PHARMACOLOGICAL STUDIES ON GINGER. II. PRESSOR ACTION OF (6)-SHOGAOL IN ANESTHETIZED RATS, OR HINDQUARTERS, TAIL AND MESENTERIC VASCULAR BEDS OF RATS

MAMORU SUEKAWA, MASAKI ABURADA AND EIKICHI HOSOYA

Tsumura Research Institute for Pharmacology, Ami-cho, Ibaragi. 300-11, Japan

(Received May 16, 1986)

When (6)-shogaol (0.5 mg/kg, i.v.) was administered to rats, blood pressure showed a tri-phasic response which was comprised of a rapid fall, followed by a rise and a delayed fall. The rapid fall, which followed immediately after injection of (6)-shogaol, disappeared with the use of atropine and vagotomy. The marked rise, which occurred after the rapid fall, was not affected by alpha-adrenoceptor blockades, Ca antagonists and ganglion blockade. However, a combination of alpha-adrenoceptor blockade and Ca antagonist inhibited this pressor response. In hindquarters perfused with a nutrient solution, (6)-shogaol (10^-5 g)-induced peripheral pressor response was also not affected by alpha-adrenoceptor blockades and Ca antagonists, but was inhibited by the combination of an alpha-adrenoceptor blockade and a Ca antagonist. Furthermore, this peripheral pressor response was eliminated by the removal of Ca ion from the perfusate. (6)-Shogaol did not exhibit a pressor response in an artery and a vein of the tail or an artery of the femur perfused with a nutrient solution. (6)-Shogaol-induced peripheral pressor response in hindquarters was markedly potentiated during the perfusion of norepinephrine (5 x 10^-6 g/ml), but this potentiation was prevented by pretreatment with reserpine (5 mg/kg, i.p.). Moreover, repeated injections of (6)-shogaol caused a tachyphylaxis in mesenteric and tail vascular beds and a slight tachyphylaxis in hindquarters.

Keywords — (6)-shogaol; blood pressure; tri-phasic response; pressor response; Ca ion; skeletal muscle; tachyphylaxis

INTRODUCTION

As a crude drug, ginger (Zingiber officinale Roscoe) is useful in Chinese medicine. (6)-Shogaol, a pungent component of ginger, is contained in semi-dried ginger, but is rarely found in fresh ginger. In a general pharmacological study, it was found that (6)-shogaol has analgesic and antipyretic properties. In addition, it also exhibits an inhibitory effect on the central nervous system and causes a tri-phasic response in blood pressure and bradycardia in rats.1 Although (6)-shogaol causes changes in the blood pressure of rats, only few pharmacological studies have been carried out to investigate the effect of ginger (or its constituents) on blood pressure in rabbits.2 3

Doi has shown that zingerone causes a fall in blood pressure in rabbits2) and Kasahara et al. have shown that Zingiber officinale extract causes hypertension and hypotension in rats.3) The mechanism by which ginger induces these changes is unclear. This study is aimed to assay changes in blood pressure induced by (6)-shogaol in rats. In order to gain further insight into the pressor response of blood pressure, the effects of various drugs (calcium entry blockers, alpha-adrenoceptor blockades, etc.) on (6)-shogaol-induced pressor response in anesthetized rats were evaluated, and in particular, the effect of (6)-shogaol on the perfused tail and the isolated vessels of the femur and tail was determined.

MATERIALS AND METHODS

In this experiment, (6)-shogaol was suspended in 2% tween 80.

Drugs — Phentolamine mesylate (CIBA), hexamethonium bromide (Tokyo Kasei), prazosin hydrochloride (Taito Pfizer), yohimbine hydrochloride (Sigma), propranolol hydrochloride (Sumimoto Kagaku), diltiazem hydrochloride (Tanabe), indomethacin (Sigma), atropine sulfate (Wako), norepinephrine (Sankyo), lysin vasopressine (Sigma), tetracain hydrochloride (Kyorin), tolazoline (Yamanouchi), verapamil hydrochloride (Tanabe) and reserpine (Daichi) were used in this study.

Animals — Male Wistar strain rats were purchased from Charles River Japan Inc. In all experiments, rats weighing 300–400 g were used.

Effects of Various Drugs on the Changes of Blood Pressure Induced by (6)-Shogaol in Anesthe-

tized Rats — Rats were anesthetized intraperitoneally (i.p.) with sodium pentobarbital (50 mg/kg). Blood pressure was recorded from a cannula inserted through the femoral artery by means of a pressure transducer (Nihon Kohden: MPU-0.5). Heart rate was continuously counted with a tachometer (Nihon Kohden: AT-600 G) triggered by pressure pulses. Recordings of these parameters were made on a minipoly (Nihon Kohden: RM-6100). Drugs were administered through a cannula inserted into the femoral vein. If required, a vagotomy was also performed.

Effects of Various Drugs on Pressor Response Induced by (6)-Shogaol in Hindquarters Vascular Beds — After being anesthetized with sodium pentobarbital (50 mg/kg, i.p.), the aorta and vena cava were exposed by an abdominal incision. The aorta was cannulated toward the hindquarters with a polyethylene tube just below the left kidney, and the vena cava was sectioned to allow drainage of the perfusion fluid. The hindquarters vascular beds were perfused with aerated (95% O₂, 5% CO₂) Krebs-Henseleit solution at 37 °C, at a constant flow of 2 ml/min using a peristaltic minipump (Ato: 1220). After the perfusion was initiated, cardiac arrest was induced by cardiac puncture of sodium pentobarbital. Perfusion pressure was measured from side-arm in the inflow tubing connected to a pressure transducer (Nihon Kohden: MPU-0.5), and recorded on a minipoly (Nihon Kohden: RM-6100). (6)-Shogaol was injected with a micro-syringe directly into the perfusion circuit through a rubber tube connecting the transducer and the cannula and various drugs were added to the perfusate.

Effect of (6)-Shogaol on Femoral Artery Perfused with Nutrient Solution — After being anesthetized with sodium pentobarbital, the rat aorta was exposed by an abdominal incision. A cannula was introduced through the abdominal aorta to the vicinity of the external iliac arteries. As shown in Fig. 1, all branches up to the muscle branch and all other arteries were ligated. Moreover, the femoral vein was also cannulated centrally. In order to drain the perfusion fluid, the femoral artery was sectioned at the muscle branch. The femoral artery was perfused with aerated (95% O₂, 5% CO₂) Krebs-Henseleit solution at 37 °C, at a constant flow of 1 ml/min using a peristaltic minipump. Moreover, the hindquarters in another rat was perfused with nutrient solution at a constant flow of 2 ml/min, and the vena cava was sectioned to allow drainage of the perfusion fluid. Perfusion pressure was measured from a side-arm in the inflow tubing connected to a pressure transducer and was recorded on a minipoly. In both preparations, pressor responses were induced by injection of noradrenaline (10⁻⁵ g) and vasopressin (0.02 IU). (6)-Shogaol was added to the perfusate and perfused simultaneously in the preparations.

Effect of (6)-Shogaol on the Perfused Artery, Vein and Vascular Beds of Tail — After being anesthetized with sodium pentobarbital, about 2 cm of the tail artery near the proximal part was exposed, and the artery cannulated peripherally. The tail veins were sectioned to allow drainage of the perfusion fluid. After the perfusion was initiated, cardiac arrest was induced by a cardiac puncture with sodium pentobarbital. On the other hand, in some experiments, the tail was sectioned. Here, the proximal half of the tail artery was exposed and all collateral vessels were ligated. A cannula was peripherally introduced into the artery. Peripheral site of the artery was sectioned for drainage of the perfusate. Then, a vein was also cannulated centrally at the midpoint of the tail.

FIG. 1. The Shrama of Hindquarter's Artery in Rat Perfused with Nutrient Solution
1, cannula; 2, aorta; 3, external iliac artery; 4, femoral artery; 5, muscle branch; 6, ligation.
Three preparations, namely an artery, a vein and vascular beds of the tail were perfused with nutrient solution at 37 °C via peristaltic minipump at a constant flow of 1 ml/min. Pressure responses were measured with a pressure transducer from a side-arm in the inflow tubing and (6)-shogaol was directly injected with a micro syringe (10 μl) into the perfusion circuit through a rubber tube connecting the transducer and a cannula. In all preparations, a pressor response was induced using norepinephrine (10⁻⁶ g).

**Effect of (6)-Shogaol on Pressor Response Induced by Electrical Stimulation of the Perfused Tail Artery** — The experiment was performed according to Chevillard's method.⁴ After a blow to the head of the rat, a proximal part (about 3 cm) of the tail artery was excised after all collateral vessels were ligated and cannulated at both ends. The artery was mounted between bipolar circular platinum electrodes and perfused with aerated Krebs-Henseleit solution at 37 °C, at a constant flow (0.8 ml/min) using a peristaltic minipump. Moreover, this preparation was superfused in a bath (5 ml) with the same nutrient solution (a constant flow: 1 ml/min). Perfusion pressure was measured from a side-arm in the inflow tubing connected to a pressure transducer and recorded on a minipoly. The intramural sympathetic nerves were stimulated at supramaximal voltage for 40 s with pulses of 2 ms duration at 10 Hz. The intervals between stimulations were 10 min. (6)-Shogaol was either directly injected into the perfusion circuit through a rubber tube or added to the perfusate or the bath.

**Effect of (6)-Shogaol on an Isolated Artery in Tail** — After a fatal blow was given to the head, the proximal part (about 3 cm) of the caudal artery was excised and cut helically. The artery was mounted in a 20 ml organ bath (37 °C) containing Krebs solution which was aerated by gas (95% O₂, 5% CO₂) and measured isometrically with a Force-Displacement transducer (San-ei Sokki: 45196A).

**Effect of (6)-Shogaol on a Portal Vein** — After a fatal blow was given to the head, a portal vein was excised. The portal vein was cut helically and mounted in a 20 ml organ bath (37 °C) containing Krebs-Henseleit solution which was aerated by gas (95% O₂, 5% CO₂). Spontaneous contractile responses of the portal vein were isometrically measured with a Force-Displacement transducer.

**Effect of Repeated Injections of (6)-Shogaol on a Mesenteric, a Tail and Hindquarters Vascular Beds** — After being anesthetized with sodium pentobarbital, the superior mesenteric artery was exposed by an abdominal incision. A cannula was then introduced into the artery. The abdominal aorta and tail artery were also cannulated. These three vascular beds were perfused with a nutrient solution, in the same way as in the method described above. (6)-Shogaol was injected repeatedly into these vascular beds.

The components of the nutrient solutions used in the study were as follows:

- Krebs-Henseleit solution (g/l): NaCl 6.9, KCl 0.35, CaCl₂ 0.19, MgSO₄ 0.15, KH₂PO₄ 0.14, NaHCO₃ 2.1, glucose 1.1
- Krebs solution (g/l): NaCl 6.8, KCl 0.33, MgSO₄ 0.12, KH₂PO₄ 0.14, NaHCO₃ 2.1, glucose 1.1

**Statistics** — The results obtained were expressed as the mean ± S.E., and the statistical significance was assessed using Student's t-test or paired t-test.

**RESULTS**

**Effect of Atropine and Vagotomy on a Rapid Fall in Blood Pressure Induced by (6)-Shogaol in Anesthetized Rats**

When (6)-shogaol (0.5 mg/kg, i.v.) was administered to rats, a rapid fall in blood pressure resulted just after its administration. This rapid fall disappeared by the administration of atropine and by vagotomy (Fig. 2). Furthermore, it was noted that atropine and vagotomy also caused bradycardia induced by (6)-shogaol to disappear.

**Effects of Various Drugs on Pressor Response of Blood Pressure Induced by (6)-Shogaol in Anesthetized Rats**

(6)-Shogaol (0.5 mg/kg, i.v.) caused a marked pressor response after a rapid fall in blood pressure in rats. We investigated the effects of various drugs on (6)-shogaol-induced pressor response. It was found that (6)-shogaol-induced pressor response was slightly reduced by phentolamine (5 mg/kg), prazosin (1 mg/kg), verapamil (1 mg/kg), diltiazem (10 mg/kg), hexamethonium (10 mg/kg) and propranolol (1 mg/kg), and was slightly increased by atropine (10 mg/kg) and yohimbine (1 mg/kg). Indomethacin (1 mg/kg) had no effect on this pressor...
response due to (6)-shogaol. Moreover, the effects of a combined treatment of drugs which slightly inhibited this pressor response were examined. Combinations of phenolamine and propranolol, hexamethonium and diltiazem, or phenolamine and verapamil showed a marked inhibition on the pressor response induced by (6)-shogaol (Table I).

**Effects of Various Drugs on (6)-Shogaol-Induced Pressor Response in Perfused Hindquarters**

When (6)-shogaol (10^{-5} g) was injected into the vascular beds of hindquarters which had been perfused with a nutrient solution, a pressor response was caused in the perfusion pressure. However, due to the repeated injection of (6)-shogaol, (6)-shogaol-induced pressor response gradually decreased. Therefore, the effects of various drugs on (6)-shogaol-induced pressor response were assessed using the method shown in Fig. 3. (6)-Shogaol-induced peripheral pressor response in hindquarters was virtually unaffected by phenolamine (10^{-5} g), prazosin (10^{-5} g), yohimbine (10^{-5} g), tolazolin (10^{-5} g), propranolol (10^{-5} g), tetracain (10^{-5} g) and indomethacin (10^{-5} g). However, this pressor response was inhibited by combinations of phenolamine (10^{-5} g) and diltiazem (10^{-5} g) or propranolol (10^{-5} g) and tetracain (10^{-5} g), but not by a combination of diltiazem (10^{-5} g) and verapamil (10^{-5} g). Moreover, this pressor response was markedly inhibited or eliminated by perfusion fluid containing zero Ca and 1 mM ethylenediamine tetraacetic acid (Table II).

In other experiments, it was demonstrated that when a nutrient solution containing norepinephrine (10^{-6} g/ml) was perfused into hind-

---

**TABLE I. Effects of Various Drug’s Treatments on Pressor Response to (6)-Shogaol in Rats**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Treatment (Δ mmHg) Pre.</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolamine</td>
<td>5</td>
<td>5</td>
<td>53.8 ± 5.3</td>
<td>46.6 ± 7.5</td>
</tr>
<tr>
<td>Propranolol</td>
<td>1</td>
<td>4</td>
<td>44.4 ± 4.1</td>
<td>32.8 ± 2.4</td>
</tr>
<tr>
<td>Verapamil</td>
<td>1</td>
<td>4</td>
<td>40.6 ± 4.6</td>
<td>34.4 ± 3.4</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>10</td>
<td>4</td>
<td>56.6 ± 5.7</td>
<td>38.6 ± 6.5</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>10</td>
<td>6</td>
<td>59.2 ± 4.9</td>
<td>45.8 ± 3.5</td>
</tr>
<tr>
<td>Prazosin</td>
<td>1</td>
<td>4</td>
<td>51.0 ± 2.7</td>
<td>42.0 ± 6.4</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>1</td>
<td>4</td>
<td>39.4 ± 4.3</td>
<td>49.4 ± 2.1</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1</td>
<td>5</td>
<td>53.2 ± 3.9</td>
<td>53.6 ± 4.1</td>
</tr>
<tr>
<td>Atropine</td>
<td>10</td>
<td>4</td>
<td>62.6 ± 3.7</td>
<td>69.6 ± 7.3</td>
</tr>
<tr>
<td>Phenolamine + propranolol</td>
<td>5, 1</td>
<td>6</td>
<td>50.0 ± 7.0</td>
<td>21.6 ± 2.1 a)</td>
</tr>
<tr>
<td>Hexamethonium + diltiazem</td>
<td>10, 10</td>
<td>4</td>
<td>65.6 ± 4.8</td>
<td>27.6 ± 5.1 a)</td>
</tr>
<tr>
<td>Phenolamine + verapamil</td>
<td>5, 1</td>
<td>5</td>
<td>61.6 ± 3.5</td>
<td>39.0 ± 5.3 a)</td>
</tr>
</tbody>
</table>

a) p < 0.01, compared with Pre.
### TABLE II. Effects of Various Drug’s Treatments on (6)-Shogaol-Induced Pressor Response in Hindquarters Vascular Beds of Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>N</th>
<th>Pressor response(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(6)-Shogaol alone</td>
<td>$10^{-6}$ g</td>
<td>10</td>
<td>87.1 ± 1.8</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>$10^{-5}$ g/ml</td>
<td>5</td>
<td>84.7 ± 5.5</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>$10^{-5}$ g/ml</td>
<td>4</td>
<td>86.7 ± 3.1</td>
</tr>
<tr>
<td>Prazosin</td>
<td>$10^{-5}$ g/ml</td>
<td>5</td>
<td>84.8 ± 10.3</td>
</tr>
<tr>
<td>Tolazoline</td>
<td>$10^{-5}$ g/ml</td>
<td>7</td>
<td>89.5 ± 4.3</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>$10^{-5}$ g/ml</td>
<td>7</td>
<td>87.5 ± 2.7</td>
</tr>
<tr>
<td>Propanolol</td>
<td>$10^{-5}$ g/ml</td>
<td>6</td>
<td>81.6 ± 4.3</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>$10^{-5}$ g/ml</td>
<td>5</td>
<td>89.4 ± 4.3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>$10^{-5}$ g/ml</td>
<td>4</td>
<td>95.3 ± 4.7</td>
</tr>
<tr>
<td>Phentolamine + diltiazem</td>
<td>$10^{-5}$, $10^{-5}$ g/ml</td>
<td>4</td>
<td>71.2 ± 3.5&lt;sup&gt;a)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yohimbine + tolazoline</td>
<td>$10^{-5}$, $10^{-5}$ g/ml</td>
<td>4</td>
<td>77.8 ± 3.2</td>
</tr>
<tr>
<td>Propanolol + diltiazem</td>
<td>$10^{-5}$, $10^{-5}$ g/ml</td>
<td>4</td>
<td>48.3 ± 6.3&lt;sup&gt;a)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Propanolol + tetracaine</td>
<td>$10^{-5}$, $10^{-5}$ g/ml</td>
<td>4</td>
<td>68.2 ± 2.9&lt;sup&gt;a)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diltiazem + tetracaine</td>
<td>$10^{-5}$, $10^{-5}$ g/ml</td>
<td>4</td>
<td>85.1 ± 2.0</td>
</tr>
<tr>
<td>Diltiazem + verapamil</td>
<td>$10^{-5}$, $10^{-5}$ g/ml</td>
<td>5</td>
<td>85.3 ± 7.2</td>
</tr>
<tr>
<td>Ca-free (1 mM EDTA)</td>
<td></td>
<td>8</td>
<td>11.6 ± 3.1&lt;sup&gt;a)&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Perfusion pressors are calculated as shown Fig. 3, and showed as means ± S. E. a) p < 0.05, compared with (6)-shogaol alone.

quartetrs, the pressor response induced by (6)-shogaol ($5 \times 10^{-6}$ g) was markedly potentiated. Conversely, when a nutrient solution containing (6)-shogaol ($10^{-5}$ g/ml) was perfused into the hindquarters vascular beds, norepinephrine ($10^{-6}$ g)-induced pressor response was inhibited (Fig. 4). Moreover, reserpine (5 mg/kg, i.p.) was found to prevent the potentiation of the pressor response of (6)-shogaol (Fig. 5).

**Effect of (6)-Shogaol on the Femoral Artery and Vascular Beds of Hindquarters**

Norepinephrine ($10^{-5}$ g) and vasopressin (0.02 IU) exhibited pressor responses in both preparations. When the nutrient solution containing (6)-shogaol ($10^{-5}$ g/ml) was simultaneously perfused into these preparations, (6)-shogaol showed a pressor response on the perfusion pressures of the hindquarters, but not on the perfusion pressure of the femoral artery (Fig. 6).

**Effect of (6)-Shogaol on an Artery, a Vein and Vascular Beds of the Tail**

Norepinephrine ($10^{-6}$ g) showed a pressor response in all three preparations of the tail. (6)-Shogaol ($10^{-6}$ to $10^{-4}$ g) exhibited a pressor response on the vascular beds of the tail in a dose-dependent manner. However, (6)-shogaol did not cause a pressor response in either of the artery or the vein in the tail (Fig. 7).

**Effects of (6)-Shogaol on the Perfused and Superfused Tail Artery**

When norepinephrine ($10^{-6}$ g) and KCl ($5 \times 10^{-6}$ g) were injected into the perfusion circuit, and when field stimulation was done, the isolated tail artery, which was perfused and superfused with nutrient solution, exhibited a pressor response. However, (6)-shogaol ($10^{-8}$ to $10^{-4}$ g), which was injected into the perfusion circuit, had no effect on this preparation (Fig. 8).

Moreover, the effects of (6)-shogaol on vaso-

---

**FIG. 3. Effects of Various Drug’s Treatment on (6)-Shogaol-Induced Peripheral Pressor Response in Hindquarters Vascular Beds of Rat**

(6)-Shogaol-induced pressor response in normal solution represents (6)-shogaol alone in Table II.
FIG. 4. Effects of Norepinephrine on (6)-Shogaol-Induced Peripheral Pressor Response in Hindquarters of Rats

(A) Pressor response of (6)-shogaol \((5 \times 10^{-6} \text{ g})\) in hindquarters of rats perfused with norepinephrine \((10^{-6} \text{ g/ml})\). \((n = 8)\). (B) Pressor response of norepinephrine \((5 \times 10^{-7} \text{ g})\) in hindquarters of rats perfused with (6)-shogaol \((10^{-5} \text{ g/ml})\). \((n = 7)\).

\(a, p < 0.01; b, p < 0.05; c, p < 0.05; d, p < 0.001\).

FIG. 5. Effect of Norepinephrine on (6)-Shogaol-Induced Pressor Response in Hindquarters of Rat Pretreated with Reserpine

P.P., perfusion pressure.
FIG. 6. Effect of (6)-Shogaol on Perfusion Pressure in Femoral Artery and Hindquarters Vascular Beds of Rat Perfused with Nutrient Solution
(A) femoral artery; (B) hindquarters vascular beds. P.P., perfusion pressure.

FIG. 7. Effect of (6)-Shogaol on Perfusion Pressure of Vascular Beds, Artery and Vein in Rat Tails
I, vascular beds; II, alone artery; III, alone vein.

- norepinephrine \(10^{-6}\) g;
- (6)-shogaol \(10^{-6}\) g;
- (6)-shogaol \(10^{-5}\) g;
- (6)-shogaol \(10^{-4}\) g.

Constrictor responses induced by field stimulation were also examined. When nutrient solution containing (6)-shogaol \(10^{-5}\) g/ml was intraluminally perfused, vasoconstrictor responses induced by field stimulation were inhibited, but were restored by perfusion of (6)-shogaol-free solution (Fig. 9). Similarly, vasoconstrictor responses induced by field stimulation were also inhibited by injection of (6)-shogaol \(10^{-6}\) to \(10^{-4}\) g into the superfused stream (Fig. 10).

Thus, while (6)-shogaol did not show a pressor response in the perfused and superfused tail artery, it inhibited vasoconstrictor responses induced by field stimulation.

Effects of (6)-Shogaol on the Helical Strips of the Isolated Tail Artery

(6)-Shogaol \(10^{-6}\) g also showed no effect on the helical strips of the isolated tail artery. On contractile responses induced by KCl (75 mM) and norepinephrine \(10^{-6}\) M, the vehicle showed a slight inhibition to phasic and tonic contractions induced by KCl and tonic contraction induced by norepinephrine, and (6)-shogaol \(10^{-5}\) g/ml showed a significant inhibition to phasic and tonic contractions induced by both KCl and norepinephrine (Table III).

Effects of (6)-Shogaol on Spontaneous Contractile Responses of a Portal Vein

(6)-Shogaol increased the spontaneous contractile responses of a portal vein at \(10^{-6}\) to \(10^{-6}\) g/ml in a concentration-dependent manner but inhibited the contractile responses at \(10^{-5}\) g/ml (Table IV).

Effects of Repeated Injections of (6)-Shogaol on a Mesenteric, a Tail and Hindquarters Vascular Beds

When (6)-shogaol \(10^{-6}\) g was repeatedly injected into the mesenteric, tail and hindquarters vascular beds, tachyphylaxis was observed in the mesenteric and tail vascular beds. However, (6)-shogaol exhibited only a slight tachyphylaxis on hindquarters vascular beds and a marked tachyphylaxis in the mesenteric vascular beds (Fig. 11).
**Fig. 8.** Effect of (6)-Shogaol on Perfusion Pressure in the Isolated Tail Artery of Rat Perfused and Superfused with Nutrient Solution

Drugs were injected into the perfusion circuit.

ES, electrical stimulation.

**Fig. 9.** Effect of (6)-Shogaol on Pressor Response in the Isolated Tail Artery of Rat Induced by Electrical Stimulation

(6)-Shogaol was administered into the intra-artery.

ES, electrical stimulation; P.P., perfusion pressure.

**Fig. 10.** Effect of (6)-Shogaol on Pressor Response in the Isolated Artery of Rat Induced by Electrical Stimulation

(6)-Shogaol was administered into the perfused bath.

E.S., electrical stimulation; P.P., perfusion pressure.
TABLE III. Effects of (6)-Shogaol on Contractions of Artery Strips in Rat Tail Induced by KCl and Norepinephrine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vehicle</th>
<th>KCl (75 mM) (6)-Shogaol 10⁻⁸ g/ml</th>
<th>Vehicle</th>
<th>Norepinephrine (10⁻⁶ M) (6)-Shogaol 10⁻⁸ g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>100</td>
<td>89.3±1.87</td>
<td>47.2±7.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.4±3.11</td>
<td>48.0±6.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 min after</td>
<td>100</td>
<td>86.0±3.05</td>
<td>53.2±10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.9±13.0</td>
<td>31.3±14.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents a change of per cent, as the mean ± S. E.  a) p < 0.05.

TABLE IV. Effects of (6)-Shogaol on Spontaneous Contraction of the Isolated Rat Portal Vein

<table>
<thead>
<tr>
<th>Concentration (g/ml)</th>
<th>(6)-Shogaol (%)</th>
<th>Isoproterenol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td></td>
<td>91.0±4.63</td>
</tr>
<tr>
<td>10⁻⁹</td>
<td></td>
<td>65.3±8.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>122.4±2.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.3±3.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>127.3±6.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>143.5±9.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>44.9±10.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are represented as mean ± S.E. (n = 4).  a) Significantly different from control, p < 0.05.

FIG. 11. Effect of Repeated Injections of (6)-Shogaol on Perfusion Pressure of Hindquarters, Tail and Mesenteric Vascular Beds in Rat

P.P., perfusion pressure; S, (6)-shogaol 10⁻⁵ g.

DISCUSSION

In anesthetized rats, (6)-shogaol produced a tri-phasic response in blood pressure and bradycardia. The tri-phasic response was comprised of a rapid fall, followed by a rise and a delayed fall. The rapid fall in blood pressure and bradycardia, which resulted immediately after the administration of (6)-shogaol, disappeared with the use
of atropine or vagotomy. Accordingly, it seems that the rapid fall in blood pressure and bradycardia was caused by vagal stimulating effect. (6)-Shogaol-induced pressor response, after a rapid fall in blood pressure, was only slightly inhibited when treated with either phentolamine, prazosin, hexamethonium, propranolol, diltiazem or verapamil, but not with indomethacin. Thus, the marked pressor response induced by (6)-shogaol was only slightly inhibited when treated with one of the various blockades. However, when treated with a combination of the blockades which displayed a slight inhibitory effect, the pressor response by (6)-shogaol was reduced significantly. This suggested that (6)-shogaol-induced pressor response on blood pressure may be caused by a complicated mechanism.

In order to elucidate the mechanism of the pressor response caused by (6)-shogaol, the effects of (6)-shogaol on the hindquarters and tail vascular beds were examined. It was found that (6)-shogaol caused a peripheral pressor response on perfusion pressure of hindquarters perfused with nutrient solution. While drugs such as phentolamine, prazosin, tolazolin, yohimbine, diltiazem, propranolol and tetracain did not have any inhibitory effect on this pressor response induced by (6)-shogaol, the combination of phentolamine and diltiazem, or propranolol and diltiazem did inhibit this peripheral pressor response. Moreover, this peripheral pressor response disappeared when the Ca ion was eliminated from the perfusate but persisted when treated with a combination of diltiazem and verapamil. These results indicate that (6)-shogaol may cause a peripheral pressor response due to its non-adrenergic effect and that (6)-shogaol-induced pressor response may not be caused by the effect of voltage-dependent Ca influx into the vascular smooth muscle, despite dependence on extracellular Ca ion. Recently, it has been shown that Ca antagonists inhibited the contractile response induced by alpha-2 adrenoceptor activation but not by alpha-1 adrenoceptor activation and that gamma-adrenoreceptors resisted the effect of phentolamine. Although (6)-shogaol-induced pressor response was not affected by Ca antagonists and phentolamine, it is unlikely that it is caused by the activation of alpha-1, of gamma-adrenoreceptor. This is because, with the exception of portal veins, (6)-shogaol has no effect on vascular smooth muscle, as described later.

Although (6)-shogaol caused a peripheral pressor response in the hindquarters and tail vascular beds, the artery and vein of the tail, or the artery of the femur were virtually unaffected by (6)-shogaol. Conversely, (6)-shogaol displayed an inhibitory effect on contractile responses of the tail artery induced by KCl, norepinephrine or electrical stimulation. Yamaguchi et al. have shown that the pressor effect of exogenous catecholamine was predominantly mediated by alpha-2 adrenoceptor whereas a response by sympathetic stimulation was due to the activation of alpha-1 adrenoceptors in the region of the vascular neuro-effector junctions. It is also known that norepinephrine-induced phasic component of the mesenteric artery in rats is due to intracellular Ca release and that the tonic component is completely dependent on Ca influx. Similarly, KCl-induced contraction is also caused by Ca-influx. Accordingly, the results obtained in the present experiments suggest that (6)-shogaol-induced peripheral pressor response may not be produced by direct effect on vascular smooth muscle, but that (6)-shogaol may in fact cause a relaxant effect on the vascular smooth muscle due to the inhibition of Ca influx.

If (6)-shogaol does not result in the contraction of vascular smooth muscle, what kind of actions are caused by (6)-shogaol-induced peripheral response? The peripheral pressor response induced by (6)-shogaol was the result of the well connected the artery and the vein. Furthermore, changes in perfusion pressure in hindquarters reflect changes in the caliber of resistant arterioles. Therefore, we assumed that (6)-shogaol might have an effect on the resistant vessels. It is known that an arteriole has many independent pacemakers which induce rhythmic contraction. This phenomenon is also evident in portal veins. Since the structure of a portal vein is similar to that of an arteriole, portal veins have been used as experimental models for resistant vessels. In portal veins in rats, (6)-shogaol enhanced spontaneous contractile responses at 10^-8 to 10^-6 g/ml in a concentration-dependent manner, but were inhibited at 10^-5 g/ml. These results indicated that (6)-shogaol may cause a peripheral pressor response due to its action on the resistance vessel system. However, a high concentration of (6)-shogaol which inhibited

NII-Electronic Library Service
contractile responses of the portal vein caused a presor response in the hindquarters perfused with a nutrient solution. Thus, although (6)-shogaol may cause a peripheral presor response by its effect on resistance vessels, (6)-shogaol-induced peripheral presor response may also be caused by an another factor.

(6)-Shogaol-induced peripheral presor response in the hindquarters was potentiated markedly when perfused with norepinephrine. However, while catecholamine depletion by reserpine supressed the potentiation, (6)-shogaol-induced presor response was not eliminated by reserpine. This suggests that the peripheral presor response of (6)-shogaol potentiared by norepinephrine may due to the activation of alpha-adrenergic nerves, and that (6)-shogaol-induced peripheral presor response may be concerned with adrenergic nerve ends.

When (6)-shogaol was administered repeatedely to the hindquarters, tail and mesenteric vascular beds of rats, tachyphylaxis was observed in the mesenteric and tail vascular beds. However, (6)-shogaol exhibited only a sligh tachyphylaxis on the hindquarters vascular beds.

From the results described above, we supposed that (6)-shogaol may cause a peripheral presor response by releasing an unknown active substance from nerve ends via a Ca channel which is not affected by Ca antagonists such as diltiazem and verapamil and that the action site of the active substance may be located on resistance vessels of skeletal muscle vascular beds. This will be the focus of our future investigation.

REFERENCES