Synephrine, a Component of Evodiae Fructus, Constricts Isolated Rat Aorta via Adrenergic and Serotonergic Receptors

Tomoko Hibino¹*, Mitsutoshi Yuzurihara¹, Yoshio Kase¹, and Atsushi Takeda²

¹Tsumura Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan
²Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka City, Shizuoka 422-8526, Japan

Received March 5, 2009; Accepted July 21, 2009

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 α₁-receptor antagonist), BRL15572 [5-hydroxytryptamine (5-HT)₁D antagonist], and ketanserin (5-HT₂A antagonist), but its vasoconstrictive effects were not inhibited by pretreatment with SB216641 (5-HT₁B 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 α₁-receptors and serotonergic (5-HT₁D and 5-HT₂A) receptors, synephrine may be one of the important components in the constrictive effects of Evodiae Fructus.

Keywords: Evodiae Fructus, synephrine, adrenergic α₁, 5-hydroxytryptamine (5-HT)₁D, 5-HT₂A

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 (evodiamine, 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 β₁-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
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-HT₁B antagonist, were purchased from Tocris (Bristol, UK). Ketanserin (5-HT₂A antagonist), prazosin (adrenergic α₁-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 ± 1°C and relative humidity of 55 ± 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 × 10⁻⁵ 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^{-9} 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 constrictive 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 ± 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 ± 1.5%, N = 9). A statistically significant difference was observed at the concentration of 3 × 10^{-6} g/mL.

The effects of adrenergic α₁- 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 pretreatment with an α₁ 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^{-7} 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 serotoninergic (5-HT₁B, 5-HT₁D, and 5-HT₂A) 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-HT₁D antagonist BRL15572 at 1 × 10^{-6} – 1 × 10^{-5} mol/L (Fig. 2b).

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

<table>
<thead>
<tr>
<th><em>Evodiae Fructus</em> (g/mL)</th>
<th>Constriction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10^{-6}</td>
<td>10.7 ± 6.3</td>
</tr>
<tr>
<td>3 × 10^{-6}</td>
<td>36.4 ± 7.8*</td>
</tr>
<tr>
<td>1 × 10^{-5}</td>
<td>79.3 ± 6.5**</td>
</tr>
<tr>
<td>3 × 10^{-5}</td>
<td>88.8 ± 4.8**</td>
</tr>
<tr>
<td>1 × 10^{-4}</td>
<td>97.9 ± 3.5**</td>
</tr>
<tr>
<td>3 × 10^{-4}</td>
<td>93.6 ± 2.8**</td>
</tr>
</tbody>
</table>

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 ± S.E.M. of four to seven determinations.

**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 α₁-antagonist, 3 × 10^{-10} mol/L), open square: *Evodiae Fructus* + prazosin (1 × 10^{-9} mol/L), open diamond: *Evodiae Fructus* + prazosin (3 × 10^{-8} 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 ± S.E.M. of four to eleven determinations.
significant differences were observed at $1 \times 10^{-6} - 1 \times 10^{-5} \text{ mol/L}$ of BRL15572 for constriction induced by $3 \times 10^{-6}$ and $1 \times 10^{-5} \text{ g/mL of Evodiae Fructus}$ and $3 \times 10^{-6}$ and $1 \times 10^{-5} \text{ mol/L of BRL15572}$ for constriction induced by $3 \times 10^{-5}$ and $1 \times 10^{-4} \text{ g/mL of Evodiae Fructus}$. Similarly, the constrictive effects of Evodiae Fructus were concentration-dependently antagonized by pretreatment with the 5-HT$_{2A}$ antagonist ketanserin at $1 \times 10^{-8} - 1 \times 10^{-7} \text{ mol/L}$ (Fig. 2c). Statistically signifi-
concentration of 3 × 10^{-7} \pm 3 × 10^{-5} \text{ mol/L} of Evodiae Fructus. The effects of Evodiae Fructus were not antagonized by pretreatment with the 5-HT \text{IB} antagonist SB216641 at 1 × 10^{-6} \text{ mol/L (Fig. 2a).}

The structural formula of synephrine is shown in Fig. 1B. The effects of adrenergic \( \alpha \)-receptors on the constrictive effects of synephrine were determined

(Fig. 5). The constrictive effects of synephrine (1 × 10^{-7} \text{–} 3 × 10^{-5} \text{ mol/L}) were concentration-dependently inhibited by pretreatment with the \( \alpha \)-agonist prazosin at 3 × 10^{-10} \text{–} 3 × 10^{-7} \text{ mol/L (Fig. 5a). Statistically significant differences were observed at prazosin concentrations of 1 × 10^{-8} \text{ and 3 × 10^{-7}} \text{ mol/L at 1 × 10^{-6} and 3 × 10^{-5}} \text{ mol/L of synephrine, respectively, and 3 × 10^{-9}} \text{ mol/L prazosin at 1 × 10^{-3}} \text{ mol/L of synephrine. However, the constrictive effects were not inhibited by pretreatment with the \( \beta \)-agonist propranolol at 1 × 10^{-6} \text{ mol/L (Fig. 5b).}

The effects of pretreatment with 5-HT \text{IB}, 5-HT \text{ID}, and 5-HT \text{IA} antagonists on the constrictive effects of synephrine are shown in Fig. 6. The constrictive effects of synephrine (1 × 10^{-7} \text{–} 3 × 10^{-5} \text{ mol/L}) were concentration-dependently antagonized by pretreatment with the 5-HT \text{ID} antagonist BRL15572 at the concentrations of 1 × 10^{-6} \text{–} 1 × 10^{-5} \text{ mol/L (Fig. 6b). Statistically significant differences were observed at the concentrations of 1 × 10^{-6} \text{–} 1 × 10^{-5} \text{ mol/L BRL15572 at 1 × 10^{-6} and 3 × 10^{-5} 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^{-4} 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 \text{IA} antagonist ketanserin at 1 × 10^{-4} \text{–} 1 × 10^{-2} \text{ mol/L (Fig. 6c). Statistically significant differences were observed at 1 × 10^{-4} \text{–} 1 × 10^{-2} \text{ mol/L ketanserin for the constrictions induced by 1 × 10^{-6} \text{ mol/L synephrine and 3 × 10^{-4} and 1 × 10^{-2} \text{ mol/L ketanserin for the constrictions induced by 3 × 10^{-6} mol/L synephrine. The effects of synephrine were not antagonized by pretreatment with the 5-HT \text{IB} antagonist SB216641 at 1 × 10^{-4} \text{ 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 serotoninergic receptor antagonists on the constrictive effects of synephrine (3 × 10^{-6} and 1 × 10^{-5} \text{ mol/L}) were compared to those on the constrictive effects of 5-HT (3 × 10^{-7} and 1 × 10^{-6} \text{ mol/L}) and phenylephrine (3 × 10^{-8} and 1 × 10^{-7} \text{ mol/L}) (Table 3). The constrictive effects of phenylephrine were significantly antagonized by pretreatment with prazosin (1 × 10^{-7} \text{ mol/L}). Also, the constrictive effects
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 × 10⁻⁵ g/mL) concentration-dependently 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 α₁-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 α₁-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 α₁-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-HT₁B, 5-HT₁D, and 5-HT₂A act on vascular smooth muscle (10, 13). 5-HT₁B and 5-HT₁D 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-HT₂A (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-HT₁B, 5-HT₁D, and 5-HT₂A) on the constrictive effects of Evodiae Fructus. The constrictive effects of Evodiae Fructus were competitively antagonized by pretreatment with ketanserin, a 5-HT₂A antagonist. Also, the constrictive effects were antagonized by pretreatment with BRL15572, a 5-HT₁D antagonist, but pretreatment with SB216641, a 5-HT₁B antagonist, did not affect the constrictive effects. These results suggest that the
constrictive effects of Evodiae Fructus were related to serotonergic 5-HT$_{2A}$ receptors. We determined the antagonistic effects of ketanserin at the concentrations of $1 \times 10^{-8} - 3 \times 10^{-7}$ 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 $1 \times 10^{-7}$ mol/L inhibited not only 5-HT$_{2A}$ receptors but also adrenergic $a_1$ receptors (data not shown). Although, ketanserin at the concentrations of $1 \times 10^{-8} - 3 \times 10^{-7}$ mol/L does not inhibit adrenergic $a_1$ 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 $a_1$-antagonist prazosin, although pretreatment with the adrenergic $\beta$-antagonist propranolol, did not affect the constrictive effects. These results suggest that synephrine stimulates $a_1$-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.
The vasoconstrictive effects of one of the potent and important components of *Evodiae Fructus* constricts the rat aorta by the same mechanism as receptor and serotonergic 5-HT. Also, pA\_1 × 10\^-1\_L constricted the isolated rat aorta via the adrenergic and synephrine. The pA\_2 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>
<thead>
<tr>
<th>Compound</th>
<th>(mol/L)</th>
<th>Control (%)</th>
<th>Prazosin (1 × 10^-6 mol/L) (%)</th>
<th>SB216641 (1 × 10^-6 mol/L) (%)</th>
<th>BRL15572 (1 × 10^-6 mol/L) (%)</th>
<th>Ketanserin (1 × 10^-6 mol/L) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synephrine</td>
<td>3 × 10^-6</td>
<td>78.0 ± 5.0</td>
<td>33.4 ± 12.0*</td>
<td>82.7 ± 4.3</td>
<td>59.9 ± 7.8</td>
<td>66.4 ± 10.4</td>
</tr>
<tr>
<td></td>
<td>1 × 10^-5</td>
<td>91.7 ± 2.1</td>
<td>64.6 ± 8.6*</td>
<td>92.3 ± 3.5</td>
<td>79.3 ± 5.1</td>
<td>83.1 ± 4.0</td>
</tr>
<tr>
<td>5-HT</td>
<td>3 × 10^-7</td>
<td>73.3 ± 4.6</td>
<td>76.2 ± 7.7</td>
<td>6.6 ± 2.0**</td>
<td>69.2 ± 4.1</td>
<td>4.8 ± 0.6**</td>
</tr>
<tr>
<td></td>
<td>1 × 10^-6</td>
<td>80.9 ± 5.3</td>
<td>84.5 ± 4.8</td>
<td>25.9 ± 11.5**</td>
<td>83.6 ± 2.0</td>
<td>9.5 ± 2.4**</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>3 × 10^-8</td>
<td>84.2 ± 3.5</td>
<td>36.6 ± 13.1**</td>
<td>90.3 ± 3.3</td>
<td>61.8 ± 4.5</td>
<td>78.2 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>1 × 10^-7</td>
<td>94.3 ± 3.2</td>
<td>63.2 ± 10.1*</td>
<td>85.1 ± 4.3</td>
<td>77.5 ± 2.6</td>
<td>86.2 ± 3.6</td>
</tr>
</tbody>
</table>

Effects of prazosin (adrenergic α\_1-antagonist, 1 × 10\^-6 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\^-6 mol/L) were determined on vasoconstriction induced by synephrine (3 × 10\^-4 and 1 × 10\^-5 mol/L), 5-HT (3 × 10\^-7 and 1 × 10\^-6 mol/L), and phenylephrine (3 × 10\^-4 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 pA\_2 values of prazosin against *Evodiae Fructus* and synephrine were nearly equal. Also, pA\_2 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*.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>pA_2</th>
<th>slope</th>
<th>pA_2</th>
<th>slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Evodiae Fructus</em></td>
<td>9.15 ± 0.10</td>
<td>0.95 ± 0.06</td>
<td>7.91 ± 0.03</td>
<td>0.83 ± 0.06</td>
</tr>
<tr>
<td>Synephrine</td>
<td>9.38 ± 0.12</td>
<td>1.01 ± 0.11</td>
<td>8.23 ± 0.14</td>
<td>0.89 ± 0.05</td>
</tr>
</tbody>
</table>

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 pA\_2 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

The constrictive effects of *Evodiae Fructus* and synephrine were competitively antagonized with prazosin and ketanserin. The pA\_2 values of prazosin against *Evodiae Fructus* and synephrine were nearly equal. Also, pA\_2 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*.

### References

Vasoconstrictive Effect of Synephrine


