Effect of Alismol on Adrenergic Mechanism in Isolated Rabbit Ear Artery

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Abstract—Effects of alismol, a sesquiterpenoid isolated from the rhizome of Alisma orientale, on adrenergic mechanisms were examined in the isolated rabbit ear artery. Alismol (10^{-6} to 10^{-4} M) inhibited the contraction of isolated rabbit ear artery by electrical stimulation of the perivascular nerves. The inhibition was concentration-dependent; at a concentration of 10^{-4} M, the inhibition was 90% (n=8). Treatment with 10^{-4} M alismol inhibited the increase in 3H-noradrenaline (3H-NAd) release induced by electrical stimulation by 63±6%. Alismol at 10^{-4} M did not affect the neuronal uptake of 3H-NAd in the artery. Alismol at 10^{-4} M slightly inhibited contractions induced by exogenously administered NAd. These results demonstrate that alismol inhibits the adrenergic neuro-effector mechanisms in rabbit ear artery, and they suggest that alismol acts primarily on nerve terminals and inhibits their responses to electrical stimulation by interfering with NAd release.

Materials and Methods
Male albino rabbits weighing 2 to 2.5 kg were bled to death by severing both carotid arteries, and the ear artery was removed. After the excess fat and connective tissue were removed, the artery was cut into helical strips, 1–2 mm wide and 20–25 mm long. Each preparation was suspended in an organ bath (10 ml) and subjected to approximately 0.5 g initial tension, and responses were recorded isometrically via a force-displacement transducer (Nihon Kohden SB-1T, Tokyo, Japan) on a polygraph (Nihon Kohden RJG-4128, Tokyo, Japan). The physiological salt solution contained (mM): NaCl, 118; KCl, 4.7; KH_{2}PO_{4}, 1.2; MgSO_{4}, 1.2; CaCl_{2}, 2.5; NaHCO_{3}, 23.3; glucose, 10.0; and 10^{-6} M propranolol to block β-adrenoceptors. The medium was maintained at pH 7.4 at 37°C and constantly equilibrated with a 95% O_{2} and 5% CO_{2} gas mixture.

For electrical perivascular nerve stimulation, the strip was fixed to the parallel platinum electrodes to investigate the contractions induced by the electrical stimulation.
After stabilization for 1 hr, the perivascular nerve was stimulated by pulses of 50 Hz in frequency, 0.3 msec in duration and 50 V in intensity for 10 sec.

The superfusion technique reported by Su and Bevan (7) was used to investigate the release of $^3$H-NAd induced by electrical stimulation of the perivascular nerve of the rabbit ear artery. Each strip was transferred into an incubation medium containing (-)-[7-3H]-NAd (New England Nuclear) at $5.3 \times 10^{-7}$ M (10 $\mu$Ci/ml) and incubated for 1 hr to uptake the $^3$H-NAd into the perivascular nerves. The incubation medium also contained 0.57 mM ascorbic acid and 0.04 mM EDTA to prevent auto-oxidation of NAd and pargyline ($10^{-5}$ M) to inhibit monoamine oxidase. The strips were suspended between two parallel platinum wire electrodes and superfused with the physiological salt solution containing 0.57 mM ascorbic acid, 0.04 mM EDTA, $10^{-5}$ M pargyline and $10^{-5}$ M cocaine to inhibit reuptake of NAd into the nerves. The preparations were stimulated by pulses of 50 Hz in frequency, 0.3 msec in duration and 50 V in intensity for 30 sec. The flow rate of the perfused solution was adjusted to 2 ml/min with a peristaltic infusion pump. To assay the labeled compounds, the fluid over the tissue was collected at intervals of 1 or 5 min, and 2 ml of each fraction was added to a scintillation vial containing 15 ml of scintillator fluid (1300 ml toluene, 700 ml triton, 0.8 g 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP), and 10.0 g 2,5-diphenyloxazole (PPO)). To determine the amount of $^3$H-NAd remaining in the tissue, each strip was solubilized in a tissue solubilizer (solute 350, Packard) after the superfusion and then mixed with the scintillator fluid; the radioactivity was determined using a liquid scintillation counter (Aloka LSC-910, Tokyo, Japan). The initial $^3$H-NAd content in the tissue was calculated by adding together the total $^3$H-NAd released into the perfusate and the amount remaining in the tissue. The amount of $^3$H-NAd released was expressed as the fractional release, the percentage of $^3$H-NAd remaining in the tissue after each washout interval.

Drugs used were: (-)-noradrenaline bitartrate (Sigma), propranolol hydrochloride (Sigma), ethylenediaminetetraacetic acid 2Na (EDTA) (Dojindo Laboratories), pargyline hydrochloride (Merck). Other compounds were obtained from Wako Pure Chemical Industries. Alismol was isolated from Alismatis Rhizoma using the method of Yamahara et al. (5).

Statistical analysis was performed by Dunnett's method (8), and each value was expressed or plotted as the mean±S.E.

**Results**

Effect of alismol on contractile responses to electrical perivascular nerve stimulation: The contractions induced by electrical perivascular nerve stimulation were inhibited in a concentration-dependent manner by treatment with alismol at $10^{-6}$, $10^{-5}$, $3 \times 10^{-5}$ and $10^{-4}$ M by about 2.5%, 15%, 28% and 90% (n=8), respectively (Fig. 1 and Table 1). After washing of the tissue, the responses tended to recover.

Effects of alismol on release of $^3$H-NAd by electrical perivascular nerve stimulation and on neural uptake of $^3$H-NAd: After the tissue was washed for 45 min, and spontaneous $^3$H-NAd release had stabilized, electrical stimulation of the artery facilitated the release of $^3$H-NAd from 0.125±0.027 to 0.937±0.107 (%) (n=4) at maximum efflux.

Since the inhibitory effects of alismol ($10^{-4}$ M) on the contraction in response to electrical

![Fig. 1. Effect of alismol on contractile responses to electrical perivascular nerve stimulation (50 Hz, 0.3 msec duration, 50 V, for 10 sec) in rabbit ear artery. Dots indicate nerve stimulation.](image-url)
Table 1. Inhibitory effect of alismol on the contractile response to electrical perivascular nerve stimulation (50 Hz, 0.3 msec duration, 50V, for 10 sec) on the isolated rabbit ear artery

| Concentration of alismol (M) | n  | % inhibition mean±S.E. |
|-----------------------------|----|-----------------------|
| 1 x 10^-6                   | 8  | 2.6±0.8               |
| 1 x 10^-5                   | 8  | 14.8±4.3              |
| 3 x 10^-5                   | 8  | 27.6±6.7              |
| 1 x 10^-4                   | 8  | 89.9±7.3*             |

Controls are responses induced by electrical perivascular nerve stimulation of the artery in the absence of alismol. Asterisk denotes significant difference from the control at P<0.01.

Table 2. Effect of alismol on the uptake of 3H-NAd in the isolated ear artery of the rabbit

| Groups              | n  | 3H-NAd uptake (nmol/g wet tissue) |
|---------------------|----|----------------------------------|
| Control             | 9  | 15.8±1.5                         |
| Alismol (10^-4 M)   | 9  | 15.2±1.4                         |

Each preparation was incubated in the incubation medium with 5.3x10^-7 M 3H-NAd for 60 min, and then solubilized by tissue solubilizer. Solubilized samples were mixed with scintillator, and radioactivity was determined.

Fig. 2. Effect of alismol on 3H-NAd release to electrical perivascular nerve stimulation (50 Hz, 0.3 msec duration, 50 V, for 30 sec) in rabbit ear artery. Arrows indicate nerve stimulation. Ordinate indicates fractional release (%) per min. Fractions during the stimulation period were collected at intervals of 1 min for 5 min, and at other fractions during the resting period were collected at intervals of 5 min.

perivascular nerve stimulation was about 90%, this concentration was used in the following experiments.

The increase in 3H-NAd release induced by electrical stimulation was inhibited by 63±6% (n=4) by treatment with alismol (10^-4 M) (Fig. 2). This inhibitory action was recovered after washing for 45 min.

After incubation of the artery with 3H-NAd (5.3x10^-7 M) for 1 hr, the uptake of 3H-NAd was 15.8±1.5 nmol/g (n=9). Alismol at 10^-4 M had no significant effect on the 3H-NAd uptake (Table 2).

Effect of alismol on contractile responses to NAd: In the presence of propranolol (10^-6 M), NAd (10^-8 to 10^-4 M) caused concentration-dependent contractions. Pretreatment for 10 min with alismol (10^-4 M) slightly inhibited the contractions by about 20% inhibition (Fig. 3). The values for EC50 were 7x10^-7 M
Fig. 3. Effect of alismol on NAd-induced contractions in rabbit ear artery. Tissues were pretreated for 10 min with alismol, and then NAd was added cumulatively in the presence of 10^{-6} M propranolol. The mean contraction induced by NAd at 10^{-4} M in the control was taken to be 100%. Each value represents the mean±S.E. of 8 experiments. Asterisks denote significant differences from the control at P<0.01.

Discussion

Alismol, isolated from the rhizome of *Alisma orientale*, has been shown to inhibit the angiotensin I and CaCl_2-induced contractions in rabbit thoracic aorta (5, 6). In the present experiments, the effects of alismol on the adrenergic neuro-effector mechanisms in rabbit ear artery were examined.

It has been reported that contractions induced by electrical stimulation of perivascular nerves in the arteries are due to release of endogenous NAd from the adrenergic nerve terminals (7, 9). Since the degree of inhibition was dependent on alismol concentration, it has been suggested that alismol may act either by inhibiting NAd release from presynaptic nerve terminals or by postsynaptic blocking effects.

Therefore, the effect of alismol on the electrically stimulated release of ^3H-NAd from nerve terminals and vasorelaxant effects of alismol on contractions in response to exogenously added NAd were examined. The results indicated that alismol (10^{-4} M) attenuated the increase of ^3H-NAd release by about 63%, which is less effective than the inhibitory effect (90%) of alismol on the contractile responses to electrical stimulation. Alismol (10^{-4} M) also inhibited NAd-induced contractions by about 20%, but it is also less effective than that of alismol on the contraction to electrical nerve stimulation. These results suggested that alismol acts primarily on nerve terminals and inhibits their response to electrical stimulation by interfering with NAd release. However, the comparison between the degree of inhibition in the electrical stimulation-induced responses and that of the NAd-release also suggested that the effect of alismol is magnified by these presynaptic and postsynaptic actions.

It is known that guanethidine, a neuron blocker, acts on nerve terminals to inhibit the NAd release induced by nerve stimulation (10-12). In addition, guanethidine has been shown to inhibit the neuronal uptake of ^3H-NAd (12-14). Since alismol had no effect on the uptake of ^3H-NAd, the inhibitory mechanisms of alismol may be different from that of guanethidine.

In summary, the present experiments demonstrate that alismol inhibits adrenergic neuro-effector mechanisms in rabbit ear artery and suggest that the mechanisms of such inhibition is mainly due to inhibition of NAd release.

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