

Cinnamaldehyde Induces Endothelium-Dependent and -Independent Vasorelaxant Action on Isolated Rat Aorta

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The vasorelaxant effect of cinnamaldehyde, one of the major oil components in Cinnamomi Cortex, was studied using isolated rat aorta. Cinnamaldehyde at final concentrations of 1 μ M to 1 mM showed dose-dependent relaxation of the rat aorta contracted by treatment with prostaglandin F_{2 α} , norepinephrine or KCl. In addition, cinnamaldehyde relaxed prostaglandin F_{2 α} -precontracted aortic rings with endothelium and without endothelium, with the latter being significantly less sensitive than the former. Relaxation induced by cinnamaldehyde with endothelium was significantly inhibited by N^G-nitro-L-arginine methyl ester (L-NAME), while nonselective cyclooxygenase inhibitor (indomethacin), β -adrenergic receptor blocker (propranolol), an inhibitor of phosphodiesterase (theophylline), a delayed rectifier K⁺ channel blocker (tetraethyl ammonium chloride), or an ATP-sensitive K⁺ channel blocker (glibenclamide) did not reduce the relaxation induced by cinnamaldehyde with endothelium treated by L-NAME. Conversely, aorta pretreatment with L-NAME and theophylline increased the relaxation by cinnamaldehyde significantly compared to aorta pretreatment with only L-NAME. Furthermore, cinnamaldehyde significantly inhibited Ca²⁺-induced contraction. These results suggested that the vasorelaxant effects of cinnamaldehyde were derived from both endothelium-dependent and -independent effects. Endothelium-dependent relaxation is affected by nitric oxide, and one of the mechanisms of endothelium-independent relaxation is thought to be influenced by the blocking of Ca²⁺ channels.

Key words cinnamaldehyde; endothelium-dependent relaxation; nitric oxide; endothelium-independent relaxation; theophylline; rat aorta

Cinnamomi Cortex is a crude drug used therapeutically in Asia and Europe. Its main component is cinnamaldehyde. There are many reports on the pharmacological effects of Cinnamomi Cortex. Mainly, the sedative effect of decreasing spontaneous motor activity,¹⁾ anti-inflammatory effects related to cyclooxygenase-2,²⁾ and antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*³⁾ have been reported. In addition, in oriental medicine, Cinnamomi Cortex is often used to improve blood circulation. In regard to the circulatory system, a catecholamine-releasing effect of cinnamaldehyde,⁴⁾ a reducing effect on platelet aggregation due to suppression of the release of arachidonic acid from platelets,⁵⁾ and an inhibitory effect of collagen-induced platelet aggregation⁶⁾ have been reported. However, reports concerning the direct effect against vasomotion by components of Cinnamomi Cortex are few, except in relation to cinnamtannin.⁷⁾ In the present study, the vasorelaxant effects of cinnamaldehyde were studied by the organ bath method, and the mechanisms of vasorelaxation were evaluated.

MATERIALS AND METHODS

Drugs and Chemicals Analytical grades of the following reagents were purchased: cinnamaldehyde, prostaglandin F_{2 α} (PGF_{2 α}), norepinephrine (NE), acetylcholine, sodium nitroprusside dehydrate (SNP), methylene blue trihydrate, theophylline, propranolol hydrochloride, indometacin, tetraethylammonium chloride (TEA), glibenclamide and verapamil hydrochloride, all from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). N^G-nitro-L-arginine methylester (L-NAME) was from Sigma (St. Louis, MO, U.S.A.) and pentobarbital sodium salt was from Tokyo Chemical Ind. (Tokyo, Japan).

Animals Male Wistar rats weighing 370–420 g were purchased from Japan SLC (Shizuoka, Japan). They were kept in an animal room at an ambient temperature of 23 \pm 1 $^{\circ}$ C under a 12-h dark–light cycle. Experimental protocols met the “Guidelines for Animal Experimentation” approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

Preparation of Rat Aorta Rats were anesthetized (50 mg/kg i.p. pentobarbital) and sacrificed by cutting their abdominal aorta. Fats and connective tissues were removed from a section of the thoracic aorta, and 3-mm-wide aortic rings were prepared. For an endothelium-free aorta, the endothelial lining of each ring was removed by pressing the ring and rolling it gently onto a filter paper a few times. The endothelium was considered to be intact when relaxation induced by 1 μ M of acetylcholine was over 20% of the maximal tension obtained by 60 mM KCl-induced contraction, and the removal of the endothelium was confirmed by the absence of acetylcholine-induced relaxation.

Vasodilative Effect of Cinnamaldehyde on Isolated Aortic Rings The aortic rings were mounted on steel hooks in a Magnus chamber (Kishimoto UC-5TD, Kyoto, Japan). One end of the aorta was attached to a force-displacement transducer (Kishimoto UM-203) so that its isometric contraction could be recorded (NEC RECTI-HORIZ-8K, Tokyo, Japan). The baths were filled with 5 ml of Krebs solution containing the following (mM): NaCl 120, KCl 4.7, NaHCO₃ 25.0, KH₂PO₄ 1.2, MgSO₄·7H₂O 1.2, CaCl₂ 2.5, and glucose 10.0. The solution was maintained at 37 $^{\circ}$ C and bubbled continuously with 5% CO₂ in O₂ at pH 7.4. The rings were equilibrated for 45 min at an initial resting tension of 1 g. During this period, the Krebs solution in the bath was

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replaced every 15 min. 60 mM of KCl was added to the bath to contract the aortic strips. After the contraction reached a plateau, 1 μ M of acetylcholine chloride was added. When the endothelium was removed, the acetylcholine chloride-induced relaxation disappeared. Then the Krebs solution was again replaced every 15 min for 60 min.

Each aortic strip with endothelium was contracted by treatment with PGF_{2 α} (5 μ M), NE (0.1 μ M) or KCl (60 mM). When the contraction reached a plateau, cinnamaldehyde was added to the bath in cumulatively increasing doses of 1 μ M—1 mM. Relaxation was expressed as percentage of the decrease in maximal tension obtained by PGF_{2 α} , NE or KCl-induced contraction.

To study endothelium-dependent relaxation, aortic strips with and without endothelium were contracted by treatment with PGF_{2 α} . When the contraction reached a plateau, cinnamaldehyde (1 μ M—1 mM) and SNP (1 nM—0.1 mM) were added to the bath cumulatively. Furthermore, to study endothelium-dependent relaxation with nitric oxide/cGMP, aortic strips with endothelium treated by L-NAME (0.1 mM) and methylene blue (10 μ M) for 60 min were contracted by treatment with PGF_{2 α} . When the contraction reached a plateau, cinnamaldehyde and SNP were added to the bath by the same schedule as mentioned above.

To study the relaxation caused by cinnamaldehyde, except NO-dependent relaxation, aortic strips with endothelium treated by L-NAME were used. They were pretreated with various inhibitors (10 μ M propranolol, 0.1 mM theophylline, 1 mM TEA, 10 μ M glibenclamide and 10 μ M indometacin) for 15 min and contracted by treatment with PGF_{2 α} . When the contraction reached a plateau, cinnamaldehyde (1 μ M—1 mM) was added to the bath cumulatively. Each inhibitor alone at the concentrations used had no effect on vasoconstriction.

Contraction Experiments The effect of cinnamaldehyde on contractions induced by the cumulative addition of calcium was studied. Ca²⁺-induced contractions were elicited by the cumulative addition of CaCl₂ (1 μ M—3 mM) to Ca²⁺-depleted 60 mM K⁺-containing Krebs solution for 10 min after the aorta strip had been suspended in Ca²⁺-depleted Krebs solution. CaCl₂ was added directly to the bath fluid. Afterwards, using the same aortic rings, this procedure was repeated 10 min after the addition to the bath of 0.1 mM or 1 mM of cinnamaldehyde, or 1 μ M of verapamil. Changes in contractile tension were expressed as percentage of the maximum tension obtained in the control curve.

Statistical Analysis Differences between specific means were tested by two-way repeated-measures ANOVA with *post hoc* analysis using the Bonferroni *t* test. A value of *p* < 0.05 was accepted as statistically significant.

RESULTS

Figure 1 shows that cinnamaldehyde caused dose-dependent vasorelaxation in PGF_{2 α} -, NE- and KCl-induced contraction, reaching a maximum of 90.0 \pm 2.4%, 60.6 \pm 8.2%, 36.5 \pm 8.8% at 1 mM of cinnamaldehyde, respectively (mean \pm S.E., *n* = 7). Cinnamaldehyde caused dose-dependent vasorelaxation of the aorta with endothelium, without endothelium, and with endothelium treated by L-NAME in PGF_{2 α} -induced contraction. But the aorta with endothelium

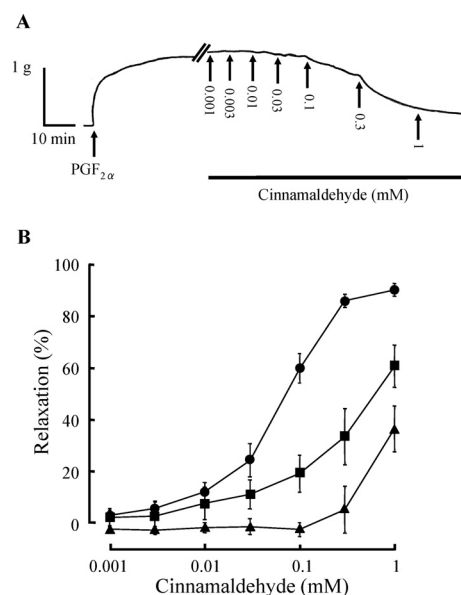


Fig. 1. Typical Recording (A) and Dose-Dependent Vasodilative Effect (B) of Cinnamaldehyde on Rat Aorta Strips

The aorta was contracted by 5 μ M PGF_{2 α} (●), 0.1 μ M NE (■) or 60 mM KCl (▲), and the vasodilative effect of decrease was examined. Values are indicated as percentage of decrease in maximal tension by each of the vasoconstrictors. Vertical bars indicate S.E. (*n* = 7).

dilated significantly compared to those without endothelium and with endothelium treated by L-NAME, reaching a maximum of 42.0 \pm 4.7%, 18.8 \pm 6.3%, and 22.2 \pm 3.1% at 0.1 mM of cinnamaldehyde, respectively (Fig. 2A, mean \pm S.E., *n* = 5—7). SNP also caused vasorelaxation of the aorta with endothelium, without endothelium, and with endothelium treated by L-NAME in PGF_{2 α} -induced contraction. There were no statistically significant differences among the three groups (Fig. 2B). Furthermore, concerning aorta pretreatment with methylene blue (10 μ M), vasorelaxation by cinnamaldehyde and SNP was decreased significantly compared to the aorta with endothelium not pretreated with methylene blue (Fig. 2).

The vasorelaxant effect of cinnamaldehyde was examined with various compounds reported as inhibitors of vasodilatation. Vasorelaxation was not affected by propranolol, TEA, glibenclamide and indometacin (Fig. 3, *n* = 6—8). There were no statistically significant inhibitory effects between the control group pretreated only with L-NAME and groups treated with various compounds reported as inhibitors of vasodilatation. But in the aorta treated with theophylline, the vasorelaxant effect of cinnamaldehyde was increased significantly compared to the control group pretreated only with L-NAME (Fig. 3, *n* = 8, *p* < 0.01 vs. control).

To evaluate the effect of the possible calcium antagonism of cinnamaldehyde, a series of experiments was performed, based on contracting the rat aortic preparations with increasing calcium concentrations in the presence and absence of different cinnamaldehyde concentrations. As shown in Fig. 4, the calcium response curve was significantly shifted to the right by 0.1 and 1 mM of cinnamaldehyde and by 1 μ M of verapamil (*n* = 6—7).

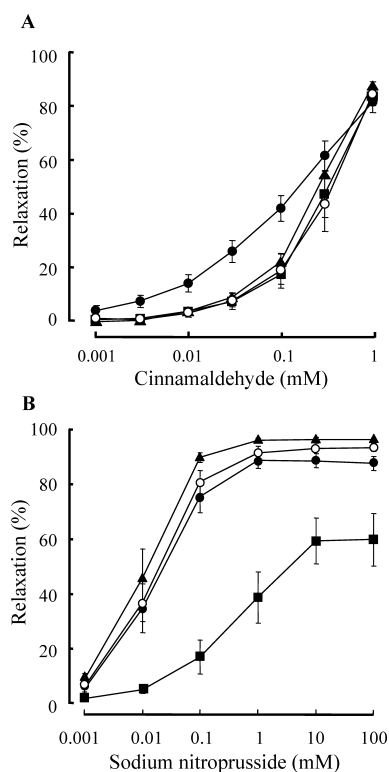


Fig. 2. Vasodilative Effects of Cinnamaldehyde (A) and SNP (B) on Endothelium-Denuded (○), Endothelium-Intact Aorta (●), Presence of 0.1 mM L-NAME (▲) and 10 μ M Methylene Blue (■)

The aorta was contracted by 5 μ M $\text{PGF}_{2\alpha}$, and the vasodilative effects of cinnamaldehyde and SNP were examined. Values are indicated as percentage of decrease in maximal tension by each of the vasoconstrictors. Vertical bars indicate S.E. Differences between endothelium-intact and endothelium-denuded were statistically significant (A, $p < 0.05$; $n = 5-7$). Differences between endothelium-intact and rings treated with methylene blue were statistically significant by two-way repeated-measures ANOVA (A, $p < 0.05$; B, $p < 0.01$; $n = 7$).

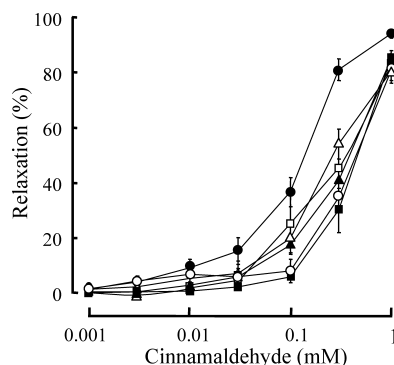


Fig. 3. Influence of Various Inhibitors on the Vasodilative Effect of Cinnamaldehyde

The aorta was treated with L-NAME and various inhibitors: 0.1 mM theophylline (○), 0.01 mM indometacin (Δ), 0.01 mM propranolol (□), 1 mM tetraethyl ammonium chloride (TEA, ■), 0.01 mM glibenclamide (◊). The aorta was contracted by 5 μ M $\text{PGF}_{2\alpha}$. Values are indicated as percentage of decrease in maximal tension by each of the vasoconstrictors. Vertical bars indicate S.E. Differences between rings treated with only L-NAME (control: ▲) and theophylline were statistically significant by two-way repeated-measures ANOVA ($p < 0.01$, $n = 6-8$).

DISCUSSION

Cinnamaldehyde is the main component of Cinnamomi Cortex. Cinnamomi Cortex is a crude drug used therapeutically in Asia and Europe. Even now it is used for a variety of diseases such as infectious disease, arthritis, cardiovascular

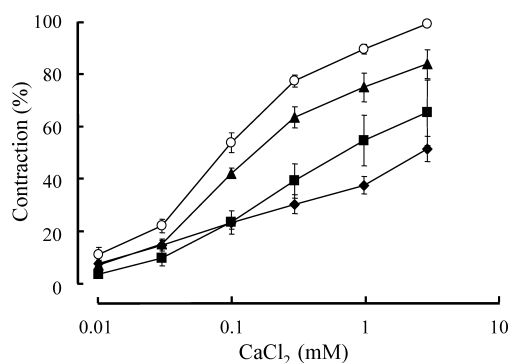


Fig. 4. Calcium Concentration-Contraction Curves of Isolated Rat Aorta in the Absence (○) and Presence of 0.1 mM (▲), 1.0 mM Cinnamaldehyde (■) and 1 μ M Verapamil (◆)

The aorta was contracted by 60 mM KCl. The differences between control and rings treated with 0.1 mM cinnamaldehyde, 1.0 mM cinnamaldehyde, and verapamil were statistically significant by two-way repeated-measures ANOVA ($p < 0.05$, $n = 6-7$).

diseases, and so on. Cinnamomi Cortex contains 1–3% oil, and the main component of this oil is cinnamaldehyde, which constitutes about 90% of the oil.⁸ As for other components of Cinnamomi Cortex, there are terpenoids and tannins, etc. The main active component is cinnamaldehyde, and it is thought to play a crucial role in the effect of Cinnamomi Cortex.

Mechanisms of the formulae containing Cinnamomi Cortex have been reported in regards to circulatory disease. Keishibukuryogan is reported to improve the microcirculation of the bulbar conjunctiva,⁹ to protect vasofunction in a model of diabetic rat,¹⁰ to decrease atherosclerosis in a model of hypercholesterolemic rabbit,¹¹ and so on. Concerning the mechanisms of these vasoprotective effects, keishibukuryogan is reported to exert an improvement effect on hemorheological factors^{9,12} as well as an anti-oxidant effect.¹³ As part of this formula, Cinnamomi Cortex is thought to have various effects on anti-platelet aggregation,^{5,6} inhibition of thromboxane A₂,¹⁴ to have a vasorelaxation effect,⁷ and so on.

As mentioned above, there are many reports concerning the effects of Cinnamomi Cortex on blood circulation, but there is no report about the effect of cinnamaldehyde on vasomotion. In this study, we demonstrated that cinnamaldehyde exerted both endothelium-dependent and -independent relaxant effects. As for endothelium-dependent relaxation, nitric oxide (NO) has been reported as an endothelium-derived relaxing factor (EDRF).^{15,16} The vasorelaxant effects of cinnamaldehyde without endothelium and with endothelium treated with L-NAME became weaker than that with endothelium. In a word, the endothelium-dependent relaxing effects of cinnamaldehyde must result from EDRF/NO. Recently, in cultured cells, cinnamaldehyde was reported to stimulate endothelial NO synthase.¹⁷ Now, we have also established that cinnamaldehyde had the same effect in an isolated vessel.

As EDRF/NO, NO is reported to lead the vasorelaxation through cGMP.¹⁸ In this study, the cinnamaldehyde-induced relaxation with endothelium treated by methylene blue, which inhibits guanylate cyclase, decreased compared to control. It is reported that cGMP inhibits the adherence of leukocytes¹⁹ and the activation of platelets.²⁰ Also, the concentration of cGMP in whole blood is related to the degree of

arteriosclerosis.²¹⁾ Based on these reports, it is suggested that the vasorelaxant effect by cGMP has vasoprotective effects.

Because cinnamaldehyde had a vasorelaxant effect without endothelium, it was clear that cinnamaldehyde had an endothelium-independent relaxant effect as well. To study this mechanism, we evaluated the effect of various inhibitors on cinnamaldehyde-induced relaxation, and found that none of them decreased the vasorelaxant effect of cinnamaldehyde.

Because the vasoconstrictive effect by the administration of calcium was inhibited by cinnamaldehyde, as one of the mechanisms in the endothelium-independent relaxant effect, it was thought that cinnamaldehyde had an inhibitory effect on calcium influx to smooth muscle cells. The inhibitory effect of calcium influx not only has an antihypertensive effect but also an anti-arteriosclerotic effect.^{22,23)}

But there was also a report that cinnamaldehyde had a vasorelaxant effect similar to that of papaverine.²⁴⁾ In addition, the relaxation effect by cinnamaldehyde was most remarkable in PGF_{2 α} -induced contraction in this study, strongly suggesting that cinnamaldehyde affects the function of the PGF_{2 α} -receptor.

Recently, in terms of crude drug components, galloylglucose²⁵⁾ and arecoline,²⁶⁾ etc., have been reported to possess an endothelium-dependent vasodilative effect, and perillaldehyde²⁷⁾ and olleuropeoside,²⁸⁾ etc., to have an endothelium-independent effect. However, a component of crude drugs having both effects has as yet not been reported. The endothelium-dependent and -independent relaxant effects of cinnamaldehyde that were revealed in the present study possibly have different actions against vascular injury. These effects are thought to play important roles in formulae containing Cinnamomi Cortex.

In this study, the relaxation induced by cinnamaldehyde treated with L-NAME increased under the condition of pretreatment with theophylline, a phosphodiesterase inhibitor reported to have antagonistic effects on adenosine receptor and inhibitory effects on intracellular Ca²⁺ release.²⁹⁾ We used high-dose theophylline treatment preceding the administration of cinnamaldehyde to vessels. As the concentration of cAMP was thought to increase sufficiently in smooth muscle, cinnamaldehyde could not increase the cAMP concentration any further. So it is thought that there is positive interaction between theophylline and cinnamaldehyde in terms of the vasorelaxant effect, rather than participation in the phosphodiesterase inhibitory effect.

As mentioned above, cinnamaldehyde induces both endothelium-dependent and -independent relaxation, and these effects are thought to be related to the vasoprotective effect of formulae containing Cinnamomi Cortex. To continue with this line of reasoning, *in vivo* studies of the effects of cinnamaldehyde on the circulatory system are now called for.

Acknowledgements This study was supported by a Grant-in-Aid for the Funds for Comprehensive Research on

Aging and Health from the Japanese Ministry of Health and Welfare. This work was also supported by Grants-in-Aid for the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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