Endothelium-Dependent Potentiation of Prostaglandin $F_{2\alpha}$ -Induced Contractions by $(\pm)$ -[6]-Gingerol Is Inhibited by Cyclooxygenase- but Not Lipoxygenase-Inhibitors in Mouse Mesenteric Veins

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The mechanism of potentiation of prostaglandin (PG) $F_{2\alpha}$ -induced contraction of mouse mesenteric veins by $(\pm)$ -[6]-gingerol was investigated *in vitro*. $(\pm)$ -[6]-Gingerol (0.3 mM) potentiated the maximal contraction response elicited by $PGF_{2\alpha}$ (0.28 mM) in the presence of intact vascular endothelium, but not in its absence (de-endothelialized preparations). The potentiating effect was completely inhibited by cyclooxygenase inhibitors (0.2 mM aspirin and 0.2 mM indomethacin) and partly by calcium antagonists (2 μ M verapamil, 8 nM nitrendipine and 1 μ M ryanodine), but not inhibited by nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor and ONO-3708, a thromboxane (TX) A_2 antagonist. The potentiation by $(\pm)$ -[6]-gingerol is also observed in mesenteric veins of streptozotocin-diabetic mice where the enhancement of $PGF_{2\alpha}$ -induced contraction is caused mainly by activation of lipoxygenase. The potentiation of $PGF_{2\alpha}$ -induced contraction by $(\pm)$ -[6]-gingerol may be caused by a cyclooxygenase-dependent release of vasoconstrictors, other than $PGF_{2\alpha}$ and TXA_2 , or by inhibiting vasorelaxants released from endothelial cells of mouse mesenteric veins.

Key words $(\pm)$ -[6]-gingerol; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced contraction; endothelium; mouse mesenteric vein

Gingerols are the pungent constituents of the rhizomes of crude ginger, *Zingiber officinale* ROSCOE (a traditional Japanese Sino-medicine, a Kampo medicine). Gingerols exhibit various pharmacological actions in the cardiovascular system,^{1,2)} positive inotropic effects on isolated guinea pig atria,³⁾ and potent anti-platelet aggregating effects in human plasma.⁴⁾ We have previously demonstrated that $(\pm)$ -[6]-gingerol potentiates prostaglandin (PG) $F_{2\alpha}$, PGE_2 and PGI_2 -induced contractions and inhibits PGD_2 , thromboxane (TX) A_2 and leukotriene (LT)-induced contractions in the mesenteric veins of mice.⁵⁾ Furthermore, we have reported that $PGF_{2\alpha}$ -induced contraction of mesenteric veins in diabetic mice is enhanced in an endothelium-dependent manner, mainly caused by the activation of lipoxygenase.^{6,7)} In the present study, we investigated the mechanism of potentiation by $(\pm)$ -[6]-gingerol on $PGF_{2\alpha}$ -induced contractions of mouse mesenteric veins. We also investigated whether $(\pm)$ -[6]-gingerol potentiated the $PGF_{2\alpha}$ contraction enhanced in streptozotocin (STZ)-diabetic mice where the lipoxygenase pathway is mainly activated.

MATERIALS AND METHODS

Animals and Preparation Male ddY mice (7–10 weeks old, 28–40 g) and STZ-diabetic ddY male mice (10 weeks old, 31–37 g) were used. Mice, 4 weeks old, were injected with 150 mg/kg of STZ into the tail vein, and were used 6 weeks after injection. The blood glucose levels of STZ-diabetic mice were higher than 150 mg/dl after fasting for 12–14 h before each experiment. The mesenteric vein was isolated rapidly, after killing by decapitation under light ether anesthesia, and adhering fat and connective tissues removed, as described previously.⁵⁾ A longitudinal segment (2–3 mm below the portal vein, 10 mm long) was prepared. The segment was tied at both ends with silk threads and suspended in an organ bath (5 ml) filled with Krebs solution (37 °C): 122 mM NaCl, 5.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM

$MgCl_2$, 15.5 mM $NaHCO_3$ and 11.5 mM glucose. The solution was continuously gassed with 95% O_2 and 5% CO_2 . One end of the preparation was tied to the base of the tissue bath, while the other end was connected to a transducer (U-gauge, type UL-2-240; Minebea, Nagano). The tissue was allowed to equilibrate for 30–60 min, loaded with a passive tension of 95 mg, and developed an optically active tension when challenged with $PGF_{2\alpha}$. Changes in isometric contraction were recorded by a pen-recorder (Recti-Horiz-8K; NEC-San-ei, Tokyo) connected to an amplifier (6L5, NEC-San-ei) and a biophysigraph 180 system (NEC-San-ei). A single dose of $PGF_{2\alpha}$ (0.28 mM) was applied to the organ bath while $(\pm)$ -[6]-gingerol (0.3 mM) was applied to the bath 5 min before the application of $PGF_{2\alpha}$. After washing out and recovery, the preparation was again challenged with $PGF_{2\alpha}$ alone. The pre- and post-control responses were averaged to give the control response. The change in $PGF_{2\alpha}$ responses produced by $(\pm)$ -[6]-gingerol in STZ-diabetic mice was compared with that in age-matched normal mice.

Drugs The following compounds were used: $(\pm)$ -[6]-gingerol (a gift from Professor T. Shioiri, Dept. of Chemistry, Nagoya City University), $PGF_{2\alpha}$ (Kaken Seiyaku, Kyoto), verapamil (Knoll, Ludwigshafen, Germany), nitrendipine (Yoshitomi Seiyaku, Osaka), ryanodine and aspirin (Wako Pure Chemical, Osaka), a TXA_2 antagonist ONO-3708 (Ono Pharmaceutical Co., Ltd., Osaka),⁸⁾ STZ, indomethacin and nordihydroguaiaretic acid (NDGA) (Sigma, St. Louis, MO, U.S.A.). $(\pm)$ -[6]-Gingerol and $PGF_{2\alpha}$ were dissolved in distilled water following brief sonication. Nitrendipine was dissolved in 20% *N,N*-dimethylformamide, while indomethacin and NDGA were dissolved in 0.15% ethanol following brief sonication; aspirin was dissolved in distilled water.

Data Analysis The contractile responses were expressed as changes in the absolute tension in mg, and the maximal contraction elicited by $PGF_{2\alpha}$ (0.28 mM) was taken as 100%. Statistical analyses were performed using a paired or unpaired Student's *t*-test.

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Table 1. Lack of Potentiating Effect of ($\pm$)-[6]-Gingerol (0.3 mM) on PGF_{2 α} (0.28 mM)-Induced Contractions by Removing the Endothelial Cells of Mouse Mesenteric Veins

($\pm$)-[6]-Gingerol	mg tension (% contraction)	
	Normal	De-endothelialized
–	6.6 $\pm$ 0.1 (100)	7.1 $\pm$ 1.4 (100)
+	8.4 $\pm$ 0.3 (128 $\pm$ 4)	7.3 $\pm$ 1.5 (102 $\pm$ 1)

The values are the mean $\pm$ S.E.M. ($n=3-12$). * $p<0.05$; significance of difference determined by paired Student's t -test. N.S.; not significant.

Table 2. Inhibitory Effects of Various Calcium Antagonists on the Potentiation of PGF_{2 α} -Induced Contractions by ($\pm$)-[6]-Gingerol in Mouse Mesenteric Veins

Compounds	Concentration of calcium antagonist (μ M)	% potentiation
($\pm$)-[6]-Gingerol (0.3 mM)		30 $\pm$ 3
+ Verapamil	2	15 $\pm$ 2**
+ Nitrendipine	0.008	17 $\pm$ 2**
+ Ryanodine	1	16 $\pm$ 1**

The maximal contraction elicited by PGF_{2 α} (0.28 mM) was taken as 100%. Calcium antagonists were applied for 10–30 min. The values are the mean $\pm$ S.E.M. ($n=3-7$). ** $p<0.01$; significantly different from ($\pm$)-[6]-gingerol alone determined by unpaired Student's t -test.

RESULTS

Lack of Potentiating Effect of ($\pm$)-[6]-Gingerol on PGF_{2 α} -Induced Contractions Following Removal of the Endothelial Cells of Mesenteric Veins ($\pm$)-[6]-Gingerol (0.3 mM) alone caused a transient relaxation but did not induce direct contraction effects in mouse mesenteric veins. The maximal contraction for PGF_{2 α} was obtained at a concentration of 0.28 mM (data not shown), as we have reported previously.⁵⁾ The contraction elicited by PGF_{2 α} (0.28 mM) was significantly potentiated up to 28% by ($\pm$)-[6]-gingerol (0.3 mM) in intact veins (Table 1). To clarify whether the endothelial cells are involved in the mechanism of potentiation by ($\pm$)-[6]-gingerol, we compared the effects of ($\pm$)-[6]-gingerol on PGF_{2 α} -induced contractions between mesenteric veins, with and without endothelium. Removal of the endothelium was confirmed by the lack of relaxation response to acetylcholine (60 μ M).⁹⁾ When the endothelium was removed, the extent of contraction responses elicited by PGF_{2 α} tended to increase slightly, but did not significantly change from those in intact preparations, and then the potentiating effect by ($\pm$)-[6]-gingerol disappeared completely (Table 1). These results indicate that the potentiating action of ($\pm$)-[6]-gingerol is dependent on the presence of intact endothelial cells.

Calcium Antagonists Partially Depress the Potentiation of PGF_{2 α} -Induced Contractions by ($\pm$)-[6]-Gingerol in Mouse Mesenteric Veins We used calcium antagonists to examine whether ($\pm$)-[6]-gingerol produces its potentiating effect by releasing vasoconstrictors or by inhibiting the release of vasorelaxants from the endothelial cells. The voltage-dependent calcium antagonists, verapamil (2 μ M) and nitrendipine (8 nM), depressed partially the potentiating effect

Table 3. Effects of Cyclooxygenase Inhibitors and a Lipoxigenase Inhibitor on the Potentiation of PGF_{2 α} -Induced Contractions by ($\pm$)-[6]-Gingerol in Mouse Mesenteric Veins

Compounds	Concentration of inhibitor (mM)	% potentiation
($\pm$)-[6]-Gingerol (0.3 mM)		35 $\pm$ 10
+ Aspirin	0.2	5 $\pm$ 2*
+ Indomethacin	0.2	–1 $\pm$ 3*
+ NDGA	0.03	29 $\pm$ 3

The maximal contraction elicited by PGF_{2 α} (0.28 mM) was taken as 100%. Inhibitors were applied for 1 h. The values are the mean $\pm$ S.E.M. ($n=4-5$). * $p<0.05$; significantly different from ($\pm$)-[6]-gingerol alone determined by unpaired Student's t -test.

Table 4. Potentiating Effect of ($\pm$)-[6]-Gingerol (0.3 mM) on PGF_{2 α} (0.28 mM)-Induced Contractions in Mesenteric Veins of STZ-Diabetic Mice

($\pm$)-[6]-Gingerol	mg tension (% contraction)	
	Age-matched normal	STZ-diabetic
–	5.6 $\pm$ 0.5 (100)	12.4 $\pm$ 2.3 (100)
+	7.4 $\pm$ 0.9 (130 $\pm$ 3)	15.3 $\pm$ 2.4 (126 $\pm$ 6)

The values are the mean $\pm$ S.E.M. ($n=3-5$). ** $p<0.01$ and * $p<0.05$; significance of difference determined by paired Student's t -test.

of ($\pm$)-[6]-gingerol from 30 $\pm$ 3% to 15 $\pm$ 2% and 17 $\pm$ 2%, respectively (Table 2). The potentiation by ($\pm$)-[6]-gingerol was also depressed significantly, but not completely to 16 $\pm$ 1% by treatment with ryanodine (1 μ M), which is widely used as a potent inhibitor of Ca²⁺ release from sarcoplasmic reticulum.¹⁰⁾ These results suggest that ($\pm$)-[6]-gingerol may produce its potentiating effect by releasing vasoconstrictors or by inhibiting vasorelaxants released from the endothelial cells.

Cyclooxygenase Inhibitors, but Not a Lipoxigenase Inhibitor, Depress the Potentiation of PGF_{2 α} -Induced Contractions by ($\pm$)-[6]-Gingerol in Mouse Mesenteric Veins

To examine whether metabolites of arachidonic acid contribute to the mechanism of potentiation by ($\pm$)-[6]-gingerol on PGF_{2 α} -induced contractions, inhibitors of cyclooxygenase and lipoxigenase were used. Inhibitors of cyclooxygenase, aspirin (0.2 mM) and indomethacin (0.2 mM), markedly reduced the endothelium-dependent potentiating response. On the other hand, an inhibitor of lipoxigenase, NDGA (0.03 mM), did not affect the potentiating effect (Table 3). A TXA₂ antagonist, ONO-3708 (3 nM), also did not affect the potentiating effect (data not shown). These results suggest the cyclooxygenase-dependent release of vasoconstrictors, other than PGF_{2 α} and TXA₂, or vasorelaxants from endothelial cells may contribute to the mechanism of potentiation by ($\pm$)-[6]-gingerol. The possibility of PGF_{2 α} being a candidate vasoconstrictor is refuted because the maximal response, but not the affinity, in the PGF_{2 α} concentration–response curve is potentiated by ($\pm$)-[6]-gingerol.⁵⁾

Potentiation Effect of ($\pm$)-[6]-Gingerol on PGF_{2 α} -Induced Contractions in Mesenteric Veins of STZ-Diabetic Mice We compared the potentiating effects of ($\pm$)-[6]-gingerol on PGF_{2 α} -induced contractions in mesenteric veins of STZ-diabetic (10-weeks old) and normal age-matched mice.

PGF_{2α} (0.28 mM)-induced contractions were two times greater in the veins of STZ-diabetic mice than in normal mice (Table 4), as shown in our previous paper.⁶⁾ The potentiation percentages by (±)-[6]-gingerol did not change between STZ-diabetic (26±6%) and normal age-matched (30±3%) mice. These results demonstrate that the potentiation mechanism by (±)-[6]-gingerol works even in the diabetic state.

DISCUSSION

The potentiating effect of (±)-[6]-gingerol on PGF_{2α}-induced contractions of normal mouse mesenteric veins was produced in an endothelium-dependent manner, because the effect disappeared completely in de-endothelialized preparations. It is well known that the endothelial cells modulate the responses of vascular smooth muscle to endogenous vasoconstrictors^{11–13)} and vasorelaxants (endothelium-dependent relaxing factor, vascular endothelial NO).^{9,14)} (±)-[6]-Gingerol may produce its potentiating effect, by releasing vasoconstrictors or by inhibiting vasorelaxants released from the endothelial cells due to the increase in intracellular Ca²⁺ concentrations because two types of calcium antagonists significantly but partially depressed the potentiation by (±)-[6]-gingerol. (±)-[6]-Gingerol actually depresses the amplitude of spontaneous Ca²⁺ spikes in mouse portal vein,¹⁵⁾ suggesting that (±)-[6]-gingerol obstructs Ca²⁺ influx through the smooth muscle cell membrane. Activation of Ca²⁺-ATPase and Ca²⁺-pumping of fragmented sarcoplasmic reticulum by (±)-[8]-gingerol, as characterized in guinea pig heart muscle,¹⁾ may be involved in the (±)-[6]-gingerol release mechanism of vasoconstrictors or in the inhibiting mechanism of vasorelaxants released from the endothelium. The present data support the idea that the potentiation by (±)-[6]-gingerol was partially depressed by treatment with ryanosine, a potent inhibitor of Ca²⁺ release from the sarcoplasmic reticulum.

The present results, furthermore, demonstrate that cyclooxygenase-dependent vasoconstrictors, except for TXA₂, released from endothelial cells may contribute to the mechanism of potentiation by (±)-[6]-gingerol, because a TXA₂ antagonist ONO-3708 had no effect on the potentiation. The possibility of PGF_{2α} as a candidate vasoconstrictor is also refuted because the maximal response, but not the affinity, in the PGF_{2α} concentration–response curve is potentiated by (±)-[6]-gingerol.⁵⁾ (±)-[6]-Gingerol is reported to be an inhibitor of PG synthetase and 5-lipoxygenase *in vitro*.^{16–18)} However, the present potentiation mechanism is not explained by the inhibition PG synthetase, because the potentiation was actually caused through the cyclooxygenase-activating pathway which was completely suppressed by indomethacin and aspirin. The concentrations of cyclooxygenase inhibitors used were high but the suppression of the potentiation may not be due to the Ca²⁺ antagonistic action pro-

duced by these high concentrations because Ca²⁺ antagonists in the present study incompletely suppressed the potentiation. Recently, it has been reported that inhibition of NO formation results in enhanced *de novo* synthesis of cyclooxygenase-2 in rat peritoneal macrophages.¹⁹⁾ The potentiation by (±)-[6]-gingerol was still observed in mesenteric veins of STZ-diabetic mice where the enhancement of PGF_{2α} response is mainly due to the activation of lipoxygenase rather than the activation of cyclooxygenase.⁷⁾ The enhancement of PGF_{2α} response in the diabetic state is suppressed by lipoxygenase inhibitors but not by cyclooxygenase inhibitors. Therefore, the present result also suggests the involvement of other mechanisms in addition to the cyclooxygenase-activating pathway.

In conclusion, the potentiation mechanism of PGF_{2α}-induced contraction by (±)-[6]-gingerol may be caused by cyclooxygenase-dependent release of vasoconstrictors, other than PGF_{2α} and TXA₂, and/or by inhibiting vasorelaxants released from endothelial cells of mouse mesenteric veins.

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