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Note

Newly Synthesized Oleylgingerol and Oleylshogaol Activate TRPV1 Ion Channels

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The oleyl moiety in vanilloids is important in activating vanilloid receptor 1 (TRPV1), but there was no ingredient of ginger containing the oleyl moiety in the natural form. We synthesized oleylgingerol and oleylshogaol and then evaluated their potential to activate a rat TRPV1 channel. Oleylgingerol is a stronger TRPV1 agonist than natural gingerols, but oleylshogaol is a weaker agonist than natural shogaols. The difference in structure between oleylgingerol and oleylshogaol is only the hydroxy group at carbon-5. This hydroxy group might have an important role in activating a TRPV1 channel.

Key words: transient receptor potential vanilloid subtype 1 (TRPV1); oleylgingerol; oleylshogaol

Ginger (*Zingiber officinale* ROSCOE) is used traditionally in China and Japan as a food having many biological activities.[1)] The components to which the pungency of ginger can be attributed are gingerols and shogaols. Gingerols are present in raw ginger and mainly comprise [6]-, [8]-, and [10]-gingerols.[2)] Shogaols, dehydration products of gingerols, are present in steamed ginger and mainly comprise [6]-, [8]-, and [10]-shogaols.[2)] These compounds are phenolic substances and possess a vanillyl moiety (see Fig. 1); therefore, these are types of vanilloids. Recently, the receptor for vanilloids was cloned and named TRPV1 (transient receptor potential vanilloid subtype 1), which is a nonselective cation channel.[3)] The most famous vanilloid is capsaicin, a pungent ingredient of red pepper (*Capsicum* fruits). Capsaicin strongly activates a TRPV1 channel and then increases the intracellular Ca^{2+} concentration.[4)] [6]-Gingerol and [8]-gingerol activate a native or recombinant TRPV1.[5,6)] We have reported that other natural gingerols and shogaols also activated TRPV1 channels, but their potency is not so strong as that of capsaicin.[7)]

Previous study of the structure-activity relationship among capsaicin-related compounds has demonstrated that olvanil, which has a oleyl moiety, was a potent agonist of TRPV1.[8,9)] We reported that olvanilate, an ester of vanillyl alcohol and oleic acid, also strongly activated TRPV1.[9)] Endogenous agonists for TRPV1 were reported to be endovanilloids, such as *N*-oleoyldopamine.[10)] One endocannabinoid, oleylethanolamine, also activates TRPV1.[11)] Thus the oleyl moiety in vanilloids is important in opening the TRPV1 channel. In nature, there is no gingerol nor shogaol containing the oleyl moiety. We think that the new synthetic analogs containing oleyl moiety will prove to be stronger agonists. In this experiment, we synthesized two new compounds (oleylgingerol and oleylshogaol), and then evaluated their potential to activate a rat TRPV1 ion channel.

Oleylgingerol was synthesized according to the methods of Wada *et al.*,[12)] with a slight modification. In brief, oleylgingerol was obtained by aldol addition of zingerone in potassium *t*-butoxide with oleylaldehyde prepared by oxidation of oleyl alcohol with pyridinium chlorochromate. Oleylshogaol was synthesized by dehydration of oleylgingerol with *p*-toluenesulfonic acid under heating. The structures of these compounds were confirmed by spectroscopic data as follows: oleylgingerol; HR-FABMS m/z [M^+] calcd for $C_{29}H_{48}O_4$ 460.3554, found 460.3557; IR ν_{max} (film) 3431, 3003, 2924, 2854, 1705, 1605, 1516, 1462, 1371, 1271, 1153, 1124, 1036, 814, 723 cm^{-1}; UV λ_{max} (MeOH) 282 nm ($\varepsilon = 2600$), 210 nm ($\varepsilon = 7000$); ^{1}H NMR ($CDCl_3$, 399.65 MHz, TMS as the internal standard) δ 6.82 (1H, d, $J = 7.8$ Hz, H-5′), δ 6.68 (1H, d, $J = 2.0$ Hz, H-2′), δ 6.65 (1H, dd, $J = 7.8$, 2.0 Hz, H-6′), δ 5.53 (1H, bs, 4′-O*H*), δ 5.34 (2H, m, H-13, 14), δ 4.02 (1H, m, H-5), δ 3.87 (3H, s, OCH_3), δ 2.93 (1H, bs, 5-O*H*), δ 2.83 (2H, t, $J = 7.2$ Hz, H-1), δ 2.73 (2H, t, $J = 7.2$ Hz, H-2), δ 2.56 (1H, dd, $J = 17.6$, 2.8 Hz, H-4a), δ 2.48 (1H, dd, $J = 17.6$, 8.8 Hz, H-4b), δ 2.01 (4H, m, H-12, 15), δ 1.2

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Abbreviation: TRPV1, transient receptor potential vanilloid subtype 1

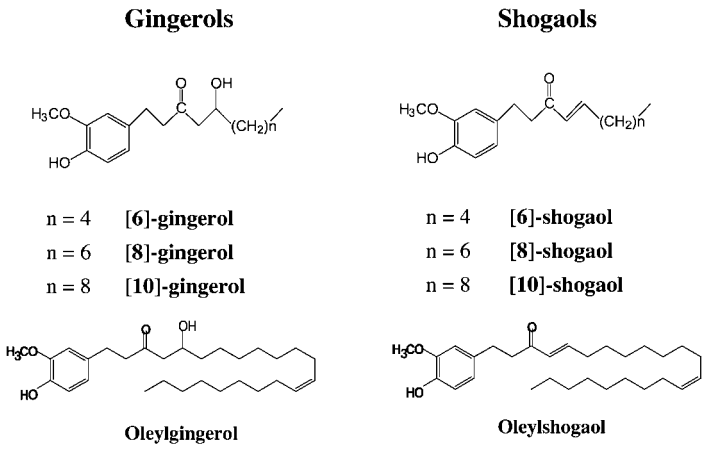

Fig. 1. Chemical Structure of Vanilloids.
The four gingerols and four shogaols used in this experiment were illustrated.

to 1.5 (24H, H-6 to 11, 16 to 21), δ 0.88 (3H, t, $J =$ 6.8 Hz, H-22); ^{13}C NMR ($CDCl_3$, 100.40 MHz) δ 211.4 (C-3), δ 146.5 (C-3′), δ 144.0 (C-4′), δ 132.6 (C-1′), δ 130.0 (C-13), δ 129.8 (C-14), δ 120.7 (C-6′), δ 114.4 (C-5′), δ 111.0 (C-2′), δ 67.7 (C-5), δ 55.9 (3′-O*C*H$_3$), δ 49.4 (C-4), δ 45.5 (C-2), δ 36.5 (C-6), δ 31.9, 29.8, 29.8, 29.5, 29.5, 29.5, 29.3, 29.3, 29.3, 29.2, 27.2, 27.2, 25.5, 22.7 (C-1, 7 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20 or 21), δ 14.1 (C-22): oleylshogaol; HR-FABMS m/z [M$^+$] calcd for $C_{29}H_{46}O_3$ 442.3448, found 442.3467; IR ν_{max} (film) 3552, 3433, 3003, 2926, 2854, 1670, 1628, 1516, 1462, 1365, 1271, 1236, 1207, 1151, 1120, 1036, 978, 852, 814, 723, 628.7, 557.3 cm^{-1}; UV λ_{max} (MeOH) 280 nm ($\varepsilon = 2500$), 227 nm ($\varepsilon = 16200$); ^{1}H NMR ($CDCl_3$, 399.65 MHz, TMS as the internal standard) δ 6.82 (1H, d, $J =$ 8.0 Hz, H-5′), δ 6.81 (1H, dt, $J =$ 16.4. 6.8 Hz, H-5), δ 6.72 (1H, d, $J =$ 1.6 Hz, H-2′), δ 6.68 (1H, dd, $J =$ 8.0, 1.6 Hz, H-6′), δ 6.09 (1H, dt, $J =$ 16.4, 1.6 Hz, H-4), δ 5.48 (1H, bs, 4′-O*H*), δ 5.34 (2H, m, H-13, 14), δ 3.87 (3H, s, OC*H*$_3$), δ 2.85 (4H, m, H-1, 2), δ 2.19 (2H, qd, $J =$ 6.8, 1.6 Hz, H-6), δ 2.01 (4H, q, J = 6.0 Hz, H-12, 15), δ 1.44 (2H, quint, $J =$ 6.8 Hz, H-7), 1.2 to 1.4 (20H, H-8 to 11, 16–21), δ 0.88 (3H, t, $J =$ 6.4 Hz, H-22); ^{13}C NMR ($CDCl_3$, 100.40 MHz) δ 199.7 (C-3), δ 147.8 (C-5), δ 146.4 (C-3′), δ 143.9 (C-4′), δ 133.3 (C-1′), δ 130.3 (C-4), δ 130.0 (C-13), δ 129.7 (C-14), δ 120.8 (C-6′), δ 114.3 (C-5′), δ 111.1 (C-2′), δ 55.9 (3′-O*C*H$_3$), δ 42.0 (C-2), δ 32.5, 3.19, 29.9, 29.8, 29.7, 29.5, 29.3, 29.3, 29.3, 29.2, 29.2, 28.1, 27.2, 27.2, 22.7 (C-1, 6, 7 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20 or 21), δ 14.1 (C-22). The purities of oleylgingerol and oleylshogaol by HPLC–UV (280 nm) were 94.3 and 99.7% respectively. Three gingerols ([6], [8], and [10]-gingerols) and three shogaols ([6], [8], and [10]-shogaols) were obtained from Professor K. Kubota of Ochanomizu University, Tokyo, Japan.[7] Capsaicinoids were purchased or synthesized as described previously.[9] Other chemicals were purchased from Sigma (St. Louis, MO, USA) or Wako Pure Chemical Industries (Osaka, Japan).

Table 1. Hydrophobicity and TRPV1 Activation Potency among Capsaicinoids, Gingerols and Shogaols

Compounds	Log *P*	EC_{50} (μM)
[6]-gingerol	2.718	4.55**
capsaicin	3.622	0.0819*
[8]-gingerol	4.374	2.09**
[6]-shogaol	4.612	0.611**
[10]-gingerol	5.891	1.99**
[8]-shogaol	6.111	0.711**
[10]-shogaol	7.540	0.874**
olvanil	9.182	0.00686*
oleylgingerol	10.40	0.264
oleylshogaol	11.93	4.17

Hydrophobicity was evaluated by reversed-phase HPLC. TRPV1 activation potency was evaluated as the change of the intracellular Ca^{2+} concentration in HEK293 cells heterologously expressing rat TRPV1 channels. Each change of the intracellular Ca^{2+} concentration was normalized to the response activated by 10 μM capsaicin. *Indicates data reported by Morita *et al.*[9] **Indicates data reported by Iwasaki *et al.*[7]

The hydrophobicity of the compounds used in this experiment was evaluated from relative log *P* values, as described previously.[13] The log of the octanol/water partition coefficient (log *P*) of capsaicin, *N*-vanillylbutanamide, and *N*-vanillylhexanamide was measured directly using an octanol-water partition system. Other relative log *P* values were calculated from the retention time in reverse-phase HPLC (J'sphere ODS-H80, 150 mm × 4.6 i.d., YMC, Kyoto, Japan, using 80% methanol). The hydrophobicity of vanilloids depends on the length of the carbon chain and the presence of hydroxy-moiety in the side chain. The most hydrophilic compound was [6]-gingerol, and the most hydrophobic compound was the newly synthesized oleylshogaol (Table 1). In the case of the same alkyl carbon length, the hydrophobicity of the shogaols was observed to be higher than that of the gingerols.

To compare the potential of each compound with regard to TRPV1 activation, we measured the intracellular Ca^{2+} concentration in HEK293 cells hetero-

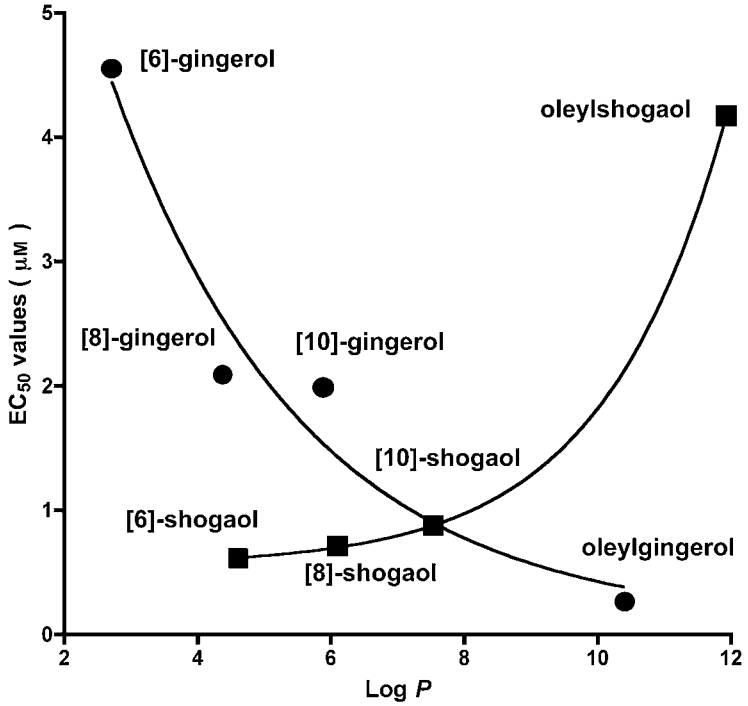

Fig. 2. Relationship between Hydrophobicity and TRPV1 Activation Potency.
Hydrophobicity and the TRPV1 activation potency were measured as described in Table 1. The log *P* and EC_{50} values were plotted for each compound of the gingerols (closed circle) and the shogaols (closed square), and the curve were fitted using Prizm4 software.

logously expressing rat TRPV1 ion channels, as described previously.[9] All test compounds were dissolved in dimethyl sulfoxide and stored at −20 °C until experiments were done. Fura-2 AM, a calcium sensitive dye, was obtained from Molecular Probes (Eugene, OR, USA). Curve fitting and parameter estimation were carried out using Prism4 (Graph Pad Software, San Diego, CA, USA).

Table 1 indicates the EC_{50} values of the tested compounds. The responses of all the compounds tested were inhibited by pretreatment with capsazepine, a specific antagonist for TRPV1 (data not shown), and no compound increased the intracellular Ca^{2+} concentration in parent HEK293 cells (data not shown), indicating that all compounds increased the intracellular Ca^{2+} concentration *via* activation of TRPV1.

Capsaicin increased the intracellular Ca^{2+} concentration in HEK293 cells expressing the rat TRPV1 channels. The values of EC_{50} for capsaicin and olvanil (Table 1) were almost the same as those reported by others.[8] [6]-, [8]-, and [10]-Gingerols also increased the intracellular Ca^{2+} concentration, but their potencies were lower than that of capsaicin. The EC_{50} value for [6]-gingerol was 4.55 μM (Table 1), almost the same value as in a previous report.[6] The newly synthesized oleylgingerol (EC_{50} = 0.264 μM) was more potent than other gingerols. In addition to gingerol-related compounds, [6]-, [8]-, and [10]-shogaols were more potent TRPV1 agonists than [6]-gingerol (Table 1).[7] Unexpectedly, the EC_{50} value for the newly synthetic oleylshogaol (4.17 μM) was higher than those of other shogaols.

To clarify the relationship between hydrophobicity (the length of the alkyl chain) and the potential for TRPV1 activation, the relative log *P* values and the EC_{50} values were plotted (Fig. 2). The EC_{50} values decreased with increases in the carbon chain length of the gingerol-related compounds (Fig. 2). The longer the alkyl chain length of the gingerol, the higher the potential to activate TRPV1 channels. Capsaicin-related compounds also exhibited the same relationship between hydrophobicity and the potential to activate TRPV1 channels, in previous experiments.[9] On the other hand, the EC_{50} values of the shogaols increased with increases in the carbon chain length (Fig. 2). The longer the alkyl chain length of the shogaol, the lower the potential to activate TRPV1 channels.

In this experiment, oleylgingerol proved to be a stronger agonist than natural gingerols, although its potential to activate TRPV1 was observed to be lower than that of capsaicin. On the other hand, oleylshogaol was a weaker agonist than natural shogaols. Among the gingerols, there was the positive relationship between hydrophobicity and potential with regard to the activation of TRPV1 channels, but there was a negative relationship among the shogaols. The reason for this opposite relationship remains obscure. The TRPV1 channel shares structural similarities with K_v-type potassium channels, including the same six-transmembrane topology.[14] Assuming helix packing for TRPV1

similar to that for potassium channels, the acyl moiety of capsaicin may bind to transmembrane domains 2 and 3 on the channel-lipid interface.[14] The presence of the oleyl moiety may be suitable for the binding of vanniloids to the TRPV1 channel, and may increase the TRPV1-activating potential,[8–11] but we report here the first evidence that the addition of the oleyl moiety to shogaol decreases the TRPV1-acrtivating potential. We confirmed the trans-configuration of the double bond at carbon-4 of oleylshogaol, suggesting that the conformation of the oleyl moiety as between oleylgingerol and oleylshogaol is similar. The difference in structure is only the hydroxy group at carbon-5. This hydroxy group may have an important role in activating a TRPV1 ion channel.

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