Goshuyuto, a Traditional Japanese Medicine, and Aqueous Extracts of *Evodiae Fructus* Constrict Isolated Rat Aorta via Adrenergic and/or Serotonergic Receptors

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Received August 29, 2008; accepted November 27, 2008; published online November 28, 2008

Effects of goshuyuto, a traditional Japanese medicine, on vascular constriction were examined using isolated strips of rat aorta. Goshuyuto (1×10⁻⁶ to 1×10⁻³ g/ml) caused constriction of aorta strips in a dose-dependent manner. The vasoconstrictive effects of goshuyuto were significantly inhibited by pretreatment with prazosin, an adrenergic α₁ receptor antagonist. The constrictive effects were partially inhibited by pretreatment with BRL15572, a 5-HT₁D antagonist, and ketanserin, a 5-HT₂A antagonist. However, the constrictive effects were not inhibited by pretreatment with SB216641, a 5-HT₁B antagonist, or propranolol, an adrenergic β receptor antagonist. In addition, aqueous extracts of *Evodiae Fructus*, one of the constituent medicinal herbs of goshuyuto, caused constriction of aorta strips strongly, but aqueous extracts of *Zizyphi Fructus*, *Ginseng Radix*, and *Zingiberis Rhizoma*, the other constituents of goshuyuto, did not have much effect on the vascular response of rat aorta strips. Also, synephrine, which is one of the ingredients of *Evodiae Fructus*, constricted the rat aorta. These results suggest that goshuyuto constricts rat aorta strips and that the mechanisms involve the adrenergic and/or serotonergic receptors. Also, it may be suggested that *Evodiae Fructus* and synephrine play the important role in the vasoconstrictive effects of goshuyuto on rat aorta strips.

Key words  goshuyuto; adrenergic α₁; 5-HT₁D; 5-HT₂A; *Evodiae Fructus*; synephrine

Goshuyuto, a traditional Japanese medicine, is composed of four medicinal herbs, *Zizyphi Fructus*, *Evodiae Fructus*, *Ginseng Radix*, and *Zingiberis Rhizoma*. Goshuyuto is used to treat migraine headache, nausea, beriberi, and heart failure. This medicine has been reported to be clinically effective in treatment of migraine headaches in Japan. Odaguchi *et al.* reported that the decrease in the number of days on which headache episodes occurred was greater in the goshuyuto group than in the placebo group (2.6±3.7 vs. 0.3±4.0 d, p=0.034), though no difference was observed with regard to the reduction in the frequency of consumption of relief medications (2.2±4.0 vs. 1.4±8.2, p=0.672).¹ They also reported that improvement in the associated symptoms was observed in more than 50% of the subjects in the goshuyuto group. In addition, autonomic nervous imbalance is implicated in chronic headache. Wakasugi *et al.* reported that goshuyuto reduced the difference in pupillary autonomic balance between the left and right eyes in patients with chronic headache.²³

It has been reported that goshuyuto increased peripheral blood flow after oral administration to normal mice at the dose of 2.0 g/kg for 7 d.¹¹ Moreover, goshuyuto was found to increase the body temperature slightly in normal rats after oral administration of 15 g/kg, and goshuyuto also prevented chlorpromazine-induced low body temperature.⁵ Goshuyuto was also reported to have dose-dependent serotonin-like contractile effects on rat stomach fundus, suggesting that goshuyuto exerts its effects on nausea and hypophagia by activating gastrointestinal motility.⁵ In addition, we elucidated that goshuyuto inhibited the platelet aggregation of guinea pigs.⁵

These results suggest that goshuyuto affects the vascular system, but there have not been any reports of pharmacological evaluation of the vascular response to goshuyuto. In the present study, we investigated the effects of goshuyuto on vascular response and its mechanism in the isolated rat aorta. Also, because goshuyuto is composed of four medicinal herbs, we examined the vascular response to aqueous extracts of each of these four medicinal herbs separately.

MATERIALS AND METHODS

Reagents and Drugs The powdered extract of goshuyuto was manufactured at our Shizuoka factory (Tsumura & Co., Tokyo, Japan). Goshuyuto is manufactured from a mixture of four medicinal herbs: 4.0 g Fructus of *Zizyphus jujube* (*Zizyphi Fructus*), 3.0 g Fructus of *Evodia rutacearcpa* (*Evodiae Fructus*), 2.0 g Radix of *Panax ginseng* (*Ginseng Radix*), and 1.5 g Rhizoma of *Zingiber officinale* (*Zingiberis Rhizoma*). The qualities of these raw materials were tested according to the Japanese Pharmacopoeia and our company’s standards. The mixture of four medicinal herbs 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% for goshuyuto. The extract was analyzed by high-performance liquid chromatography. The powdered goshuyuto extract (No. 250031010) is stored in our laboratory at constant temperature and humidity.

SB216641, a 5-hydroxytryptamine (5-HT)₁B antagonist, and BRL15572, a 5-HT₁D antagonist, were purchased from Tocris (Bristol, U.K.). Ketanserin, a 5-HT₂A antagonist, prazosin, an adrenergic α₁ antagonist, propranolol, an adrenergic β antagonist, limonin, and synephrine were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Evodiamine was purchased from Wako Pure Chemical (Osaka, Japan). Rutacarpine was purchased from LKT (MN, U.S.A.). The other reagents used for analysis were purchased from com-

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commercial sources.

Goshuyuto and the four constituent medicinal herbs were dissolved in distilled water. SB216641 and propranolol were dissolved in distilled water. BRL15572, ketanserin, prazosin, evodiamine, rutaecarpine, and synephrine were dissolved in dimethyl sulfoxide (DMSO). Final concentration of DMSO was 0.1% in the buffer solution.

**Animals** Seven- and thirteen-week-old male Wistar rats weighing 250—400 g obtained from Charles River Ltd. (Yokohama, Japan) were used. The animals were allowed free access to water and standard laboratory food (MF, Oriental Yeast, Tokyo, Japan) and kept in a facility at a temperature of 24±1 °C, relative humidity of 55±5%, and 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 (in mmol/l: NaCl 135, KCl 5.0, CaCl2 2.5, MgSO4 1.3, KH2PO4 1.2, NaHCO3 20, glucose 10, and EDTA 2Na 0.026) at pH 7.4. The aortas were cut into strips about 2.0 mm in width and 8.0 mm in length. Each 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, Japan) so that its isometric contractions could be recorded (Rika Denki Kogyo, Tokyo, Japan) via an amplifier (San-ei Instrument). The strip was equilibrated for 60 min at an initial resting tension of 2.0 g prior to the measurements of contractions.

**Measurement of Effect of Goshuyuto on Constriction of Isolated Rat Aorta Strips** Each equilibrated aorta strip was constricted by placing it in 60 mmol/l K+ solution. After 15 min, the aortas were washed with Krebs buffer three times. Then, goshuyuto at final concentrations of 3×10^{-6} to 1×10^{-3} g/ml was added to the bath in order to evaluate the vasoconstriction. The constriction strength was expressed as a percentage of the maximum tension induced by goshuyuto compared to that induced by 60 mmol/l K+ alone.

**Measurement of Effect of Evodiae Fructus on Constriction of Isolated Rat Aorta Strips** Each equilibrated aorta strip was constricted by placing it in 60 mmol/l K+ solution. After 15 min, the aortas were washed with Krebs buffer three times. Then, Evodiae Fructus at final concentrations of 1×10^{-6} to 3×10^{-4} g/ml was 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 compared to that induced by 60 mmol/l K+.

**Measurement of Effect of Ingredients of Evodiae Fructus on Relaxation of Isolated Rat Aorta Strips** Each equilibrated aorta strip was constricted by adding noradrenaline at a concentration of 1×10^{-7} mol/l in the bath. After 15 min, acetylcholine at a concentration of 1×10^{-4} mol/l was added to confirm what the endothelium of the aorta was not damaged. Then, the aortas were washed with Krebs buffer three times, and constricted by adding noradrenaline at a concentration of 1×10^{-7} mol/l again. After 15 min, each ingredient was added, and the relaxation effects were determined for 15 min. The data were expressed as a percentage of the relaxation tension induced by each ingredient compared to the maximum tension induced by 1×10^{-7} mol/l noradrenaline alone.

**Measurement of Effect of Sympetiph on Constriction of Isolated Rat Aorta Strips** Each equilibrated aorta strip was constricted by placing it in 60 mmol/l K+ solution. After 15 min, the aortas were washed with Krebs buffer three times. Then, synephrine at final concentrations of 1×10^{-7} to 3×10^{-5} mol/l was added to the bath in order to evaluate the vasoconstriction. The constriction strength was expressed as a percentage of the maximum tension induced by synephrine compared to that induced by 60 mmol/l K+.

**Statistics** Each value was expressed as the mean±S.E. 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**

Aqueous extracts of goshuyuto (3×10^{-6} to 1×10^{-3} g/ml) dose-dependently constricted the rat aorta strips, as shown in Fig. 1. The effects of each concentration were compared with the constriction induced by distilled water (12.3±1.7%, n=5). Statistically significant differences were observed at concentrations of 1×10^{-5} to 1×10^{-3} g/ml.

The effects of prazosin (adrenergic α1 antagonist) at concentrations of 1×10^{-6} to 1×10^{-5} mol/l on goshuyuto are shown in Fig. 2. The constractive curve of goshuyuto was dose-dependently shifted to the right by pretreatment with prazosin. Statistically significant differences were observed at concentrations of 3×10^{-7} g/ml of goshuyuto by prazosin (1×10^{-5}, 1×10^{-4} g/ml of goshuyuto by
Evodiae Fructus (97.6±0.9%), Ginseng Radix (19.6±1.5%), and Zingiberis Rhizoma (30.6±5.6%). However, the effects of Ginseng Radix and Zingiberis Rhizoma were considerably weak to that of Evodiae Fructus.

Moreover, Evodiae Fructus (1×10⁻⁶ to 3×10⁻⁴ g/ml) dose-dependently constricted the rat aorta strips (Fig. 3). The effects of each concentration were compared with the constriction induced by vehicle (12.3±1.7%, n=5). Statistically significant differences were observed at concentrations of 3×10⁻⁶ to 3×10⁻⁴ g/ml.

Next, we examined the effects of four ingredients of Evodiae Fructus on the aorta. Evodiamine, rutacearcalpine, and limonin relaxed the rat aorta (Table 5). The effects of each concentration were compared with the relaxation induced by vehicle (0.1% DMSO) (20.1±2.4%, n=6). The statistically
Table 4. Effects of Four Medicinal Herbs of Goshuyuto on Constriction of Isolated Rat Aorta

<table>
<thead>
<tr>
<th>Medicinal herbs</th>
<th>Constriction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3×10^{-4} g/ml</td>
</tr>
<tr>
<td>Zizyphi Fructus</td>
<td>26.4±2.5</td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>19.6±1.5*</td>
</tr>
<tr>
<td>Zingiberis Rhizoma</td>
<td>13.2±1.3</td>
</tr>
<tr>
<td>Evodiae Fructus</td>
<td>97.6±0.9**</td>
</tr>
</tbody>
</table>

Effects of Fructus of Zizyphus jujube (Zizyphi Fructus), Fructus of Evodia Rutacearpa (Evodiae Fructus), Radix of Panax ginseng (Ginseng Radix), and Rhizoma of Zingiber officinale (Zingiberis Rhizoma) were determined at doses of 3×10^{-4} g/ml and 1×10^{-3} g/ml. The constrictive strength was expressed as a percentage of the maximum tension induced by each medicinal herb to that induced by 60 mmol/l KCl. Effects of each medicinal herb at two doses on the constriction were compared with that of control (vehicle) (p<0.05, **p<0.01, Dunnett’s test). Data are expressed as mean±S.E. of three or four determinations.

Fig. 3. Effects of *Evodiae Fructus* on Construction of Isolated Rat Aorta

The constrictive strength is expressed as a percentage of the maximum tension induced by *Evodiae Fructus* to that induced by 60 mmol/l KCl. Effects of each dose of *Evodiae Fructus* on the constriction were compared with that of distilled water (p<0.05, **p<0.01, Dunnett’s test). Data are expressed as mean±S.E. from four to ten determinations.

Table 5. Effects of Ingredients of *Evodiae Fructus* on Relaxation of Isolated Rat Aorta

<table>
<thead>
<tr>
<th>Ingredient (mol/l)</th>
<th>Evodiamine (%)</th>
<th>Rutacearpine (%)</th>
<th>Limonin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3×10^{-7}</td>
<td>-11.0±3.6</td>
<td>-20.6±3.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>1×10^{-6}</td>
<td>-24.0±4.0</td>
<td>-19.0±3.4</td>
<td>-17.4±2.7</td>
</tr>
<tr>
<td>3×10^{-6}</td>
<td>-24.6±3.4</td>
<td>-34.3±8.5</td>
<td>-22.5±3.4</td>
</tr>
<tr>
<td>1×10^{-5}</td>
<td>-35.0±9.7</td>
<td>-35.8±3.5</td>
<td>-29.0±8.1</td>
</tr>
<tr>
<td>3×10^{-5}</td>
<td>-77.1±6.9**</td>
<td>-58.2±7.9**</td>
<td>-26.7±4.1</td>
</tr>
<tr>
<td>1×10^{-4}</td>
<td>N.D.</td>
<td>N.D.</td>
<td>-42.9±9.8**</td>
</tr>
</tbody>
</table>

Effects of evodiamine, and rutacearpine were determined at doses of 3×10^{-7} to 3×10^{-5} mol/l. Limonin was determined at doses of 1×10^{-8} to 1×10^{-6} mol/l. The relaxant strength was expressed as a percentage of the decreased tension induced by each dose of ingredients to the maximum tension induced by noradrenaline (1×10^{-6} mol/l). Effects of each dose of ingredients on the relaxation were compared with that of control (vehicle) (p<0.05, **p<0.01, Dunnett’s test). Data are expressed as mean±S.E. of three to five determinations. N.D. not determined.

Significant differences were observed at concentrations of 3×10^{-5} mol/l of evodiamine and rutacearpine, 1×10^{-4} mol/l of limonin. On the other hand, synephrine (1×10^{-7} to 3×10^{-5} mol/l) dose-dependently constricted the rat aorta strips (Table 6). The effects of each concentration were compared with the constriction induced by vehicle (0.1% DMSO) (6.7±1.5%, n=8). Statistically significant differences were observed at concentrations of 3×10^{-7} to 3×10^{-5} mol/l.

Table 6. Effects of Synephrine on Constriction of Isolated Rat Aorta

<table>
<thead>
<tr>
<th>Synephrine (mol/l)</th>
<th>Constriction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×10^{-7}</td>
<td>16.7±3.3</td>
</tr>
<tr>
<td>3×10^{-7}</td>
<td>24.7±7.8**</td>
</tr>
<tr>
<td>1×10^{-6}</td>
<td>56.8±9.0**</td>
</tr>
<tr>
<td>3×10^{-6}</td>
<td>70.4±6.9**</td>
</tr>
<tr>
<td>1×10^{-5}</td>
<td>86.6±5.5**</td>
</tr>
<tr>
<td>3×10^{-5}</td>
<td>88.2±0.7**</td>
</tr>
</tbody>
</table>

Effects of synephrine were determined at doses of 1×10^{-7} to 3×10^{-5} mol/l. The constrictive strength was expressed as a percentage of the maximum tension induced by each dose of synephrine to that induced by 60 mmol/l KCl. Effects of each dose of synephrine on the constriction were compared with that of control (vehicle) (**p<0.01, Dunnett’s test). Data are expressed as mean±S.E. of four to seven determinations.

DISCUSSION

In the present study undertaken to clarify the vasoconstrictive effects of goshuyuto on isolated rat aorta strips, we demonstrated that goshuyuto dose-dependently caused constriction of rat aorta strips.

In general, it is known that the adrenergic and serotonergic receptors are related to the constriction of aorta. We focused on the relation between the constrictive effects of goshuyuto and the adrenergic receptors. It is known that adrenergic \( \alpha \) receptors are related to the constriction of blood vessels.\(^{7,8}\) The constrictive effects of goshuyuto was competitively inhibited by prazosin, an adrenergic \( \alpha \) antagonist, at concentrations of 1×10^{-8} to 1×10^{-6} mol/l. Although pretreatment with propranolol, a \( \beta \) receptor antagonist, did not affect the constrictive effects of goshuyuto. It is known that the aorta strips is relaxed via adrenergic \( \beta \) receptors. These results suggest that goshuyuto may constrict the rat aorta via adrenergic \( \alpha \) receptors.

We also examined the effects of serotoninergic receptors on the constrictive effects of goshuyuto. The 5-HT receptors are currently classified into seven families, and these seven families are further classified into fourteen.\(^{9-11}\) It is generally known that 5-HT\(_{1B}\) and 5-HT\(_{1D}\) is related to the constrictive effects on vascular smooth muscle.\(^{9,12}\) Sumatriptan, which is a 5-HT\(_{1B}\)-like agonist, is used as therapy for migraine, and it is known to constrict several blood vessels.\(^{13}\) In addition, it is reported that the rat aorta is constricted in response to serotonin via activation of 5-HT\(_{3A}\).\(^{14}\) Also, it is reported that ergotamine, which is used as therapy for the aura of migraine, affect the constrictive effects of rat aorta, and that ergotamine is a partial 5-HT\(_{1A}\) receptor agonist.\(^{15}\) In our studies, the constrictive effects of goshuyuto were partially inhibited by pretreatment with BRL15572, a 5-HT\(_{1B}\) antagonist, and ketanserin, a 5-HT\(_{2A}\) antagonist. However, the constrictive effects were not inhibited by pretreatment with SB216641, a 5-HT\(_{1B}\) antagonist. Taken together, it is speculated that goshuyuto might constrict the rat aorta via serotoninergic receptors.

Moreover, 5-HT\(_{1A}\) is known to be related to anti-anxiety and antidepressive effects.\(^{16,17}\) Recently, it was reported that a 5-HT\(_{1A}\) agonist relieved migraine,\(^{18}\) but the mechanism has not been elucidated. In the present study, we tried to investigate the effects of pretreatment with 5-HT\(_{1A}\) antagonist (NAN-190) on the constrictive effects of goshuyuto, but NAN-190 antagonized not only 5-HT\(_{1A}\) receptors but also \( \alpha \) receptors in our experiments. Therefore, we did not elucidate...
the effects of goshuyuto on 5-HT$_{1A}$ receptors. Taken together, it is suggested that goshuyuto may constrict the rat aorta via adrenergic and/or serotoninergic receptors.

Goshuyuto is composed of four medicinal herbs, *Zizyphi Fructus*, *Evodiae Fructus*, *Ginseng Radix*, and *Zingiberis Rhizoma*. We examined the vasoconstrictive effects of aqueous extracts of each of the four medicinal herbs. We demonstrated that *Evodiae Fructus* strongly constricted the rat aorta. Significant differences were observed at concentrations of 3×10$^{-8}$ to 1×10$^{-3}$ g/ml. However, *Ginseng Radix* (at the dose of 3×10$^{-4}$ g/ml) showed the weak effects of constriction compared to that of *Evodiae Fructus*, and *Zizyphi Fructus* did not affect. Also, we elucidated that *Evodiae Fructus* dose-dependently constricted the rat aorta at the doses of 1×10$^{-6}$ to 3×10$^{-4}$ g/ml. Therefore, *Evodiae Fructus* is thought to play an important role in goshuyuto-induced constriction of the rat aorta strips. *Evodiae Fructus* is derived from *Evodia rutaecarpa* Bentham or *Evodia officinalis* Dode of Rutaceae, and it is reported to be effective in increasing the cerebral blood flow, breathing rate, and body temperature, and in contracting the uterus.$^{3,10,20}$ *Evodiae Fructus* contains evodiamine, dehydroevodiamine, rutacarpine, limonin, rhoetisinine, higenamine, evocarpine, and synephrine. Among the ingredients of *Evodiae Fructus*, it is reported that evodiamine, rutacarpine, dehydroevodiamine, higenamine, and evocarpine do not constrict the rat aorta but relax it.$^{21–25}$ We elucidated that evodiamine, rutacarpine, and limonin, which were ingredients of *Evodiae Fructus*, relaxed the rat aorta. These results supported the previous investigations. On the other hand, we elucidated that synephrine constricted the rat aorta. It is thought that synephrine is the important ingredient on the constrictive effects of *Evodiae Fructus*. Although most of ingredients in *Evodiae Fructus* have the relaxant effects, *Evodiae Fructus* caused constriction of the rat aorta. It is thought that synephrine may have the most potent constrictive effects in *Evodiae Fructus*, and that the other ingredient (e.g. rhoetisinine) may have the constrictive effect.

It is known that the antimigraine drugs sumatriptan and ergotamine cause vasoconstriction of several blood vessels, and that relieve migraine by constricting cerebral blood vessels.$^{13,26}$ The present study elucidated that goshuyuto constricted isolated rat aorta. Goshuyuto may relieve migraine by constricting the relaxed blood vessels like sumatriptan and ergotamine. Also, goshuyuto may constrict the aorta via adrenergic $\alpha$ receptors and/or serotoninergic (5-HT$_{1A}$ and 5-HT$_{2A}$). However, we used rat thoracic aorta in our experiments, the constrictive effects of goshuyuto on cerebral blood vessels were not demonstrated. We will examine the constrictive effects of goshuyuto on cerebral blood vessels of rabbits, dogs, or humans. It is considered that the constractive response differs among species and the sites of the blood vessels.

In conclusion, we demonstrated in this study that goshuyuto had vasoconstrictive effects on rat aorta strips, and that *Evodiae Fructus*, which was one of the constituents of goshuyuto, was involved in the vasoconstrictive effects. It may be thought that synephrine, which was one of the ingredients of *Evodiae Fructus*, was also involved in the vasoconstrictive effects. Further, the mechanism of the vasoconstrictive effects of goshuyuto involves the adrenergic and/or serotoninergic receptors.

REFERENCES