Note

[6]-Gingerol Induces Amiloride-Sensitive Sodium Absorption in the Rat Colon via the Capsaicin Receptor TRPV1 in Colonic Mucosa

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Summary [6]-Gingerol possesses various beneficial pharmacological and therapeutic properties, including anti-carcinogenic and anti-inflammatory properties and the ability to regulate intestinal contraction. Recently, our group observed that the serosal administration of [6]-gingerol stimulated electrogenic sodium absorption in the rat colon via the capsaicin receptor, TRPV1. TRPV1 is known to be expressed in both the mucosal epithelium and the muscle layers in the colon. In the present study, we assessed whether [6]-gingerol stimulated sodium absorption via TRPV1 in the colonic mucosal epithelium. We compared the effect of [6]-gingerol on TRPV1-dependent colonic sodium absorption in the colon preparation with or without muscle layer. All experiments were performed by measuring the transmural potential difference (ΔPD) in an Ussing chamber system. [6]-Gingerol induced positive ΔPD when administered to the serosal side of the colon, and this effect was significantly larger in the colon preparation without muscle layer than in that with the muscle layer. In the colon preparation without muscle layer, the [6]-gingerol-dependent induction of ΔPD was markedly suppressed by mucosal addition of amiloride, a selective inhibitor of epithelial sodium channel. ΔPD induction by [6]-gingerol was considerably diminished by capsazepine, an inhibitor of the capsaicin receptor TRPV1, but not by AP-18, an inhibitor of TRPA1. These results suggest that [6]-gingerol induces amiloride-sensitive electrogenic sodium absorption in the rat colon via TRPV1 expressed in the colonic mucosal epithelium, and that this effect is independent of TRPV1 in the colonic muscle layer.

Key Words [6]-gingerol, colon epithelium, electrogenic sodium absorption, TRPV1

The ginger plant (Zingiber officinale) is used globally not only as a spice but also as herbal medicine to treat a wide array of ailments. Gingerols, the bioactive components of ginger, exert a variety of beneficial pharmacological and therapeutic effects, including anti-carcinogenic, anti-inflammatory, and anti-emetic activities (1–4).

[6]-Gingerol exhibits anti-emetic properties in the gastrointestinal tract (3, 4). In addition, [6]-gingerol inhibits contractions of the ileum induced by the activation of the serotonin receptor 5-HT3 (5). In contrast, treatment with a single dose of [6]-gingerol has been shown to induce contractions of the ileum (6).

Studies show that contractions of the intestine correlate with mucosal ion transport. Fung et al. observed that vasoactive intestinal peptide VIP stimulates ACh-mediated longitudinal muscle contraction and induces Cl− secretion in guinea pig jejunum (7). Studies in the rat colon showed that endothelin-1 induced bowel contraction and epithelial chloride secretion (8), and that adrenomedullin modulated water and chloride ion transport, which correlated with bowel contraction (9). These contribute to the smooth movement of intestinal contents and efficient absorption of nutrients. Although [6]-gingerol has been shown to regulate contractions of gastrointestinal parts, it was unclear whether these effects are related to its effect on gastrointestinal ion transport. Recently, our group demonstrated that the serosal administration of [6]-gingerol stimulated electrogenic sodium absorption in the rat colon via the capsaicin receptor, transient receptor potential (TRP) cation channel subfamily V member 1 (TRPV1), but did not influence bumetanide-sensitive colonic electrogenic chloride or potassium secretion (10). TRPV1 is a non-selective cation channel expressed mainly in nociceptive sensory neurons and is responsible for the detection and regulation of body temperature (11). TRPV1 has been shown to be efficiently activated by [6]-gingerol in many physiological functions (12, 13).

TRPV1 is expressed in both the mucosa and muscle layer in the rat and mouse colon (14, 15). Matsumoto et al. observed that TRPV1 was localized at nerve fibers.
both in Meissner’s plexus extending to the batholateral side of the mucosal cells and at Auerbach’s plexus of the muscular layer in the mouse colon (15). In our previous study, intact colon tissue containing mucosal, submucosal, and muscle layer was used in the experiments (10). Thus, our previous results suggested at least two possibilities for the stimulation of electrogenic sodium absorption by [6]-gingerol. First, [6]-gingerol binds directly to TRPV1 in the nerve fibers of the mucosa. Second, [6]-gingerol binds to the neuronal receptors in the muscle layer and subsequently stimulates the electrogenic sodium absorption in the mucosal cells mechanically and/or chemically. However, we could not determine whether [6]-gingerol stimulated sodium absorption via TRPV1 expressed in the mucosa or muscle layer in the colon in our previous study. To address this question, we compared the effect of [6]-gingerol on TRPV1-dependent colonic sodium absorption in the colon tissue preparation with or without muscle layer by measuring the transmural potential difference in the Ussing chamber system in this study.

Materials and Methods

[6]-Gingerol, capsazepine, and AP-18 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). [6]-Gingerol was dissolved in dimethyl sulfoxide (DMSO). Capsazepine and AP-18 were dissolved in 100% ethanol. Amiloride was purchased from Enzo Life Sciences (Lausen, Switzerland) and dissolved in water.

Nine-week-old Sprague Dawley rats (CLEA Japan, Inc., Shizuoka) weighing approximately 300 g were fasted for 12 h prior to the experiments. The animals were treated in accordance with the institutional and national guidelines for the care and use of laboratory animals. The study was approved by the Animal Usage Ethics Committee of Tohoku Women’s College (approval number: 2015-01, 2017-03). Rats were anesthetized with urethane (1.0 g/kg ip). A segment of colon was isolated within 10 min after anesthesia and then rats were euthanized by overdose of urethane.

The transmural potential difference (∆PD) was measured in vitro in Ussing chambers. A segment of the rat colon was opened longitudinally on a flat sheet. The serosa, submucosa, and muscular layer were removed with fine forceps to obtain a mucosa preparation without muscle layer. To obtain a mucosa preparation with muscle layer, only the serosa layer was removed with fine forceps. The tissue was mounted vertically between Ussing chambers made from acrylic resin with an internal surface area of 0.5 cm². The bathing solution (10 mL) in each chamber was maintained at 37°C in a water-jacketed reservoir. The components of the bathing solution were 119 mM NaCl, 21 mM NaHCO₃, 2.4 mM K₂HPO₄, 0.6 mM KH₂PO₄, 1.2 mM MgCl₂, 1.2 mM CaCl₂, and 10 mM glucose, under an atmosphere of 95% O₂ and 5% CO₂ (pH 7.4). To measure ∆PD, 2% agar containing 1 M KCl bridges were placed both on the serosal and mucosal sides of the colon tissue preparation. ∆PD was continuously measured by connecting calomel half-cells to the mucosal and serosal solution using 2% agar bridges and was recorded using a high-sensitivity DC chart recorder (056-1001, Hitachi, Tokyo, Japan) (10). The ∆PD value was considered positive when cations were transported from the mucosal to the serosal side of the intestine.

Data are expressed as means±standard error (SE). Statistical comparison between two means was performed using the paired Student’s t-test. More than three mean values were compared by analysis of variance (ANOVA), followed by the Bonferroni-Dunn post hoc test using the StatView software (SAS Institute). Differences with p-values less than 0.05 were considered significant.

Results and Discussion

To determine the effect of [6]-gingerol on the ion Fig. 1. ∆PD after [6]-gingerol administration on the serosal sides of the rat colon tissue with or without muscle layer. Typical tracings of colon tissue with muscle layer (A) and without muscle layer (B). The arrows indicate the time of 10 μM [6]-gingerol administration. C shows [6]-gingerol-induced maximal ∆PD in the rat colon tissue with (+ muscle tissue) or without (mucosal tissue) muscle layer. Values are presented as mean±SE (n=4). * p<0.05 compared to + muscle tissue group.
transport in the rat colon with or without muscle layer. 10 μM [6]-gingerol was added to the serosal side of the colon tissue preparation. As shown in Fig. 1A and B, [6]-gingerol application induced an increase in ΔPD in the both types of colon preparation, which reached its maximum value within 2 min. Subsequently, ΔPD gradually returned to the basal level within 10–15 min. The [6]-gingerol-dependent induction of ΔPD was significantly higher in the colon without muscle layer (mucosal tissue) than in those with muscle layer (+ muscle tissue) (Fig. 1C). Our previous study reported that colonic transmural ΔPD evoked by [6]-gingerol was mostly due to ouabain-sensitive electrogenic sodium absorption (10). It is well known that sodium is absorbed via amiloride-sensitive sodium channels in the apical membrane of the colonic epithelium (16). Figure 2 shows that the [6]-gingerol-dependent induction of ΔPD in the mucosal tissue was markedly suppressed by mucosal addition of amiloride. This suggests that [6]-gingerol-dependent induction of electrogenic sodium absorption is markedly higher in the mucosal tissue than in the tissue with muscle.

Previously, we reported that [6]-gingerol induced colonic electrogentic sodium transport via interaction with the capsaicin receptor TRPV1 in the + muscle tissue (10). Studies show that TRPV1 is expressed in both mucosa and muscle layer in the rat colon (14). To determine whether [6]-gingerol stimulated sodium absorption in the mucosal tissue of colon via TRPV1, we pretreated the serosal side of the mucosal tissue of colon with the TRPV1 antagonist capsazepine (30 μM) for 30 min prior to the addition of [6]-gingerol. Capsazepine almost completely blocked the [6]-gingerol-stimulated electrogenic sodium absorption (Fig. 3). On the other hand, pretreatment with AP-18 (antagonist of TRPA1, another TRP family member) had no effect on the [6]-gingerol-stimulated electrogenic sodium absorption. These results suggested that [6]-gingerol induced electrogenic sodium transport via an interaction with TRPV1 expressed in the colonic epithelium.

The present study showed that [6]-gingerol induced amiloride-sensitive electrogenic sodium absorption in the rat colon via the capsaicin receptor TRPV1 but not TRPA1 in the colonic mucosa. In our previous study, we observed that the serosal administration of [6]-gingerol stimulated electrogenic sodium absorption via TRPV1 in the intact rat colon containing muscle layers. In the rat colon, TRPV1 is mainly localized in the nerve fibers in the basolateral side of the mucosa and submucosa (17) and in the mouse colon (15). TRPV1 has also been detected in the nerve fibers of the muscle layer (14, 15). Our present result suggested that the stimulation of electrogenic sodium absorption was able to occur via direct [6]-gingerol binding to TRPV1 in the mucosa. However, we cannot exclude the possibility that the muscle layer secondarily influenced the effect of [6]-gingerol on the mucosal electrogenic sodium absorption in the colon because our present result showed that the [6]-gingerol dependent induction of sodium absorption was significantly higher in the colon tissue without muscle layer than in that with muscle layer (Fig. 1). It is well known that sodium ions are absorbed via sodium channels on the apical membrane and are excreted via Na+/K+-ATPase on the basolateral membrane in the epithelium, including the colon, the distal nephron, and the respiratory epithelium (16, 18, 19). Our present study also suggests the possibility that activation of TRPV1 expressed in the colonic muscle layer secondarily decreased the vectorial transepithelial sodium transport in the mucosa, probably by mechanical and/or chemical stimulatory effect of the muscle layer. More study is needed to elucidate this possibility.

In the epithelial tissue, including colon, kidney and lung, electrogenic sodium absorption occurs via an amiloride-sensitive epithelial sodium channel (ENaC) located in the apical membrane of epithelial cells (20, 289).
It has been reported that several substances and second messengers activate ENaC by increasing its open probability (Po) and/or translocation to the apical membrane (21−23). Greenlee et al. observed that prolactin increased both Po and translocation to the apical membrane of ENaC in renal epithelial cells (22). To understand the mechanisms of [6]-gingerol on ENaC activation, examining the effect of [6]-gingerol on Po and intracellular behavior of ENaC will be interesting.

Ion transport in the colon is regulated by several second messengers. Our earlier results showed that intracellular cAMP mediated β-adrenergic stimulation of electrogenic sodium transport in the rat colon (24, 25). An increase in intracellular Ca^{2+} was also demonstrated to be involved in the upregulation of electrogenic sodium absorption by hypotonicity in renal epithelial cells (26). [6]-Gingerol was shown to induce intracellular Ca^{2+} via TRPV1 (13, 27). Shin et al. observed that capsaicin mediates saliva secretion via TRPV1 (27). In their study, the binding of capsaicin to TRPV1 stimulated chloride ion secretion via induction of intracellular Ca^{2+} in saliva secretion. Although our present study did not investigate the involvement of intracellular Ca^{2+} and the direct effect of [6]-gingerol on water transport in the colon, we hypothesize that [6]-gingerol-induced colonic sodium absorption may contribute to water retention in the body and exert anti-diarrheal effects. Additional experiments are required to elucidate the direct involvement of intracellular Ca^{2+} and other second messengers in the effect of [6]-gingerol on electrogenic sodium absorption and subsequent water absorption in the colon.

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