Reversed Effects between Crude and Processed Ginger Extracts on PGF$_{2\alpha}$-Induced Contraction in Mouse Mesenteric Veins

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Abstract—The effects of crude and processed ginger extracts and pungent components, S-(+)-[6]-gingerol and [6]-shogaol, on noradrenaline (NA)- and prostaglandin (PG) F$_{2\alpha}$-induced contraction were investigated using mouse mesenteric veins. Both spicy constituents inhibited the contractile responses to NA. Crude ginger extract and S-(+)-[6]-gingerol potentiated the PGF$_{2\alpha}$-induced contraction, whereas processed ginger extract and [6]-shogaol inhibited the contraction.

Crude ginger (Shokyo) and processed ginger (Kankyo) are used for different clinical purposes in traditional Sino-Japanese medicine. However, there are no clear reports that distinguish them with regards to the levels of extracts or main constituents. S-(+)-[6]-Gingerol and [6]-shogaol are the main pungent constituents of crude and processed ginger, respectively (1-3). Crude ginger contains 0.6-1.1% w/w S-(+)-[6]-gingerol and 0.05-0.1% w/w [6]-shogaol, whereas processed ginger contains 0.2-0.7% w/w S-(+)-[6]-gingerol and 0.3-0.7% w/w [6]-shogaol. The amount of S-(+)-[6]-gingerol equals to that of [6]-shogaol when crude ginger has been processed (steamed and dried) for 12 hours (4). During the processing, the amount of [6]-shogaol increases and that of S-(+)-[6]-gingerol decreases as a result of dehydration of the gingerols (1).

In the previous paper, we reported the important role of the 5-hydroxyl group in the aliphatic chain in a series of synthesized racemic gingerols and related compounds (5). The present study compared the effects of the extracts of ginger and the natural compounds, S-(+)-[6]-gingerol and [6]-shogaol, on the contraction induced by noradrenaline (NA) and PGF$_{2\alpha}$ in isolated mesenteric veins of mice. It is clinically useful to evaluate the hemodynamic effects of crude and processed ginger in the splanchnic area.

The isolated mesenteric veins (2-3 mm below the portal vein, 8-10 mm long, 0.2 mm diameter) of mice (ddY, male, 7-9 weeks old, 30-40 g, decapitated) were suspended in an organ chamber filled with Krebs’ solution (5 ml, 37°C, pH 7.4-7.5) of the following composition: 122 mM NaCl, 5.9 mM KCl, 15.5 mM NaHCO$_3$, 1.2 mM MgCl$_2$, 2.5 mM CaCl$_2$ and 11.5 mM glucose, and the solution was continuously gassed with a mixture of 95% O$_2$ and 5% CO$_2$. The procedures employed were essentially the same as those described by Kimura et al. (5), with minor modifications. The drugs used were the methanol extract of crude ginger (Sanwa), S-(+)-[6]-gingerol (Sanwa), (+)-[6]-gingerol (a gift from Prof. T. Shioiri, Dept. of Chemistry, Nagoya City University), water extract of processed ginger and [6]-shogaol (gifts from Dr. M. Aburada, Tsumura Juntendo Institute), PGF$_{2\alpha}$ (Kaken) and NA (Sankyo). S-(+)-[6]-Gingerol, [6]-shogaol (Fig. 1a), ginger extracts and PGF$_{2\alpha}$ were dissolved in distilled water with a sonicator (UR-20P, Tomy Seiko) just before use. The concentrations of the drugs were expressed as the final concentration in the tissue bath. The data were converted to the percentage of maximal response and statistically assayed by the paired Student’s t-test or Studentized...
Fig. 1. Chemical structures of S-(+)-[6]-gingerol and [6]-shogaol (a) and cumulative concentration-contracting curves for PGF$_{2\alpha}$ before (○) and after the addition of different concentrations of S-(+)-[6]-gingerol (○) or [6]-shogaol (▲) (b). The values are the mean percentages (n=4-27)±S.E.M. Significant differences from the control values (without gingerol or shogaol) at *: P<0.05 and **: P<0.01 by the Studentized range test.

The cumulative concentration-response relationship to PGF$_{2\alpha}$ (0.3 μM-0.3 mM) or NA (60 nM-60 μM) was obtained by directly adding the drug to the tissue bath. Each concentration was allowed to react with the muscle for 1 min. The ginger extract or pungent constituent was added to the tissue bath 5 min before the addition of PGF$_{2\alpha}$ or NA.

The methanol extract of crude ginger (10 μg/ml), S-(+)-[6]-gingerol (9-90 μg/ml), and (±)-[6]-gingerol (30-90 μg/ml) significantly potentiated the contractile response to PGF$_{2\alpha}$ and decreased the EC50 (fifty percent effective concentration) values. The former extract (10 μg/ml) was more potent than the latter two compounds on the percent maximal contraction induced by PGF$_{2\alpha}$, as shown in Table 1. The PGF$_{2\alpha}$-potentiating activities of S-(+)-[6]-gingerol was more effective than those of (±)-[6]-gingerol. On the other hand, processed ginger extract (10 μg/ml) and [6]-shogaol (8 μg/ml) similarly inhibited the PGF$_{2\alpha}$-induced contraction. S-(+)-[6]-Gingerol and [6]-shogaol significantly inhibited the contractile responses to NA and increased the EC50 values, whereas both extracts had no significant effect on NA responses (Table 1).

The effects of S-(+)-[6]-gingerol and [6]-shogaol were compared in detail. Increasing concentrations of NA (60 nM-60 μM) or PGF$_{2\alpha}$ (0.3 μM-0.3 mM) induced concentration-dependent contraction in the mouse
Table 1. Effects of ginger extracts and active components on the contractions induced by PGF$_{2\alpha}$ and NA in the isolated mesenteric veins of mice

| Compounds                  | mM (µg/ml) | EC50 (95% confidence limits; µM)$^a$ | Percent maximal contraction$^b$ |
|----------------------------|------------|--------------------------------------|---------------------------------|
|                            |            | PGF$_{2\alpha}$                      | NA                              |
| Crude ginger methanol extract | (10)       | 6.1 (4.9-7.4)                        | 134±2**                         |
| S-(+)-[6]-Gingerol          | 0.01 (3)   | 8.5 (7.5-9.8)                        | 108±2                           |
|                            | 0.03 (9)   | 5.4 (4.5-6.4)                        | -123±4**                        |
|                            | 0.1 (30)   | 5.1 (4.3-6.1)                        | -125±3**                        |
|                            | 0.3 (90)   | 3.9 (3.3-4.7)                        | -135±5**                        |
| (±)-[8]-Gingerol            | 0.1 (30)   | 6.8 (5.7-8.2)                        | -112±3**                        |
|                            | 0.3 (90)   | 5.6 (4.9-6.2)                        | 128±3**                         |
| Processed ginger extract    | (10)       | 45 (27-75)                           | 77±6**                          |
| [6]-Shogaol                 | 0.03 (8)   | 59 (48-72)                           | 81±4**                          |
|                            | 0.1 (40)   | 132 (97-177)                         | 65±5**                          |

EC50: fifty percent effective concentration, PG: prostaglandin, NA: noradrenaline. $^a$The contraction induced by PGF$_{2\alpha}$ (0.3 µM-0.3 mM) or NA (60 nM-60 µM) without the above compounds was used as the control, and the values are expressed as the concentrations inducing 50% response with confidence limits. All EC50 values were significantly different from the control value at P<0.01 by the unpaired Student's $t$-test. $^b$The contraction induced by PGF$_{2\alpha}$ (0.3 mM) or NA (60 µM) without the above compounds was used as the control (100%) and the values are expressed as mean percentages±S.E.M. (n=3-5). Statistical analysis was made using the Studentized range test or Student's $t$-test (c: paired or d: unpaired, twotailed, e: unpaired, one-tailed) at *: P<0.05 and **: P<0.01.

mesenteric veins. Both gingerol and shogaol non-competitively inhibited the contractile forces to NA (data not shown). On the other hand, the isometric contraction to increasing concentrations of PGF$_{2\alpha}$ were significantly enhanced by S-(+)-[6]-gingerol (0.01-0.3 mM) and inhibited by [6]-shogaol (0.03-0.1 mM) in a concentration-dependent manner, as evaluated by the Studentized range test after the two-way analysis of variance (Fig. 1b).

The extract from the rhizomes of ginger (Zingiber officinale) contain a series of gingerols, shogaols and a variety of diarylheptanoids (6, 7). Our results showed that crude ginger methanol extract had stronger potentiating effects on PGF$_{2\alpha}$ than S-(+)-[6]-gingerol. This result indicates that the extract contains other constituents that are more potent than S-(+)-[6]-gingerol in enhancing the contractile effects of PGF$_{2\alpha}$ (8). The potency of S-(+)-[6]-gingerol was stronger than the racemic compound.

(±)-[6]-Gingerol also potentiated the contractions induced by PGE$_2$ and the stable PG1$_2$ derivatives, PG1$_2$-Na and TRK-100, but inhibited the contractile responses to PGD$_2$, thromboxane A$_2$, leukotrienes and alpha adrenergic agonists in mouse mesenteric veins (8).

All purified components, gingerol and shogaol inhibited the responses to NA. The inhibitory action of [6]-shogaol was greater than S-(+)-[6]-gingerol. However, both extracts from crude and processed ginger showed no inhibitory effects on NA-induced contraction, indicating that the inhibitory action of S-(+)-[6]-gingerol or [6]-shogaol contained in the respective extracts may be masked by other unknown co-existing components.

In conclusion, crude ginger extract and S-(+)-[6]-gingerol potentiated the PGF$_{2\alpha}$-induced contraction, whereas processed
ginger extract and [6]-shogaol inhibited the contraction.

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