Chemical Structural Requirement in Gingerol Derivatives for Potentiation of Prostaglandin $F_{2\alpha}$ -Induced Contraction in Isolated Mesenteric Veins of Mice

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(Received September 22, 1988)

We have previously reported that $(\pm)$ -[6]- and $(\pm)$ -[8]-gingerols potentiate prostaglandin (PG) $F_{2\alpha}$ -induced muscle contraction, and that other gingerol-related derivatives do not necessarily produce the same effect. The moiety necessary for potentiation was therefore investigated. 1) $(\pm)$ -[8]-Gingerol potentiated $PGF_{2\alpha}$ -induced contraction to a greater extent than $(\pm)$ -[6]-gingerol and $(\pm)$ -hexahydrocurcumine (HHC), but [6]-shogaol produced inhibition; 2) Noradrenaline (NA)-induced contraction was inhibited in the following order: [6]-shogaol > $(\pm)$ -[8]-gingerol > [6]-gingerdione > $(\pm)$ -[6]-gingerol > S-(+)-[6]-gingerdiacetate (GDA) > [6]-dehydrogingerdione (DHG), whereas $(\pm)$ -HHC had no significant inhibitory action; 3) [6]-gingerdione had different effects on $PGF_{2\alpha}$ -induced contraction, indicating inhibition just after preparation of the solution, no effect apparent after 2 h, and potentiation after 5 h; 4) [6]-gingerdione showed a change in its chemical structure after 5 h as measured by the $FeCl_3$ reaction, and ultraviolet and 1H nuclear magnetic resonance spectra. It was concluded that the aliphatic hydroxyl group present in gingerol derivatives is necessary for potentiation of $PGF_{2\alpha}$ - and the keto group for inhibition of NA-induced contraction.

Keywords — gingerol; 5-hydroxyl group; gingerdione; keto group; prostaglandin $F_{2\alpha}$; noradrenaline; contraction potentiation; mesenteric vein

Introduction

Recently, it has been shown that gingerols have various pharmacological effects. $(\pm)$ -[6]-Gingerol and four related components have been reported to be potent inhibitors of prostanoïd synthesis and are assumed to be related to the inhibition of prostaglandin synthetase¹⁾ or cyclooxygenase and lipoxygenase enzyme activity.²⁾ Gingerols are also potent cardiotonic agents, stimulating the Ca^{2+} -pumping activity of fragmented sarcoplasmic reticulum in skeletal and cardiac muscle.³⁾ Treatment with $(\pm)$ -[6]-, $(\pm)$ -[8]- and $(\pm)$ -[10]-gingerols induces a dose-dependent positive inotropic action.⁴⁾ Previous studies on gingerols and related analogues have provided evidence for the importance and relevance of their chemical structures in the potentiation of their antihepatotoxic activity.⁵⁾ It has been demonstrated that the aliphatic chain containing a hydroxyl group is the active moiety responsible for the mutagenicity of $(\pm)$ -[6]-gingerol.⁶⁾

Our idea is in harmony with the assumption that the activities of gingerols are structurally

related. We previously found that $(\pm)$ -[6]- or $(\pm)$ -[8]-gingerol potentiated $PGF_{2\alpha}$, E_2 - and I_2 -induced contraction, but on the other hand inhibiting the contractile responses to noradrenaline.⁷⁾ Crude ginger (containing gingerol) and processed ginger (containing shogaol) were clinically used with different pharmacological purpose. Both compounds have partially different moiety in chemical structure. Therefore, the aim of the present experiments was to investigate the moiety in the gingerol structure necessary for potentiation of prostaglandin $F_{2\alpha}$ - and inhibition of noradrenaline-induced contractions.

Materials and Methods

Isolated mesenteric veins of mice (ddY strain, male, 7—8 week-old, 31—38 g weight, decapitated) were used for isometric tension recording. The longitudinal muscle strips (10 mm length; 0.3 mm diameter) were suspended in an organ chamber filled with Krebs solution (5 ml, 37 °C) of the following composition (in mM): NaCl 122, KCl 5.9, $NaHCO_3$ 15.5, $MgCl_2$

Fig. 1. Chemical Structures of Gingerols and Related Compounds
 $n=6$ and 8 represent (±)-[6]- and (±)-[8]-gingerol, respectively.

1.2, CaCl_2 2.5 and glucose 11.5, under continuous gassing with a mixture of 95% O_2 + 5% CO_2 . A resting tension of 95 mg was applied and changes in the isometric force were measured through a transducer (U-gauge, type UL-2-240, Shinkoh Tsushin) and recorded on a Linearcorde (type WR3701, Graphtec).

The drugs used were (±)-[6]-gingerol, (±)-[8]-gingerol, [6]-gingerdione, (±)-hexahydrocurcumine (gifts from Dr. T. Shioiri, Dept. of Chemistry, Nagoya City University), [6]-shogaol (a gift from Dr. M. Aburada, Tsumura Juntendo Institute), [6]-dehydrogingerdione (Sanwa) and *S*-(+)-gingerdiacetate (Sanwa). The chemical structures of these compounds are presented in Fig. 1. Prostaglandin (PG) $\text{F}_{2\alpha}$ (Kaken), E_2 (Fuji), I_2 -Na salt (Ono), Sodium (±)-4-[(1*R*, 2*R*, 3*aS*, 8*bS*)-1,2,3*a*, 8*b*-tetrahydro-2-hydroxy-1-[(3*S*, 4*RS*)-3-hydroxy-4-methyl-oct-6-yne-(*E*)-1-enyl]-

5-cyclopenta[*b*]benzofuranyl]butyrate (TRK-100, Kaken), noradrenaline (NA, Sankyo), FeCl_3 (Daichi), benzoylacetone (Nakarai) and ethanol (Wako) were also used. The gingerol derivatives were suspended homogeneously using a sonicator (UR-20P, Tomy Seiko) just before use. The concentrations of the drugs were expressed as the final concentration in the organ chamber. The data were presented as the mean percentages of the maximum contractile response and were statistically compared using paired *t* test.

In some experiments for which changes in chemical structure were investigated, the ferric chloride (FeCl_3) test, and ultraviolet (UV) and nuclear magnetic resonance (NMR) spectral data analysis were employed. Benzoylacetone (0.15 μM), (±)-[6]-gingerol (1.5 μM) and [6]-gingerdione (1.5 μM) were dissolved in ethanol: water 1:9 v/v giving clear colorless solu-

tions. To 1 ml each of these solutions, 2 drops of aqueous FeCl_3 (0.19 mM) were added and shaken for 1 min. Ethanol alone with FeCl_3 was used as the standard. The formation of red colored ferric chloride complex indicates the presence of enol. The UV spectra were obtained using Hitachi UV-VIS spectrometer. The ^1H -NMR spectra were obtained with a JEOL GX-270 Type Spectrometer at 270 MHz.

Results

Prostaglandin $\text{F}_{2\alpha}$ (0.28 mM) or noradrenaline (60 μM) induced a direct maximum contractile response in the mouse mesenteric vein. Aqueous solutions of gingerols were added to the tissue bath 5 min before the addition of

$\text{PGF}_{2\alpha}$ or NA (Fig. 2).

($\pm$)-[6]-Gingerol (0.3 mM), ($\pm$)-[8]-gingerol (0.1 mM) and ($\pm$)-HHC (0.3 mM) significantly potentiated the contraction induced by $\text{PGF}_{2\alpha}$, while [6]-DHG (0.3 mM) and *S*-(+)-[6]-GDA (0.3 mM) had no significant effect. Conversely, [6]-gingerdione (0.3 mM) and [6]-shogaol (0.1 mM) markedly inhibited the response to $\text{PGF}_{2\alpha}$. The potentiation effects recovered completely after a 30 min recovery period, whereas it took 1.5 h for recovery of the inhibitory action. On the other hand, significant inhibition of NA-induced contraction was observed with all the compounds tested, except for ($\pm$)-HHC. The inhibition of NA contractile response was recovered after 1 h (Table I).

Among the gingerols tested, [6]-gingerdione

Fig. 2. Typical Tracings of $\text{PGF}_{2\alpha}$ - and NA-Induced Contractions of Mouse Mesenteric Veins before and after Addition of ($\pm$)-[6]-Gingerol (0.3 mM)

Concentrations of $\text{PGF}_{2\alpha}$: 1, 0.3 μM ; 2, 0.9 μM ; 3, 3 μM ; 4, 9 μM ; 5, 30 μM ; 6, 90 μM ; 7, 0.3 mM.

Concentrations of NA: 1, 59 nM; 2, 177 nM; 3, 590 nM; 4, 1.77 μM ; 5, 5.9 μM ; 6, 17.7 μM ; 7, 59 μM .

TABLE I. Effects of Gingerol Analogues on $\text{PGF}_{2\alpha}$ - and NA- Induced Contractions

Compounds (mM)	% contraction	
	+ $\text{PGF}_{2\alpha}$ (0.28)	+ NA (0.06)
($\pm$)-[6]-Gingerol (0.3)	128 $\pm$ 3 ^{b)}	62 $\pm$ 4 ^{b)}
($\pm$)-[8]-Gingerol (0.1)	115 $\pm$ 2 ^{b)}	45 $\pm$ 3 ^{b)}
($\pm$)-Hexahydrocurcumine (0.3)	109 $\pm$ 3 ^{a)}	97 $\pm$ 3
[6]-Shogaol (0.1)	65 $\pm$ 5 ^{b)}	41 $\pm$ 9 ^{b)}
[6]-Gingerdione (0.3)	29 $\pm$ 3 ^{b)}	34 $\pm$ 5 ^{b)}
[6]-Dehydrogingerdione (0.3)	102 $\pm$ 3	91 $\pm$ 1 ^{b)}
S-(+)-[6]-Gingerdiacetate (0.3)	103 $\pm$ 1	75 $\pm$ 6 ^{a)}

The contraction induced by $\text{PGF}_{2\alpha}$ or NA in the absence of gingerol derivatives was taken as control=100%. The values are the mean percentages $\pm$ S.E.M. ($n=3-5$). Significant differences from the control values at a) $p < 0.05$ and b) $p < 0.01$; paired t test.

TABLE II. Different Effects of [6]-Gingerdione in Aqueous Solution on Prostaglandin- and Noradrenaline-Induced Contractions

Compounds (mM)	Time (h)		
	0	2	5
	% contraction		
[6]-Gingerdione (0.3) + $\text{PGF}_{2\alpha}$ (0.28)	29 $\pm$ 3 ^{b)}	102 $\pm$ 2	124 $\pm$ 2 ^{b)}
[6]-Gingerdione (0.3) + NA (0.06)	34 $\pm$ 5 ^{b)}	58 $\pm$ 8 ^{b)}	69 $\pm$ 8 ^{a)}

The contraction induced by $\text{PGF}_{2\alpha}$ or NA without [6]-gingerdione was taken as control=100%. The values are the mean percentages $\pm$ S.E.M. ($n=5$). Significant differences from the control values at a) $p < 0.05$ and b) $p < 0.01$; paired t test.

showed different effects on $\text{PGF}_{2\alpha}$ - or NA-induced contraction when its aqueous solution was allowed to stand for several hours at room temperature (27 °C). As shown in Table II, [6]-gingerdione just after preparation significantly inhibited $\text{PGF}_{2\alpha}$ contraction; after 2 h of incubation it produced no marked effect, but after 5 h significant potentiation of contraction was observed. Under the same conditions, [6]-gingerdione inhibited the NA-induced contractile response and the extent of inhibition weakened after a long duration of incubation (5 h).

The alcoholic solutions of benzoylacetone (0.15 μM), ($\pm$)-[6]-gingerol (1.5 μM) and [6]-gingerdione (1.5 μM) were tested with an aqueous solution of ferric chloride (0.19 mM). Benzoylacetone and [6]-gingerdione showed positive results for the presence of enol as indicated by the change in color (from colorless to red), whereas ($\pm$)-[6]-gingerol was negative (data

not shown).

In addition, absorption spectra determination for benzoylacetone (75 nM) showed maximum absorption bands at 250 nm and 310 nm. Similarly, with [6]-gingerdione (1.5 μM) absorption maxima were obtained at 235 nm and 280 nm, the same intensity of absorption being observed. Only one spectral band was noted with ($\pm$)-[6]-gingerol (1.5 μM) at a maximum of 245 nm.

In Fig. 3, the UV absorption spectra of [6]-gingerdione (3 μM) in 10% ethanol under the same experimental conditions (incubation at 27 °C for different time intervals) are compared. The measured absorbance at 235 nm decreased after several hours, while in contrast, the absorbance at 280 nm increased.

The ^1H -NMR spectra (CDCl_3 , δ ppm) of [6]-gingerdione showed proton signals of a 1, 2, 4-substituted benzene ring (6.6–6.9, 3H, m), a methoxy signal (3.9, 3H, s), benzyl proton sig-

Fig. 3. UV Absorption Spectrum for [6]-Gingerdione ($3 \mu\text{M}$) just after Preparation (0 h) and after Incubation for 2 and 5 h at 27°C

····, 0 h; ---, 2 h; —, 5 h.

nals (2.9, 2H, m), a ketone α -proton signal (2.6, 2H, m), an olefinic proton signal (5.5, 1H, s), signals for methylene groups (2.3, 2H, m; 1.2–1.4, 4H, m; 1.6, 2H, m) and a methyl signal (0.9, 3H, t). A proton signal (15.5, 1H, s) in deuterium chloroform (CDCl_3) was assigned to be an enol proton because it disappeared by the further addition of deuterium methanol (CD_3OD) or deuterium oxide (D_2O). The same situation was also observed for benzoylacetone. The gingerdione solution just prepared showed the ratio of a ketone α -proton signal to an olefinic proton signal to be 4:1. Several hours after solubilization, the ratio of the olefinic proton signal was increased.

Prostaglandin E_2 (0.28 mM), I_2 -Na salt (0.27 mM) and TRK-100 (0.24 mM) also induced direct contraction of the mouse mesenteric vein, being 34%, 102% and 25%, respectively, of the maximum contractile response to $\text{PGF}_{2\alpha}$ (0.28 mM). ($\pm$)-[6]-Gingerol (0.3 mM) caused a transient relaxation of the smooth muscle that gradually recovered and then significantly potentiated the contraction induced by prostanooids (Fig. 2 and Fig. 4 a).

On the other hand, low concentrations of ethanol had no direct effect of the tissue, although higher concentrations (1.7 mM) significantly potentiated $\text{PGF}_{2\alpha}$ -induced contraction. A tendency to augment the contractions induced by PGE_2 and TRK-100 was also observed, but this was not significant (Fig. 4 b). The potentiation effects of ($\pm$)-[6]-gingerol on prostanooid-induced contraction were reversible but those induced by ethanol were irreversible.

Discussion

We have previously reported that ($\pm$)-[6]- and ($\pm$)-[8]-gingerols induced potentiation of contraction to $\text{PGF}_{2\alpha}$ and inhibition of that produced by NA.⁷⁾ The latter effect may be attributed to the direct action on NA receptor by gingerols.^{7,8)} In the present work, the effects of other compounds related to gingerols were investigated. The potentiating effect of ($\pm$)-[8]-gingerol on $\text{PGF}_{2\alpha}$ -induced contraction was greater than that of ($\pm$)-[6]-gingerol. The diarylheptanoid, ($\pm$)-HHC, also potentiated $\text{PGF}_{2\alpha}$ but to a lesser extent compared with gingerols. No

Fig. 4. Potentiation Effects of 0.3 mM (±)-[6]-Gingerol on $\text{PGF}_{2\alpha}$, PGE_2 , PGI_2 -Na Salt and TRK-100-(a) and 1.7 mM Ethanol on $\text{PGF}_{2\alpha}$, PGE_2 and TRK-100-(b) Induced Contractions of Mouse Mesenteric Vein

The contraction in response to $\text{PGF}_{2\alpha}$ without (±)-[6]-gingerol or ethanol was taken as control=100%. The values are mean percentages ($n=3-4$) $\pm$ SEM. Significant differences at a) $p < 0.05$ and b) $p < 0.01$; paired t test.

(a) □, without; ▨, with (±)-[6]-gingerol. (b) □, without; ▨, with ethanol.

potentiation effect was observed with [6]-shogaol, [6]-DHG and *S*-(+)-[6]-GDA. Comparing the results and the chemical structures of the compounds tested, it will be noted that only those compounds containing a hydroxyl group at C-5 in the side chain potentiated $\text{PGF}_{2\alpha}$ -induced contraction.

Furthermore, contraction in response to NA was significantly inhibited in the order of activity: [6]-shogaol $\geq$ (±)-[8]-gingerol > [6]-gingerdione > (±)-[6]-gingerol > *S*-(+)-[6]-GDA > [6]-DHG. No significant inhibition of NA-induced contraction was observed with the

diarylheptanoid. It appeared that shogaols were more potent inhibitors of NA contraction than gingerols when compounds with same length of alkyl chain were compared. These results also showed that in gingerols, shortening of the side chain decreases the activity.^{5,9)} Similarly, alterations in gingerol structure, such as the substitution of acetate radical in *S*-(+)-[6]-GDA and the presence of a double bond in [6]-DHG, also reduced the inhibitory action. From the above results, it is assumed that the presence of a carbonyl group on C-3 contributes to the inhibition of NA contractile response. Suekawa *et al.* reported that repeated injection of [6]-shogaol caused tachyphylaxis in mesenteric vascular beds.¹⁰⁾ In the present experiments, repeated applications were tried when the tissues had completely recovered from previous drug effects.

The difference in responses of [6]-gingerdione is probably due to the diketones at C-3 and C-5 undergoing enolization. [6]-Gingerdione inhibited the response to NA but its potency became weaker after several hours of incubation at 27 °C. Different effects on $\text{PGF}_{2\alpha}$ -induced contraction were also observed, ranging from inhibition to potentiation after several hours of incubation.

The activated hydrogen of the keto form migrates from the carbon by an α , γ -shift to give an enol. The high enol content of 1, 3 diketones such as acetylacetone and benzoylacetone shows that the activation by 2 carbonyl groups is more effective and favors enolization more readily than simple ketones like acetone. Enols give a red color when treated with ferric chloride, forming colored ferric chloride complexes.¹¹⁾

Absorption spectra determinations for benzoylacetone show 2 absorption bands, with maximum near 247 and 310 nm, and their respective intensities differing from one solvent to another. These two maxima were associated with the enolic and ketonic forms. The intensity of the 310 nm peak in different solvents varies linearly with the total enol content, and that at 247 nm with the ketone content.¹²⁾

Our UV spectral data on benzoylacetone agree with the previous results. The absorption maxima that occur with [6]-gingerdione at 235

Fig. 5. Possible Chemical Structure of [6]-Gingerdione in Solution

and 280 nm may be associated with its ketone and enol contents, respectively, whereas only the ketone absorption peak was detected with ($\pm$)-[6]-gingerol at 245 nm. The ^1H -NMR spectrum of [6]-gingerdione solution also showed time-dependent increase of an enol type of configuration. It is therefore presumed that in aqueous solution, [6]-gingerdione changed its chemical structure after several hours of incubation, although the extent of conversion to enol and the resulting chemical structure need further elucidation. Figure 5 suggests one possible chemical structure of [6]-gingerdione in solution.

Moreover, the intensity of absorption at 235 nm decreased after 5 h while the intensity at 280 nm increased. The shift in the intensity of absorption spectrum was only slight because of its extremely low concentrations as compared with pharmacologically effective concentrations. It is possible that the conversion of diketone to enol might have increased during incubation, thus causing the observed transfer of effect from inhibition to potentiation of $\text{PGF}_{2\alpha}$ -induced contraction and the decrease in inhibition of NA-induced contractile responses.

Ethanol can differentially affect the contractile responses to a variety of prostaglandin compounds in vascular smooth muscle. Low concentrations of ethanol can enhance prostaglandin contraction in isolated blood vessels.¹³⁻¹⁵⁾ The gingerol derivatives containing the hydroxyl group in the alkyl part of the chemical structure potentiated $\text{PGF}_{2\alpha}$ in the same manner as ethanol. In addition, a significant potentiating effect of ($\pm$)-[6]-gingerol was also observed upon other types of prostanoid-induced contraction.

Thus, it seems logical to conclude that in

gingerols and related compounds, the aliphatic hydroxyl group is necessary for potentiation of the $\text{PGF}_{2\alpha}$ -induced contraction and the keto group for inhibition of NA-induced contraction.

Acknowledgment We are deeply grateful to Miss J. Ogawa for her skillful experimental assistance and to Dr. T. Shioiri (Professor, Dept. of Chemistry, Nagoya City University) for his helpful suggestions.

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