●; MA ■) and without endothelium (TA, ○; MA, □). Da represent mean ± SEM. n=4-6.

latum Linne weren' t endothelium-dependent. At high concentrations of ASR, the inhibition effect on mesenteric artery was a more sensitive response than thoracic aorta at the maximum 5-HT induced contraction.

In conclusion, the effect of water extract of ASR had potent inhibition on 5-HT induced contraction and the effect of ASR in isolated thoracic aorta and mesenteric artery showed dose-dependent inhibition but endothelium-independent response.

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