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Screening of Plant Materials for the Antagonistic Effects against Angiotensin or Bradykinin

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Angiotensin 또는 Bradykinin 길항작용 식물의 검색

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Angiotensins and bradykinin play important roles in blood pressure regulation and in other physiological and pathological conditions. The present work is aimed to investigate potential antagonists against either angiotensin or bradykinin from plant sources. Each methanol extract of selected 25 plants was solvent fractionated to three fractions. The effects of total of 75 plant samples against the contractions induced by angiotensin I or bradykinin in rat ileum were measured. The results are summerized.

It has been well documented that angiotensins and bradykinin play important roles in blood pressure regulation and in other physiological and pathological conditions.[1,2]

During the last few years, much efforts has been given to investigate the antagonists against either angiotensin or bradykinin.[3-6] Various peptide analogs of angiotensin or bradykinin have been prepared and utilized as pharmacological tools, however most of the peptide analogs are unstable at the physiological conditions to observe prolonged effects. Stable, competitive and specific antagonists against either angiotensin or bradykinin should find wide application as either pharmacological tools or therapeutic agents.

The present work is aimed to investigate potential antagonists against angiotensin or bradykinin from plant sources. Plants were selected from those with folkloric reputation. The effects of plant materials against the contractions induced by angiotensin or bradykinin in rat ileum were measured.[7]

Experimental Procedure

Plant materials

Plant materials were purchased from the local herb drug market and identified taxonomically.

Extraction and solvent fractionation of plant materials

Plant materails were extracted and fractionated as described on Fig. 1. 300 g of each plant material was extracted twice with methanol for 6 hrs. Then it was filtered off and the filtrate was concentrated under reduced pressure. The MeOH extract was partitioned between $CHCl_3$ and water. $CHCl_3$ layer on evaporation of the solvent was again partitioned with haxane and 90% MeOH. Water layer (Fr. I), 90% MeOH

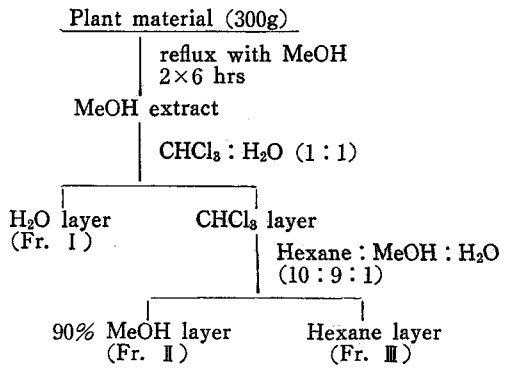

Fig. I. Extraction and solvent fractionation of plant materials.

layer (Fr. II) and Hexane layer (Fr. III) after evaporation of the solvents were used as test samples.

Rat ileum assay

Samples were tested for their effects on contractile responses of rat ileum to angiotensin I, bradykinin and acetyl choline. Male rats (Sprague-Dawley, 180~250 g) were killed by stunning and bleeding through carotid arteries. Terminal ileum was isolated. 2~3 cm segments of ileum were suspended in tissue bath fillled with

Table I. Antagonistic effects of plant preparations against angiotensin, bradykinin and acetyl choline.

Plant name (Family name)	Parts of plants used	Fr.	Conc. mg/cc	Agonists		
				AI	Bk	ACh
Acanthopanax spp. (Araliaceae)	ba	I	1	—	—	
		II	0.01	—	—	
		III	0.05	+	+	+
Achyranthes japonica (Amarantaceae)	ra	I	1	—	—	
		II	0.1	—	—	
		III	0.1	—	—	
Aconitum pseudolaeve (Ranunculaceae)	ra	I	1	+	+	+
		II	0.1	++	++	—
		III	0.1	—	—	
Alisma orientale (Alismataceae)	tu	I	1	—	—	
		II	0.1	+	+	+
		III	0.1	—	—	
Angelica gigas (Umbelliferae)	ra	I	1	—	—	
		II	0.025	+	+	+
		III	R			
Angelica koreana (Umbelliferae)	ra	I	1	—	—	
		II	R			
		III	0.01	+	+	+
Aralia continentalis (Araliaceae)	ra	I	1	—	—	
		II	0.05	++	+	+
		III	0.05	++	++	+
Astragalus membranaceus (Leguminosae)	ra	I	1	—	—	
		II	0.05	+	+	+
		III	0.05	—	+	—
Atractylodes japonica (Compositae)	rh	I	1	—	—	
		II	R			
		III	0.05	+	+	—
Carthamus tinctorius (Compositae)	fl	I	1	—	—	
		II	0.1	+	+	+
		III	0.1	—	—	

Plant name (Family name)	Parts of plants used	Fr.	Conc. mg/cc	Agonists		
				AI	Bk	ACh
Chrysanthemum indicum (Compositae)	fl	I	1	—	—	
		II	0.01	—	—	
		III	0.1	—	—	
Conioselium spp. (Umbelliferae)	rh	I	R			
		II	0.01	+	++	+
		III	R			
Crataegus pinnatifida (Rosaceae)	fr	I	1	—	—	
		II	0.05	+	+	+
		III	0.05	—	—	
Eucommiae ulmoides(Eucommiaceae)	sb	I	1	—	—	
		II	0.05	+	+	+
		III	0.05	—	—	
Fritillaria spp. (Liliaceae)	tu	I	1	—	—	
		II	0.05	++	+	++
		III	0.1	—	—	
Inula helenium (Compositae)	ra	I	1	+	+	+
		II	0.1	+	+	+
		III	0.05	++	++	++
Ledebouriella seseloides (Umbelliferae)	ra	I	1	—	—	
		II	0.1	—	—	
		III	0.05	+	—	—
Leonurus sibiricus (Labiatae)	ha	I	1	—	—	
		II	0.01	—	—	
		III	0.05	+	+	+
Lycium chinense (Solanaceae)	fr	I	1	—	—	
		II	0.1	—	+	—
		III	0.1	—	—	
Machilus thunbergii (Lauraceae)	sb	I	1	+	+	+
		II	0.1	++	++	++
		III	R			
Paeonia obovata (Ranunculaceae)	ra	I	1	—	—	
		II	0.1	—	—	
		III	0.1	—	—	
Polygonatum japonicum (Liliaceae)	rh	I	1	—	—	
		II	0.1	—	—	
		III	0.1	—	—	
Prunus persica (Rosaceae)	sm	I	1	—	—	
		II	0.1	—	—	
		III	0.1	—	—	
Rehmannia glutinosa (Scrophulariaceae)	ra	I	1	—	—	
		II	0.1	+	+	+
		III	0.1	—	—	
Scutellaria baicalensis (Labiatae)	ra	I	0.5	+	+	+
		II	0.01	—	+	—
		III	0.05	—	+	—

ba; bark, fl; flower, fr; fruit, ha; herba, ra; radix, rh; rhizome, sb; stem bark, sm; semen, tu; tuber. AI; Angiotensin I ; 20 ng/ml, BK; bradykinin; 5 ng/ml, Ach; acetyl choline; 20 ng/ml, R; caused relaxation to rat ileum, —; no effect, +; antagonistic effect, ++; strong antagonistic effect.

modified Krebs solution (10 ml)[8] at 37° and bubbled with 95% O_2-5% CO_2. The contractile responses were recorded on a kymograph. After equilibration for about 60 min., each contractile agonist angiotensin I, bradykinin or acetyl choline at final bath concentrations of about 20 ng/ml, 5 ng/ml and 20 ng/ml respectively was added. After observing the contractions, the tissue was washed several times and then was allowed to rest for 20~30 min. At the end of the resting period, 0.1ml of test sample (100 times of desired concentration in Krebs soluion) was added to the bath 1 min. prior to agonist and the effect of the test compound was observed. For those which are not easily soluble in H_2O, solution of test sample was prepared to contain 0.5% EtOH (final concentration).

Results and Discussions

Total of 75 plant preparations (Fr. I, II and III of 25 plant materials) were tested for their effects to the contractions induced by angiotensin I (AI) or bradykinin (Bk) in rat ileum. Angiotensin I is converted to angiotensin II to exert contractions, so that inhibition of the converting enzyme should also be resulted as antagonistic effect against AI in our testing system. The results are summarized in Table I. In case of preparations which itself exerted either contraction or relaxation to the muscle, the concentration was lowered as low as 0.01 mg/ml. Six preparations which showed relaxation at this concentration were not tested. Six preparations showed strong antagonistic effects and 18 preparations mild antagonistic effects against AI. Five preparations showed strong and 22 preparations showed mild effects against Bk. The effects against acetyl choline (ACh) were measured with the preparations which showed activities against either AI or Bk to see whether the activities are specific to either AI or Bk. Most of the preparations which were inhibitory against one agonist were inhibitory against the other two agonists. Seven preparations including Fr. II of *Aconitum pseudolaeve*, Fr. III of *Astragalus membranaceus*, Fr. III of *Atractylodes japonica*, Fr. III of *Ledebouriella seseloides*, Fr. II of *Lycium chinense*, Fr. II and Fr. III of *Scutellaria baicalensis* showed some specificities, however these should be further clarified. In this experiment, it is shown that plants could serve as a potential source for separation of compounds with antagonistic effects against angiotensin or bradykinin.

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