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Modifications of Capsaicin-Sensitive Neurons in Isolated Guinea Pig Ileum by [6]-Gingerol and Lafutidine

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Abstract. A segment of guinea pig ileum was used to confirm the hypothesis that [6]-gingerol and lafutidine interact with capsaicin-sensitive neurons. Addition of 30 and 100 μM [6]-gingerol (a pungent constituent of ginger) induced contraction of the ileum immediately. Like capsaicin, [6]-gingerol-induced contraction was inhibited by antagonists of the vanilloid receptor (capsazepine and ruthenium red), tetrodotoxin, and atropine. Treatment with [6]-gingerol up to 0.3 μM, which alone had no effect, enhanced 3 μM capsaicin-induced contraction, but greater than 3 μM [6]-gingerol significantly inhibited capsaicin-induced contraction. Treatment with lafutidine (a new type of antagonist of the histamine H₂ receptor), which was suggested to interact with capsaicin-sensitive neurons in vivo, also showed both stimulatory and inhibitory effects on capsaicin-induced contraction depending on the concentrations. Lafutidine alone had no effect. The enhanced contraction induced by capsaicin in the [6]-gingerol- or lafutidine-treated ileum was also inhibited by antagonists of the vanilloid receptor, tetrodotoxin, and atropine. Capsaicin and [6]-gingerol, but not lafutidine, at 30 μM stimulated [³H]choline release from the prelabeled slices of the ileum. These findings suggest that [6]-gingerol and lafutidine act on capsaicin-sensitive cholinergic neurons and modulate the contraction in isolated guinea pig ileum.

Keywords: [6]-gingerol, lafutidine, vanilloid receptor, enteric nervous system, guinea pig ileum

Introduction

Capsaicin, the major pungent constituent of hot peppers of the plant genus Capsicum, excites a subset of primary sensory neurons with somata in dorsal root ganglia or trigeminal ganglia (1, 2). A functional receptor termed the vanilloid receptor 1 (VR1), which is activated not only by vanilloids such as capsaicin but also by noxious heat and low pH, has been cloned (3). In general, capsaicin-sensitive neurons transmit noxious information perceived as pain or itching to the central nervous system. Activation of capsaicin-sensitive neurons in the peripheral tissues also evoked various responses (1, 4, 5). In the ileum, activation of primary afferent neurons by capsaicin causes release of transmitter substances from the neurons, which in turn activate myenteric cholinergic neurons and induce contraction of the ileum by activation of muscarinic acetylcholine (ACh) receptors (5 – 9).

Ginger is widely used as a spice throughout the world, and the rhizome of ginger has been used in traditional oriental medicine to ameliorate such symptoms as gastrointestinal discomforts, inflammation, and rheumatic disorders for centuries in East Asia. Gingerols including [6]- and [8]-gingerol are the pungent constituents of ginger, Zingiber officinale ROSCOE (a traditional Sino-Japanese medicine, a Kampo medicine). Gingerols exhibited various pharmacological effects in cardiovascular systems (10, 11) and showed positive inotropic effects on isolated atria and cardiac muscles (12 – 14). Zingerone, another pungent constituent of ginger, has been shown to have similar pharmacological effects as capsaicin, and thus it was suggested that capsaicin and zingerone could activate the same receptor and/or a common pathway in trigeminal ganglion neurons (15 – 17). However, there is no evidence...
concerning the effects of gingerols on the enteric nervous system. In the present study, we investigated whether [6]-gingerol activates capsaicin-sensitive neurons in the guinea pig ileum.

Lafutidine is a new type of antagonist of histamine H$_2$ receptors (18, 19). Since the protective effects of lafutidine against gastric ulcers were totally abolished by loss of function of capsaicin-sensitive sensory neurons in rats in vivo (19 – 22), it is suggested that the capsaicin-sensitive pathways are involved in gastro-protection induced by lafutidine. To confirm the hypothesis that [6]-gingerol and lafutidine interact with capsaicin-sensitive neurons, we examined the effects of two agents on the capsaicin-induced contraction and ACh release in the isolated ileum preparation from guinea pigs.

Materials and Methods

Animals and materials

Male, albino Dunkin-Hartley guinea pigs (300 – 400 g) were purchased from Takasugi Lab. Animals Co., Ltd. (Saitama). The animals were housed under controlled environmental conditions (temperature 24 ± 2°C and light between 7:00 a.m. and 7:00 p.m.) and fed commercial MF chows (Oriental Yeast Co., Ltd., Tokyo). The animals were fasted overnight before each experiment with free access to water. Animal experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

[3H]Choline chloride (79 Ci/mmol (2.92 TBq /mmol)) was purchased from Amersham (Buckinghamshire, UK). Capsaicin and ACh chloride were obtained from Wako (Osaka). Capsazepine was from RBI (Natic, MA, USA). Ruthenium red, famotidine, and ionomycin were purchased from Sigma (St. Louis, MO, USA). Hemicholinium-3 was obtained from Sigma-Aldrich (Steinheim, Germany). [6]-Gingerol and atropine sulfate were obtained from Nacalai Tesque (Kyoto). Hexamethonium chloride and tetrodotoxin were obtained from Tokyo-Kasei (Tokyo) and Sankyo (Tokyo), respectively. CP96345 ((2S,3S)-cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine) was from Pfizer, Inc. (Groton, CT, USA). Substance P and lafutidine were obtained from Peptide Institute, Inc. (Osaka) and Taiho Pharmaceutical Co., Ltd. (Tokyo), respectively. Capsaicin and [6]-gingerol were dissolved in a minimum of ethanol, and capsazepine and lafutidine were dissolved in a minimum of dimethyl sulfoxide. These agents were diluted with the indicated buffer when used, and the final concentration of ethanol or dimethyl sulfoxide in the assay was less than 0.5% (v/v). The pHs of the vehicles containing ethanol and/or dimethyl sulfoxide and the agents used in experiments were pH 6.5 – 7.4, and the vehicle (containing 0.5% ethanol or dimethyl sulfoxide) had no effect on the contraction and the [3H]choline release in the guinea pig ileum.

Isolated ileum preparation and measurement of contraction

The ileum preparation and measurement of contraction were done as described previously (23). Briefly, the whole segments of guinea pig ileum were removed in Krebs-Henseleit buffer (112.0 mM NaCl, 5.9 mM KCl, 2.0 mM CaCl$_2$, 1.2 mM MgCl$_2$, 1.2 mM NaH$_2$PO$_4$, 25.0 mM NaHCO$_3$, 11.5 mM glucose; pH 7.4). The segment of ileum was set up under a 1-g load in a 5-ml organ bath containing the buffer. The bath was maintained at 32°C and continuously bubbled with a gas mixture of 95% O$_2$ and 5% CO$_2$. Contraction was recorded using an isotonic transducer (Type 45347; NEC San-ei, Tokyo). At the start of each experiment, a maximal response to 3 μM ACh was obtained in each tissue to estimate the effects of the tested agents. To investigate the susceptibility of capsaicin- and [6]-gingerol-induced contraction to desensitization, segments of the ileum were incubated for 7 min with the indicated concentrations of capsaicin or [6]-gingerol, then washed more than 3 times with the stimulant-free buffer, and incubated with the indicated agents to measure contraction for the second time. The interval between the first exposure to the agents and the second exposure to the agents was about 30 min. Since the second responses of capsaicin and [6]-gingerol were desensitized easily in the same preparation as shown in Results, the concentration-response curves of capsaicin and [6]-gingerol were obtained in another ileal segment isolated from the same animal. In some experiments, segments of the ileum were incubated for 7 min with the antagonists or inhibitors, and then the response induced by contractive agents was measured in the presence of the respective antagonists or inhibitors. The antagonists or inhibitors were used at concentrations equal to or 2 times higher than those used in previous reports (5, 24 – 26). The maximal contraction induced by ACh was obtained at the concentration of 3 μM, and each response was expressed as a percentage of the contraction due to 3 μM ACh (% of ACh contraction). The addition of capsaicin or [6]-gingerol caused somewhat irregular responses, and thus the value of the peak contraction was used for the calculation.

[3H]Choline release from the prelabeled slices of the guinea pig ileum

The measurement of [3H]choline and [3H]ACh releases
from the prelabeled slices of the guinea pig ileum was carried out as follows: The mucosal component in the segments of guinea pig ileum was denuded by gently rubbing with wet-cotton. The segments were further cross-chopped to slices (400 × 400 μm) by hand, followed by filtration through a mesh (300 × 300 μm). The slices were washed twice with a modified Tyrode HEPEs buffer (137 mM NaCl, 5 mM KCl, 5 mM glucose, 2 mM MgSO₄, 2 mM CaCl₂, 20 mM HEPEs; pH 7.4) followed by centrifugation at 4°C (100 × g, 30 s). Several investigators reported that the tissues in a superfusion system were subjected to electrical stimulation in order to reduce endogenous acetylcholine, and then the acetylcholine stores were labeled with [³H]choline (27, 28). In the present study, the slices were incubated with 5 μM ionomycin for 30 min, and the washed slices were incubated for 60 min with [³H]choline (2.5 μCi/ml) at 37°C. [³H]Choline incorporated into the cholinergic nerve terminals is synthesized to [³H]ACh. The labeled slices were washed twice and suspended in the buffer. The labeled slices (300 – 500 μg protein) were incubated with the indicated agents for 10 min at 37°C. The assay mixture was further supplemented with 10 μM hemicholinium-3 to prevent the uptake of choline formed from [³H]ACh. The total volume was 300 μl and the reaction was terminated by the addition of 500 μl of ice-cold, Ca²⁺-,free, Mg²⁺-free Tyrode buffer containing 5 mM EGTA and 5 mM EDTA followed by centrifugation (2,000 × g, 30 s) at 4°C. The ³H content (the sum of [³H]choline and [³H]ACh) in the supernatant was estimated by liquid scintillation spectrometry. The values represented in the [³H]choline release are the ratio of released ³H content to the total incorporation of [³H]choline into the slices.

Statistical analyses

Values are presented as means ± S.E.M. for greater than 3 independent experiments. The number of experiments (n) refers to the number of experimental animals used. The statistical significance of differences between two groups was assessed using the two tailed Student’s t-test. Multiple comparisons against a single control group were made by one-way analysis of variance (ANOVA) with Dunnett’s multiple comparisons test. P<0.05 was considered statistically significant.

Results

Contraction induced by [6]-gingerol in guinea pig ileum

First, we confirmed 3 μM capsaicin-induced contraction in the whole segments of guinea pig ileum (Fig. 1, trace a). As described previously (5 – 9), the contraction induced by capsaicin was concentration-dependent. The maximal contraction was produced by the concentrations greater than 3 μM capsaicin, and that was about 20 – 30% of the contraction induced by 3 μM ACh (% of ACh contraction) in the present study. The effect of capsaicin was susceptible to desensitization, as in previous reports (5, 8). The segments of ileum were incubated with 3 μM capsaicin for 7 min and then thoroughly washed with the capsaicin-free buffer and used as capsaicin-treated ileum. Contraction of the capsaicin-treated ileum induced by the second exposure to 3 μM capsaicin was 1.2 ± 0.9% (n = 5), which was significantly lower than the control contraction (23.4 ± 4.4%, n = 8).

[6]-Gingerol showed no effect at lower concentrations (up to 3 μM), and it induced contraction in the ileum at higher concentrations greater than 30 μM (Fig. 1, traces b and c; Fig. 2A). The [6]-gingerol-induced contraction was susceptible to desensitization; the contraction induced by the first challenge of 30 μM [6]-gingerol was 13.3 ± 2.8% (n = 6), but the second challenge hardly elicited the contraction in the [6]-gingerol-treated ileum (2.0 ± 0.9%, n = 3; P<0.01). Capsazepine is a competitive antagonist, and ruthenium red is a functional and non-competitive antagonist of the vanilloid receptor (1, 2). In guinea pig ileum, both the antagonists at 10 μM inhibited not only 3 μM capsaicin- but also 30 μM [6]-gingerol-induced contraction markedly (Table 1). These antagonists for the vanilloid receptor did not inhibit the submaximal and maximal contractions induced by 100 nM and 3 μM ACh, respectively (n = 4). The contractions induced by 3 μM capsaicin and 30 μM [6]-gingerol in the control ileum were markedly inhibited by atropine (1 μM, an antagonist of muscarinic ACh receptor) and by tetrodotoxin (100 nM, an inhibitor of voltage-dependent Na⁺ channels) treatment.

Treatment of the segment of guinea pig ileum for 40 min with 20 μM indomethacin (an inhibitor of cyclooxygenases) did not modify the contractions induced by 100 μM [6]-gingerol and by 3 μM capsaicin (Table 2). Treatment with hexamethonium (100 μM, an antagonist of nicotinic ACh receptor and thus a ganglionic blocking agent) for 10 min did not show an inhibitory effect on [6]-gingerol- and capsaicin-induced contraction. Treatment with 2 μM CP96345, an antagonist for neurokinin 1 (NK₁) receptors (29), almost completely inhibited the contraction induced by 1 nM substance P, but not that by 100 μM [6]-gingerol and 3 μM capsaicin. The maximal contraction induced by 100 nM substance P was also markedly inhibited in the CP96345-treated ileum: 70 – 80% and 30 – 35% (of ACh response) in the control and the CP96345-treated ileum, respectively.
Dual effects of [6]-gingerol treatment on capsaicin-induced contraction

Treatment with [6]-gingerol at lower concentrations between 3 nM and 0.3 μM, which alone did not induce contractions, enhanced the 3 μM capsaicin-induced contractions; the contraction was significantly enhanced in the 0.3 μM [6]-gingerol-treated ileum (Fig. 1, trace b and Fig. 2A). The enhanced contraction induced by capsaicin in the [6]-gingerol-treated ileum was inhibited by the antagonists of the vanilloid receptor, atropine, and tetrodotoxin (Table 1). In addition, pretreatment with [6]-gingerol at concentrations greater than 3 μM inhibited the contraction induced by the second challenge of 3 μM capsaicin; the contraction was almost completely abolished in the 100 μM [6]-gingerol-treated ileum (Fig. 1, trace c). The submaximal contraction induced by 100 nM ACh (Fig. 1, traces f and g) and the maximal contraction by 3 μM ACh in the 0.3 μM [6]-gingerol-treated ileum were almost the same as those in the control ileum (n = 4). In the 100 μM [6]-gingerol-treated ileum, the response by 100 nM ACh was slightly (20 – 30%) inhibited, and the response by 3 μM ACh was hardly (82.4 ± 5.7%, n = 4, P = 0.054) inhibited.

Dual effects of lafutidine treatment on capsaicin-induced contraction

Next, we investigated whether lafutidine regulates capsaicin-induced contraction in isolated guinea pig ileum (Fig. 1, traces d and e). Lafutidine alone did not induce contraction in the ileum at any concentrations (Fig. 2B). Treatment with lafutidine at low concentrations from 0.1 to 1 μM enhanced the contraction induced by 3 μM capsaicin; the contraction was significantly enhanced in the 1 μM lafutidine-treated ileum. The enhanced contraction induced by capsaicin in the 1 μM lafutidine-treated ileum was almost completely eliminated by the antagonists for the vanilloid receptor, atropine, and tetrodotoxin (Table 1). Pretreatment with lafutidine at higher concentrations between 10 μM and 1 mM inhibited the contraction induced by 3 μM capsaicin (Fig. 2B). The capsaicin-induced contraction in the 100 μM lafutidine-treated ileum was significantly smaller than that in the 1 μM lafutidine-treated ileum.
Effects of capsaicin, [6]-gingerol, and lafutidine on \[^{3}H\]\textit{choline} release from the prelabeled slices of guinea pig ileum

The addition of 30 \(\mu\text{M}\) capsaicin or 30 \(\mu\text{M}\) [6]-gingerol stimulated \[^{3}H\]choline (and \[^{3}H\]ACh) release from the prelabeled slices of the ileum in the presence of 2 mM \(\text{CaCl}_2\) (Table 3). The \[^{3}H\]choline releases induced by capsaicin and [6]-gingerol in the absence of extracellular \(\text{CaCl}_2\) were markedly less than those in the presence of 2 mM \(\text{CaCl}_2\) (data not shown, \(n=4\)). The release induced by co-addition of 30 \(\mu\text{M}\) capsaicin and 30 \(\mu\text{M}\) [6]-gingerol was significant compared with the control, but was not additive by the two stimulants. Lafutidine (10 \(\mu\text{M} - 1 \text{mM}\)) alone had no effect on \[^{3}H\]choline release. Co-addition of 30 \(\mu\text{M}\) lafutidine inhibited 30 \(\mu\text{M}\) capsaicin-induced \[^{3}H\]choline release. Because of wide variations, we could not detect significant effects of the agents at low concentrations and could not study the effect of pretreatment with [6]-gingerol or lafutidine on 3 \(\mu\text{M}\) capsaicin-induced \[^{3}H\]choline release.

### Table 1. Effects of capsazepine, ruthenium red, atropine, and tetrodotoxin on capsaicin- and [6]-gingerol-induced contraction in the vehicle-, [6]-gingerol-, or lafutidine-treated ileum

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Addition</td>
<td>3 (\mu\text{M}) Capsaicin</td>
<td>30 (\mu\text{M}) [6]-Gingerol</td>
<td>3 (\mu\text{M}) Capsaicin</td>
<td>3 (\mu\text{M}) Capsaicin</td>
</tr>
<tr>
<td>Capsazepine</td>
<td>20.3 (\pm) 2.4</td>
<td>13.3 (\pm) 2.8</td>
<td>47.6 (\pm) 1.4</td>
<td>38.3 (\pm) 4.2</td>
</tr>
<tr>
<td>Ruthenium red</td>
<td>8.7 (\pm) 3.3</td>
<td>2.0 (\pm) 0.3</td>
<td>9.4 (\pm) 0.3</td>
<td>4.5 (\pm) 2.6</td>
</tr>
<tr>
<td>Atropine</td>
<td>4.3 (\pm) 1.7</td>
<td>0.2 (\pm) 0.4</td>
<td>3.2 (\pm) 1.7</td>
<td>0.8 (\pm) 0.5</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>0.6 (\pm) 0.5</td>
<td>0.1 (\pm) 0.3</td>
<td>1.1 (\pm) 0.4</td>
<td>2.3 (\pm) 2.3</td>
</tr>
</tbody>
</table>

The guinea pig ileum segments were incubated for 7 min with vehicle (None), 10 \(\mu\text{M}\) capsazepine, 10 \(\mu\text{M}\) ruthenium red, 1 \(\mu\text{M}\) atropine, or 100 \(\mu\text{M}\) tetrodotoxin. Then 3 \(\mu\text{M}\) capsaicin or 30 \(\mu\text{M}\) [6]-gingerol were added to measure contraction. In some experiments, the segments were incubated with 0.3 \(\mu\text{M}\) [6]-gingerol or 1 \(\mu\text{M}\) lafutidine for 7 min, and then the indicated concentrations of antagonists or inhibitor were added and further incubated for additional 7 min. Then 3 \(\mu\text{M}\) capsaicin was added to measure contraction. Values are each the mean \(\pm\) S.E.M. for 4 – 6 independent animals. \(^bP<0.05\), statistically significant compared with the capsaicin-induced contraction in the vehicle-treated ileum. \(^bP<0.05\), statistically significant compared with the value without the indicated antagonists or inhibitor (None).
The contraction of [6]-gingerol at lower concentrations up to 0.3 μM enhanced the 3 μM capsaicin-induced contraction, and the enhanced contraction was eliminated by antagonists of vanilloid receptors. Fourth, treatment with higher concentrations greater than 3 μM of [6]-gingerol inhibited the capsaicin-induced contraction. [6]-Gingerol at any concentrations barely modified the contraction induced by activation of muscarinic ACh receptors on smooth muscles in the ileum. There is some evidence that capsaicin and zingerone can activate the same receptor and/or a common pathway on the afferent neurons in vivo and on the trigeminal ganglion neurons in vitro (15 – 17). Although [6]-gingerol and zingerone structurally differ from capsaicin in that the two agents have a shorter hydrophobic moiety and lack an acyl-amide moiety, both have a vanillyl-like moiety as is seen in the structure of capsaicin. Thus, it is probable that [6]-gingerol activates the capsaicin-sensitive vanilloid receptor.

Barthó and Vizi (6) reported that activation of vanilloid receptors with capsaicin stimulated the release of 3H radioactivity from the myenteric plexus-longitudinal muscle preparation of the guinea pig ileum preincubated with [3H]choline. In the present study, not only capsaicin but also [6]-gingerol stimulated [3H]choline release from the prelabeled slices of the ileum (Table 3). In addition, treatment with atropine or tetrodotoxin almost completely inhibited not only capsaicin- but also [6]-gingerol-induced contractions (Table 1). These findings suggest that the contraction induced by [6]-gingerol were mediated by activation of vanilloid receptor and ACh release in the neurons and the following activation of muscarinic ACh receptors in the ileum.

### Table 2. Effects of indomethacin, hexamethonium, and CP96345 on capsaicin- and [6]-gingerol-induced contraction

<table>
<thead>
<tr>
<th>Addition</th>
<th>Capsaicin</th>
<th>[6]-Gingerol</th>
<th>Substance P</th>
</tr>
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<tbody>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (None)</td>
<td>24.3 ± 4.3</td>
<td>20.8 ± 5.4</td>
<td>Not determined</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>22.8 ± 3.6</td>
<td>19.8 ± 3.6</td>
<td>Not determined</td>
</tr>
<tr>
<td>Experiment II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (None)</td>
<td>23.3 ± 3.8</td>
<td>21.8 ± 4.5</td>
<td>20.2 ± 4.2</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>18.8 ± 3.4</td>
<td>19.9 ± 3.8</td>
<td>Not determined</td>
</tr>
<tr>
<td>CP96345</td>
<td>17.5 ± 2.8</td>
<td>18.7 ± 3.2</td>
<td>1.8 ± 1.2a</td>
</tr>
</tbody>
</table>

In Experiment I, the guinea pig ileum segments were incubated for 40 min with vehicle (None) or 20 μM indomethacin. In Experiment II, the segments were incubated for 10 min with vehicle, 100 μM hexamethonium, or 2 μM CP96345. Then 3 μM capsaicin, 100 μM [6]-gingerol, or 1 μM substance P was added to measure contraction. Values are each the mean ± S.E.M. for 3 – 4 independent animals. *P<0.05, statistically significant compared with the value without CP96345.

### Table 3. Effects of capsaicin, [6]-gingerol, and lafutidine on [3H]choline release from the prelabeled slices of guinea pig ileum

<table>
<thead>
<tr>
<th>Addition</th>
<th>[3H]Choline release (% of total)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>10.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>13.9 ± 0.7a</td>
</tr>
<tr>
<td>30 μM [6]-Gingerol</td>
<td>14.9 ± 0.8a</td>
</tr>
<tr>
<td>30 μM Lafutidine</td>
<td>10.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>10.7 ± 0.5b</td>
</tr>
</tbody>
</table>

The prelabeled slices were incubated with vehicle (None), 30 μM capsaicin, 30 μM [6]-gingerol, or 30 μM lafutidine for 10 min at 37°C. Values were calculated as percentages relative to the total incorporation of [3H]choline into the slices. Values are each the mean ± S.E.M. for 3 independent animals. *P<0.05, statistically significant compared with the control value (None). *P<0.05, statistically significant compared with the value with capsaicin.

### Discussion

#### Involvement of capsaicin-sensitive neurons on [6]-gingerol-induced contraction of guinea pig ileum

In the present study, we found that [6]-gingerol induced contraction alone and also enhanced the capsaicin-induced contraction in guinea pig ileum. The effects of [6]-gingerol appeared to be mediated by activation of vanilloid receptors on the neurons in the ileum. First, the effects of [6]-gingerol were almost completely inhibited by capsazepine and ruthenium red. Although these agents were reported to have non-specific actions such as inhibition of Ca2+ channels (30, 31), these agents at 10 μM markedly inhibited capsaicin- and [6]-gingerol-induced contractions without changing the ACh response. Second, the effect of [6]-gingerol was desensitized easily. Third, treatment of the ileum with [6]-gingerol at lower concentrations up to 0.3 μM enhanced the 3 μM capsaicin-induced contraction, and the enhanced contraction was eliminated by antagonists of vanilloid receptors. Fourth, treatment with higher concentrations greater than 3 μM of [6]-gingerol inhibited the capsaicin-induced contraction. [6]-Gingerol at any concentrations barely modified the contraction induced by activation of muscarinic ACh receptors on smooth muscles in the ileum. There is some evidence that capsaicin and zingerone can activate the same receptor and/or a common pathway on the afferent neurons in vivo and on the trigeminal ganglion neurons in vitro (15 – 17). Although [6]-gingerol and zingerone structurally differ from capsaicin in that the two agents have a shorter hydrophobic moiety and lack an acyl-amide moiety, both have a vanillyl-like moiety as is seen in the structure of capsaicin. Thus, it is probable that [6]-gingerol activates the capsaicin-sensitive vanilloid receptor.

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#### Involvement of NK1 receptors and prostanoids formation on [6]-gingerol-induced contraction

Treatment with 100 μM hexamethonium had no effect on capsaicin- and [6]-gingerol-induced contraction in the guinea pig ileum. Like capsaicin (7), the effect of [6]-gingerol did not appear to be mediated by nicotinic ganglionic neurons. The capsaicin-induced contractions in many gastrointestinal preparations are reported to be mediated through the release of neuropeptides such as neurokinins from capsaicin-sensitive neuronal structures (9, 32, 33). Barthó et al. (5) reported that the contraction evoked by capsaicin in the ileum was not reduced when NK1 or NK3 receptors were blocked separately, whereas a combined blockade of NK receptors significantly depressed the capsaicin response. Although an antagonist of the NK1 receptor alone had no effect on capsaicin- and [6]-gingerol-induced contraction in the present study, we could not exclude the involvement of other
NK receptors in the contractile responses. In addition, it should be determined whether ACh is released from the neurons having VR1 directly and/or through the possible involvement of other inter-neurons having neurokinins and other mediators.

[6]-Gingerol potentiated prostaglandins (F2αr, E2 and I2)-induced contractions in mouse mesenteric veins, although [6]-gingerol alone showed no effect (10, 11). Since it has been established that stimulation of prostanoid receptors caused contraction in the guinea pig ileum (34), prostaglandin-like compounds may be involved in [6]-gingerol-induced contraction. However, [6]-gingerol is reported to be an inhibitor of prostaglandin synthetase and 5-lipoxygenase in vitro (35, 36). As treatment with indomethacin did not modify the contraction induced by [6]-gingerol, the effects of [6]-gingerol did not appear to be due to prostaglandin(s) formation.

Possible interaction to capsaicin-sensitive neurons by lafutidine

Lafutidine is a new type antagonist of histamine H2 receptors (18, 19). Several studies including ours suggested that the capsaicin-sensitive neurons are involved in the gastro-protection induced by lafutidine in vivo (19–22). In the present study, treatment of the isolated ileum preparation with 1 μM lafutidine, which alone showed no effect, enhanced the 3 μM capsaicin-induced contraction. The enhancement of the capsaicin response induced by lafutidine was abolished by tetrodotoxin and by atropine. Onodera et al. (19) also reported that the gastro-protective effect of lafutidine in rats was decreased by intra-arterial infusion of tetrodotoxin in vivo. Treatment with lafutidine at higher concentrations (100 μM and 1 mM) showed a phenomena similar to desensitization; inhibition of the contraction induced by 3 μM capsaicin. Famotidine had no effect on the capsaicin response. The addition of 30 μM lafutidine alone did not stimulate [3H]choline release from the prelabeled slices of the ileum, but co-addition of lafutidine inhibited the capsaicin-induced [3H]choline release. Although the reasons are not clear at present, this observation may be in relation to the desensitization. The present study is the first to report the involvement of the capsaicin-sensitive neurons and/or pathway on the response induced by lafutidine in vitro (in isolated preparation), to our knowledge.

Summary and problems to be determined

Recently, Dedov et al. (37) reported that [6]-gingerol evoked intracellular Ca2+ transients in rat dorsal root ganglion neurons via VR1. In the present study, [6]-gingerol at concentrations greater than 30 μM alone induced contraction of the ileum via activation of vanilloid receptors. Treatment with [6]-gingerol or lafutidine depending on their concentrations showed dual effects on capsaicin-induced contraction of the ileum: stimulation at lower concentrations and inhibition at higher concentrations. Our conclusion is that both [6]-gingerol and lafutidine have some interactions with capsaicin-sensitive neurons and/or vanilloid receptors and thus regulate the contraction via ACh release in isolated guinea pig ileum in vitro.

Pharmacological evidence suggested the existence of vanilloid receptor subtypes with distinct characteristics (38), and a homolog of VR1 was cloned (39). [6]-Gingerol and lafutidine may interact with these putative homologs of VR1. Specifically, the molecular mechanism clarifying how lafutidine regulates the capsaicin-sensitive pathway remains to be clarified. Some natural products, which are used extensively in indigenous medicine, are proposed as novel-type vanilloid receptor agonists, and these compounds may be useful for development of ligands of vanilloid receptors (40). Further studies on screening and identification of natural products and/or endogenous compounds that can regulate the functions of vanilloid receptors are currently in progress in our laboratory.

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