Vasodilatory effects of cinnamaldehyde and its mechanism of action in the rat aorta

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Abstract: The vasodilatory effect of cinnamaldehyde was investigated for its mechanism of action using isolated rings of rat aorta. Cinnamaldehyde relaxed aortic rings precontracted with phenylephrine in a dose-dependent manner, was not affected by either the presence or removal of the endothelium. Pretreatment with NG-nitro-L-arginine methyl ester and 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one could not block vasodilation by cinnamaldehyde, indicating that nitric oxide signaling is not involved. Potassium channel blockers, such as glibenclamide, tetraethylammonium, and BaCl₂, had no effect on the relaxation produced by cinnamaldehyde. In addition, treatment with either indomethacin or propranolol did not affect cinnamaldehyde-induced vasodilatation. On the other hand, pretreatment of endothelium-denuded rings with cinnamaldehyde significantly inhibited vasoconstriction induced by endogenous vasoconstrictors, including angiotensin II, 5-hydroxytryptamine, dopamine, endothelin-1, and phenylephrine. In a Ca²⁺-free experimental setting, this natural vasodilator not only blocked Ca²⁺ influx-dependent vasoconstriction by either phenylephrine or KCl, but also inhibited phenylephrine-induced tonic contraction, which relies on intracellular Ca²⁺ release. This study shows that endothelium-independent, Ca²⁺ influx and/or an inhibitory release mechanism contributes to the vasodilatory effect of cinnamaldehyde.

Keywords: cinnamaldehyde, vasodilation, endothelium, vascular smooth muscle cell

Introduction

Cinnamomum cassia is a Chinese herbal medicine frequently used for its multiple therapeutic functions, such as enhancing immunity, eliminating the sense of coldness, relieving pain and improving blood circulation.¹ In modern pharmacological research, C. cassia has exhibited diverse actions, including antioxidative stress,² preventing mitochondrial dysfunction,³ antitumor properties,⁴ and inhibition of tau protein aggregation in Alzheimer’s disease.⁵ Cinnamaldehyde, one of the main constituents of C. cassia, is an aromatic aldehyde which has been reported to have multiple potential therapeutic activities.⁶ Ma et al found that cinnamaldehyde could decrease production of prostaglandin E₂ stimulated by interleukin-1β and could downregulate the expression of transient receptor potential vanilloid subtype 4 in the cerebral microvascular endothelial cells of the mouse, which may contribute to its antipyretic effects.⁷ Chao et al has reported that cinnamaldehyde has antioxidant and anti-inflammatory properties. Low concentrations of cinnamaldehyde can inhibit secretion of interleukin-1β, tumor necrosis factor α, and reduce reactive oxygen species in lipopolysaccharide-stimulated J774 A.1 macrophages. The phosphorylation of extracellular signal-regulated kinase 1/2 and c-Jun N-terminal kinase 1/2 induced...
by lipopolysaccharides was also inhibited.8 In addition, Liao et al found that cinnamaldehyde inhibits adhesion of tumor necrosis factor α-induced monocytes to endothelial cells, and suppresses the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 at the transcriptional level by suppressing nuclear transcription factor κB activation.9

With regard to the circulation, several studies have shown that cinnamaldehyde has antiplatelet and antithrombotic activity.10,11 In anesthetized rats, cinnamaldehyde decreased blood pressure, left ventricular systolic pressure, and rate of change of left ventricular maximum pressure (dp/dtmax).12 Cinnamaldehyde also showed a dose-dependent relaxation of the rat aorta contraction induced by noradrenaline, potassium, and prostaglandin F2α.12,13 Based on the above observations, we hypothesized that the cardiovascular effect of cinnamaldehyde may be due to signaling beyond the receptor level. In the present study, we systematically evaluated the vasodilatory effects of cinnamaldehyde in isolated rat aorta rings using pharmacological methods and explored its potential mechanism of action.

Methods and materials
Reagents
Cinnamaldehyde (C9H8O, Figure 1) was purchased from Aladdinbiotech Company (Shanghai, China). Acetylcholine, phenylephrine, NG-nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4]-oxadiazole-[4,3-a]-quinoxalin-1-one (ODQ), indomethacin, propranolol, glibenclamide, tetraethylammonium, BaCl2, angiotensin II, 5-hydroxytryptamine, dopamine, and endothelin-1 were purchased from Sigma Chemical Co (St Louis, MO). Ethyleneglycol bis (2-aminoethyl ether) tetra-acetic acid (EGTA) and other inorganic salts were all purchased from Sinopharm Chemical Reagent Co Ltd (Shanghai, China). Acetylcholine, phenylephrine, L-NAME, tetraethylammonium, propranolol, angiotensin II, 5-hydroxytryptamine, dopamine, and endothelin-1 were purchased from Sigma Chemical Co (St Louis, MO). The rats were killed by cervical dislocation and their thoracic aortas were rapidly removed and dissected in ice-cold Krebs solution (pH 7.4, containing [mM] NaCl 118, KCl 4.7, MgSO4 1.1, KH2PO4 1.2, CaCl2 1.5, NaHCO3 25, and glucose 10). The aortas were cut into 3 mm-wide ring segments after removing the surrounding connective tissue and fat. All dissection procedures were done with extreme care to protect the endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface of the aortic ring back and forth several times with a wooden toothpick. Each ring was suspended with two L-shaped stainless steel wires in a 4 mL organ bath filled with Krebs solution and maintained at 37°C. The upper wire was connected to a force displacement transducer (Grass Instruments, West Warwick, RI) and the lower one fixed at the bottom of the organ bath. The bath solution was continuously bubbled with 95% O2 and 5% CO2. The baseline load placed on the aortic ring was 2.0 g.
Examination of endothelial integrity
At the beginning of each experiment, the bath solution was replaced every 20 minutes with prewarmed and oxygenated Krebs solution. After equilibrating for 60 minutes, all aortic rings were contracted twice with KCl 60 mM to obtain a maximal response, and the rings were washed three times at 20-minute intervals with Krebs solution. After restoration of vessel tension to baseline levels, the rings were exposed to phenylephrine 10^{-6} M to test their contractile responses, and subsequently challenged with acetylcholine to verify endothelial integrity or functional removal. Thus, the endothelium was considered intact when 15%–20% relaxation (percentage of 10^{-6} M forskolin-evoked relaxation) was achieved by acetylcholine 10^{-7} M, 60% by acetylcholine 10^{-6} M, and >80% by acetylcholine 10^{-5} M in aorta rings precontracted using phenylephrine. When the endothelium was fully removed, <1% relaxation in response to acetylcholine 10^{-5} M could be recorded (Figure 2).

Vasodilation by cinnamaldehyde
The vasodilatory effect of cinnamaldehyde was tested in both endothelium-intact and endothelium-denuded rings contracted with phenylephrine 10^{-6} M. Once a plateau of phenylephrine contraction was obtained, cinnamaldehyde was applied cumulatively at concentrations of 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M. The contractions induced by forskolin 10^{-6} M was recorded and used as 100% for blood vessel relaxation.

To understand the mechanisms of relaxation, L-NAME (a nitric oxide synthase inhibitor) and indomethacin (a cyclo-oxygenase inhibitor) were preincubated with an endothelium-intact ring for 25 minutes, and cinnamaldehyde-induced relaxation was observed. Preparations without endothelium were pretreated for 25 minutes with ODQ (a soluble guanylyl cyclase inhibitor), propranolol (an adrenergic β-receptor inhibitor), glibenclamide, (a K_{ATP} blocker), tetraethylammonium (a K_{Ca} blocker), and BaCl_{2} (a K_{IR} blocker) prior to addition of phenylephrine 10^{-6} M. Concentration-dependent vasodilation by cinnamaldehyde was then examined.

Effect of cinnamaldehyde on vasoconstriction
The endothelium-denuded ring was first contracted in a concentration-dependent manner by a series of constrictors, including dopamine, 5-hydroxytryptamine, angiotensin II, K^+, endothelin-1, and phenylephrine. After washing, the ring was incubated with cinnamaldehyde 1.3 \times 10^{-5} g/mL or 5 \times 10^{-5} g/mL for 10 minutes, and the contractions induced by the vasoconstrictors were again observed. The response to 60 mM K^+ was used as 100% contraction.

Effect of cinnamaldehyde calcium influx
The aorta ring without endothelium was washed and treated with Ca^{2+}-free high-K^+ solution (containing 10^{-4} M EGTA and 60 mM KCl). The Ca^{2+}-free incubated media preparation was then cumulatively contracted with CaCl_2 at concentrations in the range 0.5–3.0 mM. The contractions induced by CaCl_2 were compared between the group treated with cinnamaldehyde 1.3 \times 10^{-5} g/mL and the controls. Contraction induced by 60 mM K^+ in normal Ca^{2+} media was used as 100%.

Effect of cinnamaldehyde on calcium release
The endothelium-denuded ring was washed and exposed to Ca^{2+}-free Krebs solution (containing 10^{-4} M EGTA) for 20 minutes. Phenylephrine 10^{-6} M was added and a small tonic contraction mainly due to the release of intracellular Ca^{2+} was observed. Comparison between the group treated with cinnamaldehyde 1.3 \times 10^{-5} g/mL and the controls was made, with contraction by 60 mM K^+ in normal Ca^{2+} media used as 100%.

Statistical analysis
All data were expressed as means ± standard error, and analyzed using one-way analysis of variance. P < 0.05 was used as the significance level for statistical tests.

Results
Cinnamaldehyde-induced relaxation
Despite a significant difference in acetylcholine-induced relaxation in aorta ring tissue with or without endothelium (Figure 2), cinnamaldehyde relaxed the blood vessels in an

![Figure 2](image-url)
endothelium-independent manner. Maximum relaxation of the vessel with and without endothelium by cinnamaldehyde was 86.78% and 85.71%, respectively, and the EC$_{50}$ was $1.16 \times 10^{-5}$ g/mL and $1.32 \times 10^{-5}$ g/mL, respectively (Figure 3A).

To verify further the involvement of nitric oxide/cyclic guanosine monophosphate signaling pathway, we pretreated endothelium-intact aortic rings with L-NAME $10^{-4}$ M or ODQ $10^{-5}$ M. Neither the nitric oxide synthase inhibitor nor the soluble guanylyl cyclase blocker affected cinnamaldehyde-induced vasodilation (Figures 3B and 3C).

To understand the involvement of the cyclooxygenase/prostaglandin I$_2$ pathway, indomethacin $10^{-5}$ M was used. The relaxation curve for cinnamaldehyde was not affected by indomethacin (Figure 3B).

**Effect of cinnamaldehyde on potassium channels and β-receptors**

To test for possible involvement of K$^+$ channels in cinnamaldehyde-induced relaxation, we preincubated endothelium-denuded rings with tetroethylammonium $3 \times 10^{-3}$ M, BaCl$_2$ $10^{-4}$ M, and glibenclamide $10^{-5}$ M, each for 25 minutes. Tetroethylammonium (Figure 4A), BaCl$_2$ (Figure 4B), and glibenclamide (Figure 4C) did not inhibit vascular relaxation by cinnamaldehyde. We also used propranolol $10^{-5}$ M to preincubate the endothelium-denuded rings, which did not inhibit vascular relaxation induced by cinnamaldehyde (Figure 4D).

**Effect of cinnamaldehyde on endogenous vasoconstrictors**

Dopamine, 5-hydroxytryptamine, angiotensin II, endothelin-1, and phenylephrine are all endogenous vasoconstrictors and play key roles in vascular tone. We wondered whether cinnamaldehyde relaxes blood vessel by blocking one of the above vasoconstrictors. We pretreated endothelium-denuded aorta rings with cinnamaldehyde at $1.3 \times 10^{-5}$ g/mL (EC$_{50}$ relaxation by cinnamaldehyde) and $5.0 \times 10^{-5}$ g/mL, and found that cinnamaldehyde exerted inhibitory effects on the contraction curves of dopamine (Figure 5A), 5-hydroxytryptamine (Figure 5B), angiotensin II (Figure 5C), and endothelin-1 (Figure 5D), and phenylephrine (Figure 5E).
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Phenylephrine contracts vascular smooth muscle mainly
by activation of receptor-operated Ca^[2+]| channels, while
KC1 mainly activates potential-dependent Ca^[2+]| channels, all of which result in calcium influx. 14 In this study,
cinnamaldehyde pretreatment significantly reduced
vasoconstriction by phenylephrine (Figure 5E) and KC1
(Figure 6B).

To confirm the aforementioned observations, we tested the
inhibitory effect of cinnamaldehyde on K^[+]|-stimulated voltage-dependent Ca^[2+]| influx during a Ca^[2+]|-free experiment.14 As
demonstrated in Figure 6A, Ca^[2+]|-induced vasoconstrictions
stimulated by K^[+]| 60 mM were significantly suppressed by
pretreatment with cinnamaldehyde 1.3 × 10^[−5]| g/mL.

When aorta rings were exposed to Ca^[2+]|-free media, addi-
tion of phenylephrine 10^[−6]| M elicited a small tonic contraction
induced mainly by intracellular Ca^[2+]| release from endoplasmic
reticulum stores.15 Pretreatment of endothelium-denuded rings
with cinnamaldehyde 5.0 × 10^[−5]| g/mL significantly reduced
phenylephrine-induced contraction under extracellular
Ca^[2+]|-free conditions (Figure 6C).

Discussion
Vascular endothelium plays a key role in maintaining normal function of the vasculature.15 Endothelial cells release
endothelium-dependent vasodilators, such as nitric oxide
and prostacyclin (prostaglandin I2), upon stimulation of
various factors in the blood stream and as a result of physiological stress.15,16 Nitric oxide is mainly formed by nitric
oxide synthase using L-arginine as a substrate. Diffusible
nitric oxide gas penetrates vascular smooth muscle and
activates soluble guanylyl cyclase which catalyzes guanos-
ine triphosphate to form cyclic guanosine monophosphate.
Cyclic guanosine monophosphate-activated protein kinase
G inhibits the Ca^[2+]| influx, reduces sensitivity of contractile
elements to Ca^[2+], and relaxes the blood vessel.14 In our
study, cinnamaldehyde-induced vasodilation was neither
affected by removal of endothelium nor by treatment with
L-NAME (Figure 3B) or ODQ (Figure 3C). These results
suggest that the vasodilatory effect of cinnamaldehyde is
not mediated through the nitric oxide/cyclic guanosine
monophosphate pathway. The cyclo-oxygenase in either
endothelial or smooth muscle cells can catalyze arachidonic
acid to endoperoxide prostaglandin H2, which is finally

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**Figure 4** Effect of cinnamaldehyde on K^[+]| channels and the β-receptor. After preincubation with tetraethylammonium 3 × 10^[−3]| M, BaCl2 10^[−4]| M, or glibenclamide 10^[−5]| M
for 25 minutes, the effects of cinnamaldehyde were not inhibited by tetraethylammonium (A, n = 6), BaCl2 (B, n = 6), or glibenclamide (C, n = 6). After preincubation with
propranolol 10^[−5]| M, the vasodilatory effect of cinnamaldehyde was not inhibited (D, n = 6).
converted into prostacyclin, which activates adenyl cyclase and elevates cyclic adenosine monophosphate levels to relax the blood vessel.\cite{18} In the current study, relaxation by cinnamaldehyde in endothelium-intact aortic rings was not affected by indomethacin (Figure 3B), which rules out the involvement of the cyclo-oxygenase pathway.

Potassium channels regulate vascular smooth muscle tone by interfering with the cellular membrane potential.\cite{19} When the K⁺ channel is activated, an efflux of K⁺ causes membrane hyperpolarization, which reduces calcium influx and attenuates vascular tone.\cite{19} At least four types of K⁺ channels were identified in arterial smooth muscle cells, ie, the voltage-dependent K⁺ (\(K_{v}\)) channel, activated by depolarizing stimuli; the Ca²⁺-activated K⁺ (\(K_{Ca}\)) channel which responds to intracellular Ca²⁺; the inward rectifier K⁺ (\(K_{IR}\)) channel which may be responsible for external K⁺-induced dilation; and the adenosine triphosphate-sensitive K⁺ (\(K_{ATP}\)) channel which responds to changes in adenosine triphosphate levels. It is known that Ba²⁺ and tetraethylammonium antagonize a broad range of K⁺ channels, and glibenclamide can block \(K_{ATP}\).\cite{20} To test for possible involvement of K⁺ channels in cinnamaldehyde-induced relaxation, we preincubated vessel preparations with tetraethylammonium \(3 \times 10^{-3}\) M (Figure 4A), BaCl₂ \(10^{-4}\) M (Figure 4B), and glibenclamide \(10^{-5}\) M (Figure 4C). We found that potassium channel blockers did not affect cinnamaldehyde-induced vasorelaxation.

Figure 5 Concentration-response curves showing the vasoconstriction of dopamine A), 5-hydroxytryptamine B), angiotensin II C), endothelin-1 D), and phenylephrine E) in the absence or presence of cinnamaldehyde (Cin). The contraction curves of all the vasoconstrictors can be inhibited by cinnamaldehyde at the indicated concentration. Notes: *P < 0.05 versus controls, #P < 0.01 versus controls, n = 6.
The adrenergic β-receptor is an important contributor to vasodilation by increasing intracellular cyclic adenosine monophosphate and activating protein kinase A. However, in our study, propranolol $10^{-5}$ M had no effect on cinnamaldehyde (Figure 4D), suggesting that vasodilation is not mediated via the adrenergic β-receptor.

Endogenous vasoconstrictors not only play a key role in maintaining vascular tension, but also serve as therapeutic targets in many pathological conditions, such as hypertension. Blood vessels pretreated with cinnamaldehyde attenuated vasoconstriction by dopamine (Figure 5A), 5-hydroxytryptamine (Figure 5B), angiotensin II (Figure 5C), endothelin-1 (Figure 5D), and phenylephrine (Figure 5E) in a concentration-dependent manner. Thus, the involvement of a secondary signaling pathway beyond specific receptor levels is speculated. It has been shown that adrenalin, angiotensin, endothelin-1, and 5-hydroxytryptamine all have G protein-coupled receptors which can manipulate the intracellular Ca$^{2+}$ concentration by interference with either Ca$^{2+}$ influx or intracellular Ca$^{2+}$ release. Indeed, calcium channels appear to play a crucial role in contraction of vascular smooth muscle.

Intracellular Ca$^{2+}$ controls smooth muscle contraction through binding with calmodulin to form a calcium-calmodulin complex. This complex further activates myosin light chain kinase to phosphorylate myosin light chains and cause muscle contraction. The intracellular Ca$^{2+}$ concentration can be regulated by extracellular Ca$^{2+}$ influx through both the voltage-dependent Ca$^{2+}$ channel and the receptor-operated Ca$^{2+}$ channel, or by intracellular Ca$^{2+}$ release from endoplasmic reticulum Ca$^{2+}$ stores. KCl mainly activates potential-dependent Ca$^{2+}$ channels to promote calcium influx and vasoconstriction. In our study, cinnamaldehyde markedly inhibited CaCl$_2$-induced contraction of vessels treated with Ca$^{2+}$-free high-K$^+$ media (Figure 6A). In addition, cinnamaldehyde also inhibited concentration-dependent contraction by K$^+$ (Figure 6B). Thus, at least, Ca$^{2+}$ influx through voltage-sensitive Ca$^{2+}$ channels is affected by cinnamaldehyde.

On the other hand, phenylephrine regulates intracellular Ca$^{2+}$ through both receptor-operated calcium channels and intracellular Ca$^{2+}$ release. As showed in Figure 5E, pretreatment with cinnamaldehyde significantly reduced phenylephrine contraction, suggesting possible interference with...
with Ca²⁺ influx through receptor-operated calcium channels. Furthermore, cinnamaldehyde also markedly inhibited small tonic contractions elicited by phenylephrine in Ca²⁺-free media-treated rings (Figure 6C), indicating a blockage of intracellular Ca²⁺ release. Taken together, we propose that cinnamaldehyde relaxes the isolated rat aorta by inhibiting both Ca²⁺ influx and Ca²⁺ release. However, further experimentation with patch clamp methods is needed to confirm this mechanism in detail.

In conclusion, the present study demonstrates that cinnamaldehyde dilates vascular smooth muscle in an endothelium-independent manner. The vasodilatory effect of cinnamaldehyde may be related to its ability to interfere with both Ca²⁺ influx and Ca²⁺ release.

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Disclosure
The authors report no conflicts of interest in this work.

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