

Full Paper

Synephrine, a Component of *Evodiae Fructus*, Constricts Isolated Rat Aorta via Adrenergic and Serotonergic ReceptorsTomoko Hibino^{1,*}, Mitsutoshi Yuzurihara¹, Yoshio Kase¹, and Atsushi Takeda²¹Tsumura Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan²Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka City, Shizuoka 422-8526, Japan

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Abstract. We investigated the effects of *Evodiae Fructus* and synephrine, one of the components of *Evodiae Fructus*, on blood vessels. We found that *Evodiae Fructus* (1×10^{-6} – 3×10^{-4} g/mL) had constrictive effects on rat aorta. The vasoconstrictive effects of *Evodiae Fructus* were significantly inhibited by pretreatment with prazosin (adrenergic α_1 -receptor antagonist), BRL15572 [5-hydroxytryptamine (5-HT)_{1D} antagonist], and ketanserin (5-HT_{2A} antagonist), but its vasoconstrictive effects were not inhibited by pretreatment with SB216641 (5-HT_{1B} antagonist) or propranolol (adrenergic β -receptor antagonist). These results suggest that *Evodiae Fructus* constricts rat aorta via adrenergic and serotonergic receptors. We also investigated the constrictive effects of synephrine on blood vessels. The vasoconstrictive effects of synephrine (1×10^{-7} – 3×10^{-5} mol/L) were significantly inhibited by pretreatment with prazosin, BRL15572, and ketanserin. However, its constrictive effects were not inhibited by pretreatment with SB216641 and propranolol. The pA₂ values of prazosin or ketanserin were nearly equal between *Evodiae Fructus* and synephrine. Because the constrictive effects of both *Evodiae Fructus* and synephrine were exerted via adrenergic α_1 -receptors and serotonergic (5-HT_{1D} and 5-HT_{2A}) receptors, synephrine may be one of the important components in the constrictive effects of *Evodiae Fructus*.

Keywords: *Evodiae Fructus*, synephrine, adrenergic α_1 , 5-hydroxytryptamine (5-HT)_{1D}, 5-HT_{2A}

Introduction

Evodiae Fructus is the dried, nearly ripe fruit of *Evodia rutaecarpa*. It is prescribed, according to traditional Chinese medical practice, for the treatment of headache, abdominal pain, dysentery, amenorrhea, and postpartum hemorrhage (1). Our previous study demonstrated that *goshuyuto*, a traditional Japanese medicine, had an antiaggregatory effect of platelets in guinea pigs and that *Evodiae Fructus* was the active substance in the components of *goshuyuto* (2). Also, we demonstrated that *goshuyuto* constricted isolated rat thoracic aorta and that the constrictive effects was due to *Evodiae Fructus* (3).

Evodiae Fructus contains various components (evodi-

amine, rutaecarpine, limonin, synephrine, and so on). In our previous studies, we found that evodiamine, rutaecarpine, and limonin did not constrict rat aorta but relaxed it (3). We also found that synephrine constricted rat aorta. Thus, it is thought that synephrine is the important ingredient in the constrictive effects of *Evodiae Fructus*.

Synephrine (1-[4-hydroxyphenyl]-2-methyl-amino-ethanol), which is found in plants including citrus species, is one of the biogenic amines. It is known to be a sympathomimetic agent. Synephrine has been reported to act on α -adrenoceptors (4) and to reduce portal pressure and elevate mean arterial pressure in sham-operated and portal hypertensive rats (5). Moreover, synephrine has been reported to have anti-obesity activity (6), exerted through its action on β_3 -adrenoceptors, which function to activate lipolysis. In addition, synephrine has been reported to have antidepressant-like effects (7). These results suggest that synephrine has various effects

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via adrenergic receptors.

We previously demonstrated that *Evodiae Fructus* and synephrine constricted isolated rat aortic strips, but the mechanism was not clear. In general, the aorta is known to be constricted via adrenergic and serotonergic receptors. In this study, we investigated whether adrenergic and serotonergic receptors are involved in the constrictive effects of *Evodiae Fructus* and synephrine on rat aorta.

Materials and Methods

Reagents and drugs

Fructus of *Evodia rutaecarpa* (*Evodiae Fructus*) cultivated in China was used. The quality of this raw material was tested according to the Japanese Pharmacopoeia and our company's standards. The powdered extract of *Evodiae Fructus* was manufactured at our Shizuoka factory (Tsumura & Co., Tokyo). *Evodiae Fructus* was extracted with purified water at 100°C for 1 h. Then, the extracted solution was concentrated by removing water via reduced pressure and spray-dried. The yield of the extract was ca. 20%. The extract was analyzed by high-performance liquid chromatography (HPLC). The powdered extract of *Evodiae Fructus* (No. 2041042010) was stored in our laboratory at constant temperature and humidity. *Evodiae Fructus* was dissolved in distilled water.

SB216641, a 5-hydroxytryptamine (5-HT)_{1B} antagonist, and BRL15572, a 5-HT_{1D} antagonist, were purchased from Tocris (Bristol, UK). Ketanserin (5-HT_{2A} antagonist), prazosin (adrenergic α_1 -antagonist), propranolol (adrenergic β -antagonist), synephrine, phenylephrine, and 5-HT were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other reagents used for analysis were purchased from commercial sources. SB216641, propranolol, phenylephrine, and 5-HT were dissolved in distilled water. Synephrine, BRL15572, ketanserin, and prazosin were dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the buffer solution was 0.1%.

Animals

Seven- and thirteen-week-old male Wistar rats weighing 250–400 g obtained from Charles River, Ltd. (Yokohama) were used. The animals were allowed free access to water and standard laboratory food (MF; Oriental Yeast, Tokyo) and kept in a facility at a temperature of $24 \pm 1^\circ\text{C}$ and relative humidity of $55 \pm 5\%$, with lights on from 07:00 to 19:00 daily. All experimental procedures were performed according to the "Guidelines for the Care and Use of Laboratory Animals" approved by the Laboratory Animal Committee

of Tsumura & Co.

Preparation of isolated rat aorta strips

Rats were killed by a blow to the head and exsanguinated. Thoracic aortas were isolated, cleaned of nonarterial tissue, and immediately immersed in Krebs solution (135 mmol/L NaCl, 5.0 mmol/L KCl, 2.5 mmol/L CaCl₂, 1.3 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 20 mmol/L NaHCO₃, 10 mmol/L glucose, and 0.026 mmol/L EDTA·2Na) at pH 7.4. The aortas were cut into helical strips about 2.0 mm in width and 8.0 mm in length. Each endothelium-intact aorta strip was mounted in an organ bath containing 20 mL Krebs solution gassed with 5% CO₂ in O₂ and maintained at 37°C. One end of the aorta was attached to a force displacement transducer (San-ei Instrument, Tokyo) so that its isometric constrictions could be recorded (Rika Denki Kogyo, Tokyo) via an amplifier (San-ei Instrument). The strip was equilibrated for 60 min at an initial resting tension of 2.0 g prior to measurement of the constriction.

Measurement of constrictive effects of *Evodiae Fructus* and synephrine

Each equilibrated aorta strip was constricted by placing it in 60 mmol/L K⁺ solution. After 15 min, the aorta was washed three times with Krebs buffer. Various concentrations of *Evodiae Fructus* (1×10^{-6} – 3×10^{-4} g/mL) or synephrine (1×10^{-7} – 3×10^{-5} mol/L) were added to the bath in order to evaluate the vasoconstriction. The constriction strength was expressed as a percentage of the maximum tension induced by *Evodiae Fructus* or synephrine compared to that induced by 60 mmol/L K⁺.

Measurement of inhibitory effects of serotonin and adrenergic antagonists on constrictive effects of *Evodiae Fructus* or synephrine

Each equilibrated aorta strip was constricted by placing it in 60 mmol/L K⁺ solution. After 15 min, the aorta was washed three times with Krebs buffer. Then SB216641 at 1×10^{-6} mol/L, BRL15572 at 1×10^{-6} – 1×10^{-5} mol/L, ketanserin at 1×10^{-8} – 1×10^{-7} mol/L, prazosin at 3×10^{-10} – 3×10^{-9} mol/L, or propranolol at 1×10^{-6} mol/L was added to the bath. After 10 min, various concentrations of *Evodiae Fructus* (1×10^{-6} – 3×10^{-4} g/mL) or synephrine (1×10^{-7} – 3×10^{-5} mol/L) were added to the bath in order to evaluate the vasoconstriction. The constriction strength was expressed as a percentage of the maximum tension induced by *Evodiae Fructus* or synephrine with or without antagonist to that induced by 60 mmol/L K⁺.

Measurement of inhibitory effects of serotonin and adrenergic antagonists on constrictive effects of synephrine or 5-HT or phenylephrine

SB216641 at 1×10^{-6} mol/L, BRL15572 at 1×10^{-6} mol/L, ketanserin at 1×10^{-8} mol/L, or prazosin at 1×10^{-9} mol/L was added to the bath. After 10 min, synephrine (3×10^{-6} and 1×10^{-5} mol/L), 5-HT (3×10^{-7} and 1×10^{-6} mol/L), or phenylephrine (3×10^{-8} and 1×10^{-7} mol/L) was added to the bath to evaluate the vasoconstriction. The constriction strength was expressed as a percentage of the maximum tension induced by synephrine, 5-HT, or phenylephrine with or without antagonist to that induced by 60 mmol/L K^+ .

Statistics

Each value was expressed as the mean \pm S.E.M. Results were statistically evaluated using a one-way analysis of variance coupled with Dunnett's test or Student's *t*-test. Significance was accepted at $P < 0.05$.

Results

Water extracts of *Evodiae Fructus* had concentration-dependent constrictive effects on the rat aorta strips at the concentrations of 1×10^{-6} – 3×10^{-4} g/mL (Table 1). The effects of each concentration were compared with the constriction induced by the vehicle ($9.6 \pm 1.5\%$, $N = 9$). A statistically significant difference was observed at the concentration of 3×10^{-6} g/mL.

The effects of adrenergic α_1 - and β -receptor antagonists on the constrictive effects of *Evodiae Fructus* (1×10^{-6} – 3×10^{-4} g/mL) on the isolated rat aorta were investigated (Fig. 1). The constrictive effects of *Evodiae Fructus* were concentration-dependently inhibited by

Table 1. Effects of *Evodiae Fructus* on constriction of isolated rat aorta

<i>Evodiae Fructus</i> (g/mL)	Constriction (%)
1×10^{-6}	10.7 ± 6.3
3×10^{-6}	$36.4 \pm 7.8^*$
1×10^{-5}	$79.3 \pm 6.5^{**}$
3×10^{-5}	$88.8 \pm 4.8^{**}$
1×10^{-4}	$97.9 \pm 3.5^{**}$
3×10^{-4}	$93.6 \pm 2.8^{**}$

Effects of *Evodiae Fructus* were determined at the concentrations of 1×10^{-6} to 3×10^{-4} g/mL. The constrictive strength is expressed as a percentage of the maximum tension induced by each concentration of *Evodiae Fructus* to that induced by 60 mmol/L K^+ . Effects of each concentration of *Evodiae Fructus* on the constriction were compared with that of the control (vehicle) ($*P < 0.05$, $**P < 0.01$, Dunnett's test). Data are expressed as the mean \pm S.E.M. of four to seven determinations.

pretreatment with an α_1 antagonist prazosin at the concentrations of 3×10^{-10} – 3×10^{-9} mol/L (Fig. 1a). Statistically significant differences were observed at prazosin concentrations of 1×10^{-9} and 3×10^{-9} mol/L for constriction induced by 1×10^{-5} g/mL of *Evodiae Fructus*. However, the constrictive effects of *Evodiae Fructus* were not inhibited by pretreatment with the β antagonist propranolol at 1×10^{-6} mol/L (Fig. 1b).

The effects of pretreatment with serotonergic (5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A}) antagonists on the constrictive effects of *Evodiae Fructus* are shown in Fig. 2. The constrictive effects of *Evodiae Fructus* (1×10^{-6} – 3×10^{-4} g/mL) were concentration-dependently antagonized by pretreatment with the 5-HT_{1D} antagonist BRL15572 at 1×10^{-6} – 1×10^{-5} mol/L (Fig. 2b). Statistically

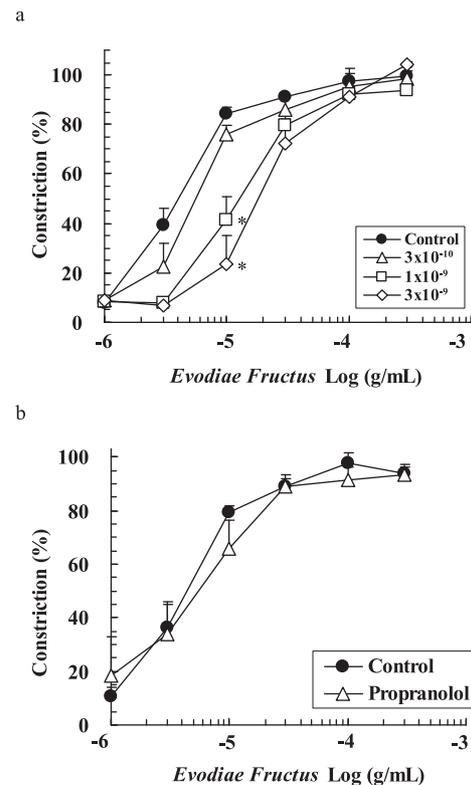
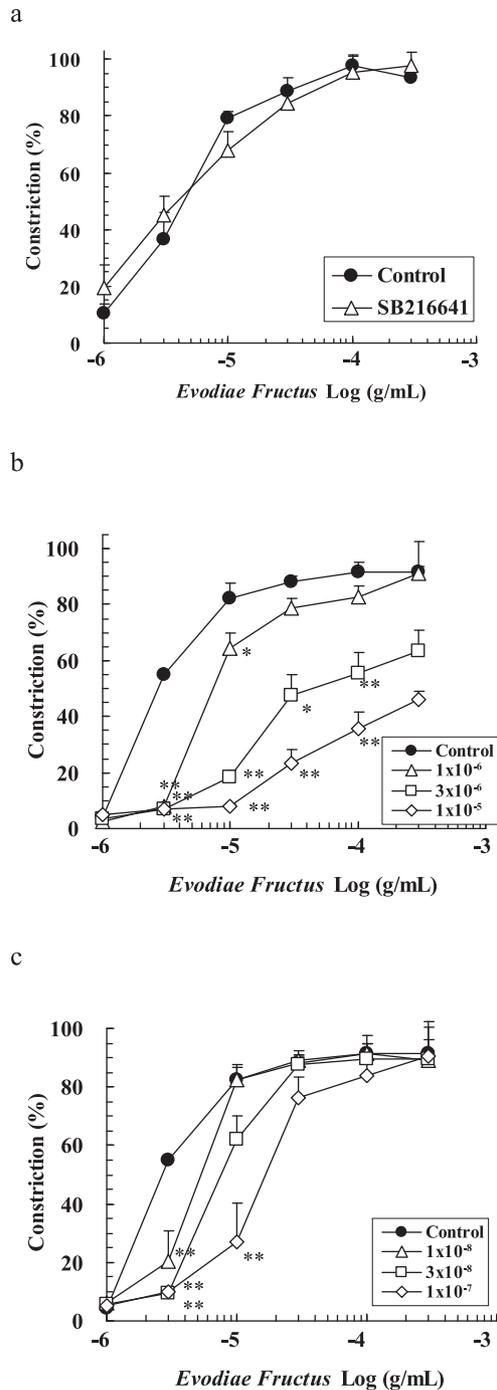


Fig. 1. Effects of adrenergic receptor antagonists on constriction of *Evodiae Fructus* in isolated rat aorta. a: Closed circle: *Evodiae Fructus* + vehicle (control), open triangle: *Evodiae Fructus* + prazosin (adrenergic α_1 -antagonist, 3×10^{-10} mol/L), open square: *Evodiae Fructus* + prazosin (1×10^{-9} mol/L), open diamond: *Evodiae Fructus* + prazosin (3×10^{-9} mol/L). b: Closed circle: *Evodiae Fructus* + vehicle (control), open triangle: *Evodiae Fructus* + propranolol (adrenergic β -antagonist, 1×10^{-6} mol/L). Prazosin or propranolol was added. After 10 min, *Evodiae Fructus* was added. The constrictive strength is expressed as a percentage of the maximum tension induced by *Evodiae Fructus* with or without antagonist to that induced by 60 mmol/L K^+ . Effects of antagonist on the constriction of *Evodiae Fructus* compared with the control ($*P < 0.05$, Dunnett's test or Student's *t*-test). Data are expressed as the mean \pm S.E.M. of four to eleven determinations.



significant differences were observed at 1×10^{-6} – 1×10^{-5} mol/L of BRL15572 for constriction induced by 3×10^{-6} and 1×10^{-5} g/mL of *Evodiae Fructus* and 3×10^{-6} and 1×10^{-5} mol/L of BRL15572 for constriction induced by 3×10^{-5} and 1×10^{-4} g/mL of *Evodiae Fructus*. Similarly, the constrictive effects of *Evodiae Fructus* were concentration-dependently antagonized by pretreatment with the 5-HT_{2A} antagonist ketanserin at 1×10^{-8} – 1×10^{-7} mol/L (Fig. 2c). Statistically signifi-

Fig. 2. Effects of 5-HT-receptor antagonists on constriction of *Evodiae Fructus* in isolated rat aorta. a: Closed circle: *Evodiae Fructus* + vehicle (control), open triangle: *Evodiae Fructus* + SB216641 (5-HT_{1B} antagonist, 1×10^{-6} mol/L). b: Closed circle: *Evodiae Fructus* + vehicle (control), open triangle: *Evodiae Fructus* + BRL15572 (5-HT_{1D} antagonist, 1×10^{-6} mol/L), open square: *Evodiae Fructus* + BRL15572 (3×10^{-6} mol/L), open diamond: *Evodiae Fructus* + BRL15572 (1×10^{-5} mol/L). c: Closed circle: *Evodiae Fructus* + vehicle (control), open triangle: *Evodiae Fructus* + ketanserin (5-HT_{2A} antagonist, 1×10^{-8} mol/L), open square: *Evodiae Fructus* + ketanserin (3×10^{-8} mol/L), open diamond: *Evodiae Fructus* + ketanserin (1×10^{-7} mol/L). SB216641, BRL15572, or ketanserin was added. After 10 min, *Evodiae Fructus* was added. The constrictive strength is expressed as a percentage of the maximum tension induced by *Evodiae Fructus* with or without antagonist to that induced by 60 mmol/L K⁺. Effects of 5-HT antagonist on the constriction of *Evodiae Fructus* were compared with control (* $P < 0.05$, ** $P < 0.01$, Dunnett's test or Student's *t*-test). Data are expressed as the mean \pm S.E.M. of four to eleven determinations.

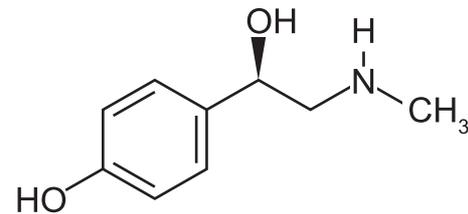


Fig. 3. The structural formula of synephrine.

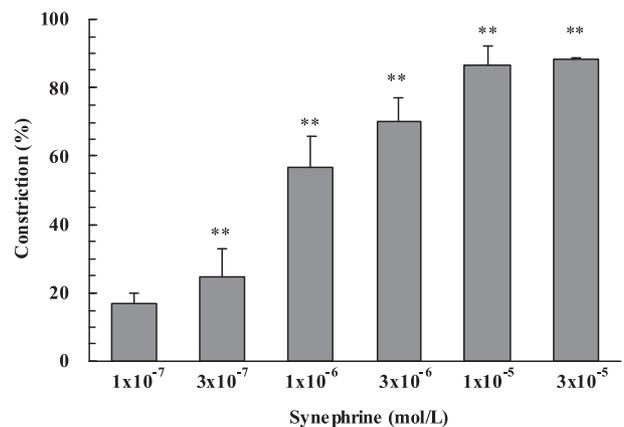


Fig. 4. Dose-dependent effects of synephrine on constriction of isolated rat aorta. Effects of synephrine were determined at the concentrations of 1×10^{-7} – 3×10^{-5} mol/L. The constrictive strength is expressed as a percentage of the maximum tension induced by synephrine to that induced by 60 mmol/L K⁺. Effects of each dose of synephrine on the constriction were compared with that of vehicle (** $P < 0.01$, Dunnett's test). Data are expressed as the mean \pm S.E.M. of four to seven determinations.

cant differences were observed for ketanserin concentrations of 1×10^{-8} – 1×10^{-7} mol/L at 3×10^{-6} g/mL of *Evodiae Fructus* and 1×10^{-7} mol/L ketanserin at

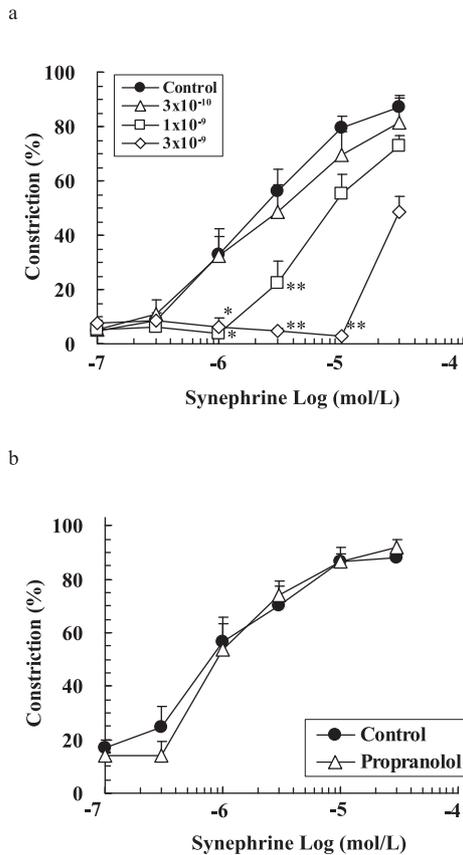


Fig. 5. Effects of adrenergic receptor antagonists on constriction of synephrine in isolated rat aorta. a: Closed circle: synephrine + vehicle (control), open triangle: synephrine + prazosin (adrenergic α_1 -antagonist, 3×10^{-10} mol/L), open square: synephrine + prazosin (1×10^{-9} mol/L), open diamond: synephrine + prazosin (3×10^{-9} mol/L). b: Closed circle: synephrine + vehicle (control), open triangle: synephrine + propranolol (adrenergic β -antagonist, 1×10^{-6} mol/L). Prazosin or propranolol was added. After 10 min, synephrine was added. The constrictive strength is expressed as a percentage of the maximum tension induced by synephrine with or without antagonist to that induced by 60 mmol/L K^+ . Effects of antagonist on the constriction of synephrine compared with the control (* $P < 0.05$, ** $P < 0.01$, Dunnett's test or Student's t -test). Data are expressed as the mean \pm S.E.M. of four to ten determinations.

1×10^{-5} g/mL of *Evodiae Fructus*. The effects of *Evodiae Fructus* were not antagonized by pretreatment with the 5-HT_{1B} antagonist SB216641 at 1×10^{-6} mol/L (Fig. 2a).

The structural formula of synephrine is shown in Fig. 3, and the constrictive effects of synephrine (1×10^{-7} – 3×10^{-5} mol/L) are shown in Fig. 4. The effects of each concentration were compared with the constriction induced by the vehicle ($6.7 \pm 1.5\%$, $N = 8$). A statistically significant difference was observed at a concentration of 3×10^{-7} mol/L.

The effects of adrenergic α_1 - and β -antagonists on the constrictive effects of synephrine were determined

(Fig. 5). The constrictive effects of synephrine (1×10^{-7} – 3×10^{-5} mol/L) were concentration-dependently inhibited by pretreatment with the α_1 antagonist prazosin at 3×10^{-10} – 3×10^{-9} mol/L (Fig. 5a). Statistically significant differences were observed at prazosin concentrations of 1×10^{-9} and 3×10^{-9} mol/L at 1×10^{-6} and 3×10^{-6} mol/L of synephrine, respectively, and 3×10^{-9} mol/L prazosin at 1×10^{-5} mol/L of synephrine. However, the constrictive effects were not inhibited by pretreatment with the β antagonist propranolol at 1×10^{-6} mol/L (Fig. 5b).

The effects of pretreatment with 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A} antagonists on the constrictive effects of synephrine are shown in Fig. 6. The constrictive effects of synephrine (1×10^{-7} – 3×10^{-5} mol/L) were concentration-dependently antagonized by pretreatment with the 5-HT_{1D} antagonist BRL15572 at the concentrations of 1×10^{-6} – 1×10^{-5} mol/L (Fig. 6b). Statistically significant differences were observed at the concentrations of 1×10^{-6} – 1×10^{-5} mol/L BRL15572 at 1×10^{-6} and 3×10^{-6} mol/L of synephrine, 3×10^{-6} and 1×10^{-5} mol/L BRL15572 at 1×10^{-5} mol/L of synephrine, and 1×10^{-5} mol/L BRL15572 at 3×10^{-5} mol/L of synephrine. Similarly, the constrictive effects of synephrine were concentration-dependently antagonized by pretreatment with the 5-HT_{2A} antagonist ketanserin at 1×10^{-8} – 1×10^{-7} mol/L (Fig. 6c). Statistically significant differences were observed at 1×10^{-8} – 1×10^{-7} mol/L ketanserin for the constriction induced by 1×10^{-6} mol/L synephrine and 3×10^{-8} and 1×10^{-7} mol/L ketanserin for the constriction induced by 3×10^{-6} mol/L synephrine. The effects of synephrine were not antagonized by pretreatment with the 5-HT_{1B} antagonist SB216641 at 1×10^{-6} mol/L (Fig. 6a).

Schild plots of prazosin against *Evodiae Fructus* (Fig. 1a) and synephrine (Fig. 5a) are shown in Fig. 7, a and b, respectively. Also, Schild plots of ketanserin against *Evodiae Fructus* (Fig. 2c) and synephrine (Fig. 6c) are shown in Fig. 7, c and d, respectively. The Schild analysis allowed us to calculate the pA_2 values of prazosin and ketanserin against *Evodiae Fructus* and synephrine, with the slopes, which were not significantly different from unity (Table 2). The pA_2 of these antagonists between *Evodiae Fructus* and synephrine were not significantly different.

Effects of adrenergic and serotonergic receptor antagonists on the constrictive effects of synephrine (3×10^{-6} and 1×10^{-5} mol/L) were compared to those on the constrictive effects of 5-HT (3×10^{-7} and 1×10^{-6} mol/L) and phenylephrine (3×10^{-8} and 1×10^{-7} mol/L) (Table 3). The constrictive effects of phenylephrine were significantly antagonized by pretreatment with prazosin (1×10^{-9} mol/L). Also, the constrictive effects

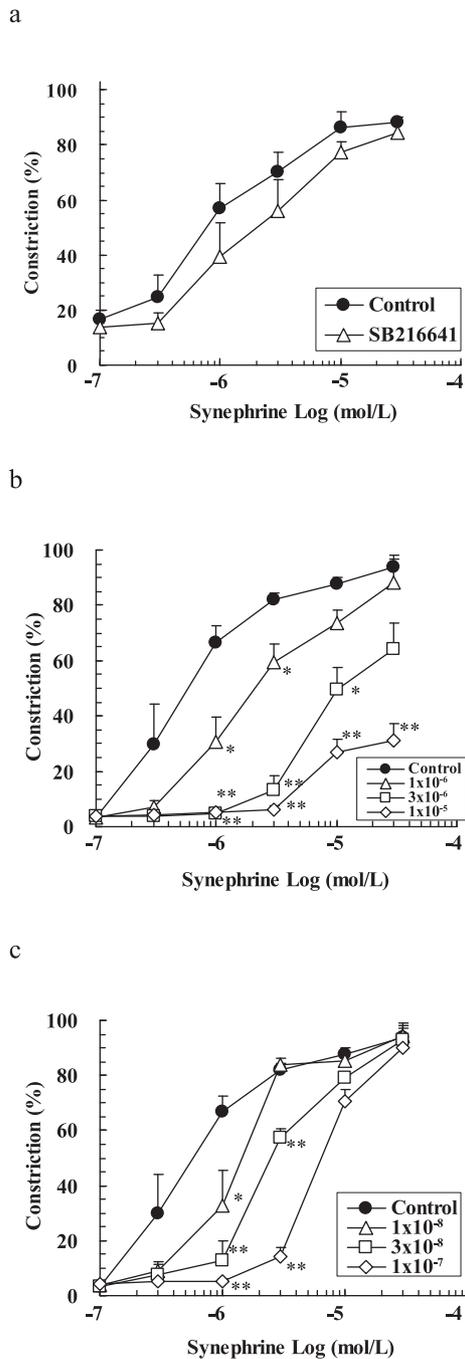


Fig. 6. Effects of 5-HT-receptor antagonists on constriction of synephrine in isolated rat aorta. a: Closed circle: synephrine + vehicle (control), open triangle: synephrine + SB216641 (5-HT_{1B} antagonist, 1×10^{-6} mol/L). b: Closed circle: synephrine + vehicle (control), open triangle: synephrine + BRL15572 (5-HT_{1D} antagonist, 1×10^{-6} mol/L), open square: synephrine + BRL15572 (3×10^{-6} mol/L), open diamond: synephrine + BRL15572 (1×10^{-5} mol/L). c: Closed circle: synephrine + vehicle (control), open triangle: synephrine + ketanserin (5-HT_{2A} antagonist, 1×10^{-8} mol/L), open square: synephrine + ketanserin (3×10^{-8} mol/L), open diamond: synephrine + ketanserin (1×10^{-7} mol/L). SB216641 or BRL15572 or ketanserin was added. After 10 min, synephrine was added. The constrictive strength is expressed as a percentage of the maximum tension induced by synephrine with or without antagonist to that induced by 60 mmol/L K⁺. Effects of 5-HT antagonist on the constriction of synephrine were compared with the control (* $P < 0.05$, ** $P < 0.01$, Dunnett's test or Student's *t*-test). Data are expressed as the mean \pm S.E.M. of four to seven determinations.

caused constriction of isolated rat thoracic aorta strips (3). However, the mechanism of the constrictive effects of *Evodiae Fructus* is not clear. In the present study undertaken to clarify the mechanism of the constrictive effects of *Evodiae Fructus* on rat aorta, we focused on the effects of adrenergic and serotonergic receptors on the constrictive effects.

It is known that adrenergic α_1 -receptors are related to the constriction of blood vessels (8, 9). The constrictive effects of *Evodiae Fructus* were competitively inhibited by pretreatment with prazosin, an adrenergic α_1 -antagonist, although pretreatment with propranolol, an adrenergic β -antagonist, did not affect the constrictive effects. In general, the aorta is relaxed via adrenergic β -receptors. These results suggest that *Evodiae Fructus* stimulates α_1 -receptors.

The 5-HT receptors are currently classified into seven families, and these seven families are further classified into fourteen (10–12). It is generally known that 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A} act on vascular smooth muscle (10, 13). 5-HT_{1B} and 5-HT_{1D} are known to be involved in constricting cerebral blood vessels (14). In addition, it is reported that the rat aorta is constricted in response to serotonin via activation of 5-HT_{2A} (15). These reports indicated that serotonergic receptors were related to vasoconstrictive effects on the blood vessels.

We investigated the effects of pretreatment with serotonergic vasoactive receptor antagonists (5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A}) on the constrictive effects of *Evodiae Fructus*. The constrictive effects of *Evodiae Fructus* were competitively antagonized by pretreatment with ketanserin, a 5-HT_{2A} antagonist. Also, the constrictive effects were antagonized by pretreatment with BRL15572, a 5-HT_{1D} antagonist, but pretreatment with SB216641, a 5-HT_{1B} antagonist, did not affect the constrictive effects. These results suggest that the

of 5-HT were significantly antagonized by pretreatment with SB216641 (1×10^{-6} mol/L) and ketanserin (1×10^{-8} mol/L). The constrictive effects of synephrine were significantly antagonized by pretreatment with prazosin, like those of phenylephrine.

Discussion

We previously demonstrated that *Evodiae Fructus* (1×10^{-6} – 3×10^{-4} g/mL) concentration-dependently

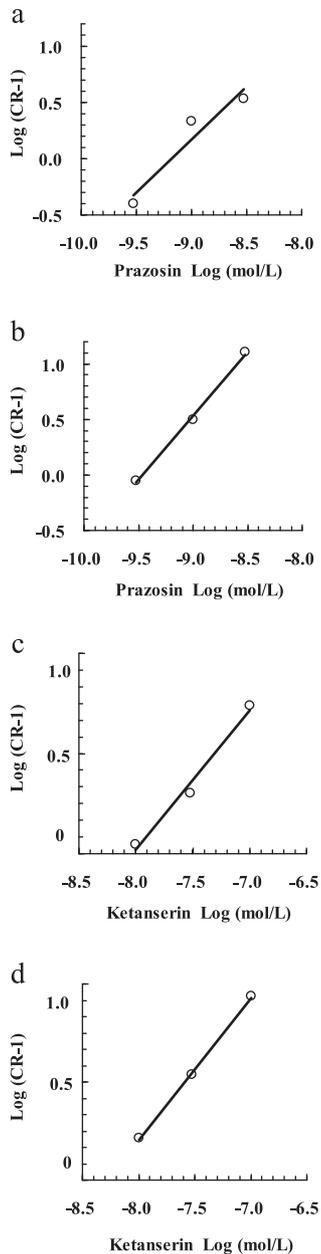


Fig. 7. Schild plots for the interaction of prazosin and ketanserin with *Evodiae Fructus* and synephrine. The Schild lines of prazosin at the concentrations of 3×10^{-10} , 1×10^{-9} , and 3×10^{-9} mol/L for *Evodiae Fructus* (a) and synephrine (b) are shown. Also, the Schild lines of ketanserin at the concentrations of 1×10^{-8} , 3×10^{-8} , and 1×10^{-7} mol/L for *Evodiae Fructus* (c) and synephrine (d) are shown. Schild plots were made by plotting the logarithm [concentration ratio (CR) – 1] against the logarithm of the molar concentration of prazosin or ketanserin. A linear regression was performed on mean data points of four to seven determinations.

constrictive effects of *Evodiae Fructus* were related to serotonergic 5-HT_{1D} and 5-HT_{2A} receptors. We determined the antagonistic effects of ketanserin at the concentrations of 1×10^{-8} – 1×10^{-7} mol/L on the constrictive effects of *Evodiae Fructus* and synephrine, as shown

in Fig. 2c and Fig. 6c. We elucidated that ketanserin at a concentration of 1×10^{-7} mol/L inhibited not only 5-HT_{2A} receptors but also adrenergic α_1 receptors (data not shown). Although, ketanserin at the concentrations of 1×10^{-8} – 3×10^{-7} mol/L does not inhibit adrenergic α_1 receptors but does inhibit 5-HT_{2A} receptors. Therefore, the constrictive effects of *Evodiae Fructus* were thought to be related to serotonergic 5-HT_{2A}.

On the other hand, *Evodiae Fructus* contains many components (evodiamine, dehydroevodiamine, rutaecarpine, limonin, higenamine, evocarpine, synephrine, and so on). Among the ingredients of *Evodiae Fructus*, it was reported that evodiamine (16, 17), rutaecarpine (17, 18), dehydroevodiamine (17), higenamine (19), and evocarpine (20) do not constrict the rat aorta but relax it instead. Our previous study demonstrated that evodiamine, rutaecarpine, and limonin, ingredients of *Evodiae Fructus*, showed relaxant effects on the rat aorta (3). These results for evodiamine and rutaecarpine supported the previously reported observations (16 – 18). On the other hand, synephrine showed the constrictive effects on the rat aorta. Although most components of *Evodiae Fructus* have relaxant effects, *Evodiae Fructus* caused constriction of the rat aorta. In future studies, we will investigate the constrictive effects of other components of *Evodiae Fructus*. However, the content of synephrine in *Evodiae Fructus* is only 0.46% – 3.42% (21). Therefore, synephrine is thought to be a potent and important component of the constrictive effects of *Evodiae Fructus*.

We examined the effects of adrenergic receptors on the constrictive effects of synephrine. The constrictive effects of synephrine were competitively inhibited by pretreatment with the adrenergic α_1 -antagonist prazosin, although pretreatment with the adrenergic β -antagonist propranolol, did not affect the constrictive effects. These results suggest that synephrine stimulates α_1 -receptors.

We also investigated the effects of pretreatment with serotonergic vasoactive-related receptor antagonists (5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A}) on the constrictive effects of synephrine. The constrictive effects of synephrine were competitively antagonized by pretreatment with a 5-HT_{2A} antagonist. Also, the constrictive effects of synephrine were antagonized by pretreatment with a 5-HT_{1D} antagonist, but pretreatment with a 5-HT_{1B} antagonist had no effect. These results suggest that synephrine also stimulates serotonergic 5-HT_{1D}, and 5-HT_{2A} receptors. Moreover, synephrine had effects on the constriction of rat aorta, like phenylephrine did. It may be thought that synephrine affected both adrenergic and serotonergic receptors and that the effects of synephrine on adrenergic receptors were more potent than the effects on serotonergic receptors.

Table 2. Schild analysis of the antagonistic effects of prazosin and ketanserin against *Evodiae Fructus* and synephrine

	Prazosin		Ketanserin	
	pA ₂	slope	pA ₂	slope
<i>Evodiae Fructus</i>	9.15 ± 0.10	0.95 ± 0.06	7.91 ± 0.03	0.83 ± 0.06
Synephrine	9.38 ± 0.12	1.01 ± 0.11	8.23 ± 0.14	0.89 ± 0.05

The inhibitory effects of three concentrations of prazosin (adrenergic α_1 -antagonist) were determined on the constrictive effects of *Evodiae Fructus* or synephrine. Similarly, the effects of three concentrations of ketanserin (5-HT_{2A} antagonist) were determined on the constrictive effects of *Evodiae Fructus* or synephrine. The pA₂ and slope were obtained by the Schild plots shown in Fig. 7. Data are expressed as the mean ± S.E.M. of four to seven determinations.

Table 3. Effects of adrenergic α_1 - and serotonergic antagonists on constriction induced by synephrine, 5-HT, and phenylephrine

Compound	(mol/L)	Control (%)	Prazosin (1 × 10 ⁻⁹ mol/L) (%)	SB216641 (1 × 10 ⁻⁶ mol/L) (%)	BRL15572 (1 × 10 ⁻⁶ mol/L) (%)	Ketanserin (1 × 10 ⁻⁸ mol/L) (%)
Synephrine	3 × 10 ⁻⁶	78.0 ± 5.0	33.4 ± 12.0*	82.7 ± 4.3	59.9 ± 7.8	66.4 ± 10.4
	1 × 10 ⁻⁵	91.7 ± 2.1	64.6 ± 8.6*	92.3 ± 3.5	79.3 ± 5.1	83.1 ± 4.0
5-HT	3 × 10 ⁻⁷	73.3 ± 4.6	76.2 ± 7.7	6.6 ± 2.0**	69.2 ± 4.1	4.8 ± 0.6**
	1 × 10 ⁻⁶	80.9 ± 5.3	84.5 ± 4.8	25.9 ± 11.5**	83.6 ± 2.0	9.5 ± 2.4**
Phenylephrine	3 × 10 ⁻⁸	84.2 ± 3.5	36.6 ± 13.1**	90.3 ± 3.3	61.8 ± 4.5	78.2 ± 4.6
	1 × 10 ⁻⁷	94.3 ± 3.2	63.2 ± 10.1*	85.1 ± 4.3	77.5 ± 2.6	86.2 ± 3.6

Effects of prazosin (adrenergic α_1 -antagonist, 1 × 10⁻⁹ mol/L), SB216641 (5-HT_{1B} antagonist, 1 × 10⁻⁶ mol/L), BRL15572 (5-HT_{1D} antagonist, 1 × 10⁻⁶ mol/L), and ketanserin (5-HT_{2A} antagonist, 1 × 10⁻⁸ mol/L) were determined on vasoconstriction induced by synephrine (3 × 10⁻⁶ and 1 × 10⁻⁵ mol/L), 5-HT (3 × 10⁻⁷ and 1 × 10⁻⁶ mol/L), and phenylephrine (3 × 10⁻⁸ and 1 × 10⁻⁷ mol/L). The constrictive strength is expressed as a percentage of the maximum tension induced by each concentration of vasoconstrictor to that induced by 60 mmol/L K⁺. Effects of each concentration of antagonist on the constriction were compared with that of the control (without antagonist) (*P < 0.05, **P < 0.01, Dunnett's test). Data are expressed as the mean ± S.E.M. of four to seven determinations.

The constrictive effects of *Evodiae Fructus* and synephrine were competitively antagonized with prazosin and ketanserin. The pA₂ values of prazosin against *Evodiae Fructus* and synephrine were nearly equal. Also, pA₂ values of ketanserin against *Evodiae Fructus* and synephrine were nearly equal. Therefore, it is thought that the mechanism for the inhibitory effects of these antagonists were the same for *Evodiae Fructus* and synephrine.

In conclusion, we found that *Evodiae Fructus* constricted the isolated rat aorta via the adrenergic α_1 -receptor and serotonergic 5-HT_{1D} and 5-HT_{2A} receptors and that synephrine, one of the components of *Evodiae Fructus*, also constricted the isolated rat aorta via the adrenergic α_1 -receptor and serotonergic 5-HT_{1D} and 5-HT_{2A} receptors. These results suggest that synephrine constricts the rat aorta by the same mechanism as *Evodiae Fructus*. It is thought that synephrine may be one of the potent and important components of the vasoconstrictive effects of *Evodiae Fructus*.

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