Communications to the Editor

[Chem. Pharm. Bull. 34(10)4422—4424(1986)]

THE ACTIVE PRINCIPLE OF ALISMATIS RHIZOMA WHICH INHIBITS CONTRACTILE RESPONSES IN AORTA

Johji Yamahara,* Hisashi Matsuda, Hideki Murakami and Hajime Fujimura
Kyoto Pharmaceutical University, Misasagi Yamashina-ku, Kyoto 607, Japan

The acetone extract of Alismatis Rhizoma inhibited contractile responses to angiotensin I(AI) in rabbit thoracic aortic strips. Studies were conducted to identify the active principle involved. It was found to be alismol. Alismol acted dose-dependently.

KEYWORDS ——— Alismatis Rhizoma; biologically active principle; alismol; contractile response; angiotensin I; rabbit aortic strip

Alismatis Rhizoma, the rhizoma of Alisma oriental Juzepuk (Alismataceae), has been used clinically as a diuretic. In Chinese medicine, it is said that the diuretic action is due to the alisol A, B and potassium salts which are present in abundance.[1-3]

In order to discover new drugs affecting the rennin-angiotensin system, which is closely related to water balance and blood pressure control, extracts of herbal medicines and other general plants including Alismatis Rhizoma were examined for their inhibitory effects on contractile responses to angiotensin I(AI) in the thoracic aorta of rabbit. The acetone extract of Alismatis Rhizoma was found to be active in this system and further, alismol was determined to be one of the active principles.

MATERIALS AND METHODS

1) Fractionation procedure for the acetone extract of Alismatis Rhizoma

Alismatis Rhizoma was obtained from local markets in Osaka and cut into segments of approximately 5mm. They were soaked 3 times in 50% MeOH or acetone and filtered. After filtration, the 50% MeOH and acetone extracts were obtained by concentration of the filtered solution under reduced pressure at below 40 °C.

The acetone extract of Alismatis Rhizoma was separated into 6 fractions, Fr.1 - Fr.6, using as eluent benzene:acetone 50:1 - 20:1 in column chromatography with silica gel (Silica gel 60, Merck). Fr.2 was found to have the most activity of all the fractions. It was further separated into 3 fractions, Fr.2-1 - Fr.2-3, by reversed-phase silica gel(Silica gel 60 silanised, MercK) column chromatography using as eluent 60% MeOH. Fr.2-2 was identified as alismol,[4] based on MS, NMR and IR spectra.[5]

2) Aorta contraction method

Eight male rabbits (Kitayama Labes.) were bled to death by severing both carotid arteries, and the thoracic aorta was excised. After the excess fat and connective tissue were removed, the aorta was cut into helical strips about 2mm wide and 20 mm long. Each of the strips was fixed with a loading tension of 2g in a tissue bath

containing 25 ml of modified Kreb's medium (MK-medium) maintained at 37 °C and oxygenated with a gas mixture of 95% O_2 and 5% CO_2. The MK-medium consisted of (mM): NaCl 120.1, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.2, glucose 5.8, at pH 7.4. Tissue preparations were washed every 30 min with MK-medium for 1.5 h for stabilization and AI (10^{-9} - 3 x 10^{-7}M) was added to the bath cumulatively to obtained concentration-response curves. The preparations were washed 4 times with MK-medium at 15min intervals. The test compounds were dissolved in a small amount of ethanol then added 15 min prior to addition of AI. Responses to AI in the presence of test compounds were calculated with the maximum response to AI in the absence of test drugs taken to be 100%.

Isometric responses of tissues preparations were measured through a force transducer (Nihon Denki Sanei:45691A) and recorded on a polygraph (Nihon Deki Sanei).

3) Statistical analysis

Values were reported as means ± S.E. and Dunnett's method[6] was used for statistical analysis.

RESULTS AND DISCUSSION

The inhibitory effects of 50% MeOH and the acetone extracts of Alismatis Rhizoma, containing non-polar substances, on the contractile response to AI, were examined. The acetone extract showed a stronger inhibition of the response than the 50% MeOH extract (Fig.1). The fractions of the acetone extract, Fr.2 and Fr.2-2 (alismol) significantly inhibited AI-induced contraction. Other fractions did not significantly inhibit contraction at the concentration of 5 x 10^{-2} mg/ml. Alismol was found to be the factor responsible for the inhibition of AI contraction. Alismol inhibited responses to AI concentration-dependently (Fig.2). Captopril, an inhibitor of angiotensin converting enzyme (ACE), also inhibited the response to AI (Fig.2).

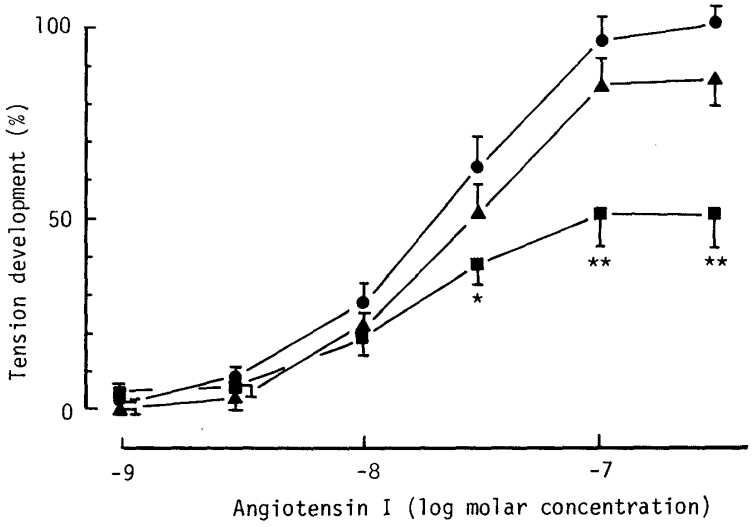

Fig.1 Effects of 50% MeOH Ext. and Acetone Ext. on the Angiotensin I-Induced Contraction in the Rabbit Aorta

—●— : Control
—▲— : 50% MeOH ext. 5 x 10^{-2}mg/ml
—■— : Acetone ext. 5 x 10^{-2}mg/ml

Each point represents the mean with S.E. of 6 experiments.
Significantly different from control at $^{*}p<0.05$, $^{**}p<0.01$.

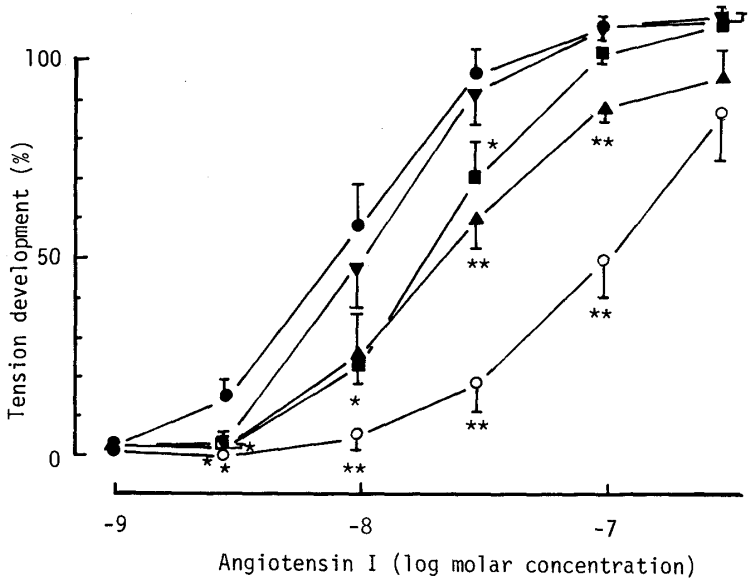

Fig.2. Effects of Alismol and Captopril on the Angiotensin I-Induced Contraction in the Rabbit Aorta

—●— : Control
—▲— : Alismol 10^{-4}M
—■— : Alismol 10^{-5}M
—▼— : Alismol 10^{-6}M
—○— : Captopril 10^{-6}M

Each point represents the mean with S.E. of 6 experiments.
Significantly different from control at $^{*}p<0.05$, $^{**}p<0.01$.

The results of these experiments show that the alismol in Alismatis Rhizoma inhibited the AI-induced contraction of smooth muscles concentration-dependently.

REFFERENCES AND NOTES

1) C.T.Hsu, Acta.Sch.Med.Univ.Gifu, 5, 485 (1957).
2) H.Hikino,T.Iwakawa,Y.Oshima,K.Nishikawa and T.Murata, Shoyakugaku Zasshi, 36, 150 (1982).
3) T.Kobayashi, Yakugaku Zasshi, 80, 1456, 1465, 1612 (1960).
4) Y.Oshima,T.Iwakawa and H.Hikino, Phytochemistry, 22, 183 (1983).
5) Spectral data for Fr.2-2, High resolution MS: Calcd for $C_{15}H_{24}O$: 220.1827. Found: 220.1801. MS m/z: 220 (M^+). IR ν_{max}^{neat} cm^{-1}: 3350, 1640. ^{1}H-NMR(400MHz,$CDCl_3$) δ : 0.96(3H,d,J=6.8Hz), 0.97(3H,d,J=6.8Hz), 1.23(3H,s), 4.68,4.74(each 1H,br s), 5.53(1H,br s). ^{13}C-NMR(75MHz,C_5D_5N) δ : 21.4(q), 21.6(q), 24.6(q), 25.3(t), 30.2(t), 37.4(t),37.6(d),40.5(t),47.3(d),55.2(d),79.6(s),106.6(t),123.2(d),148.5(s),154.3(s).
6) C.W.Dunnett, Am.Statist.Assoc.J., 50, 1096 (1955).

(Received July 29, 1986)