[6]-Gingerol Induces Electrogenic Sodium Absorption in the Rat Colon via the Capsaicin Receptor TRPV1

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Summary [6]-Gingerol possesses a variety of beneficial pharmacological and therapeutic properties, including anti-carcinogenic, anti-inflammatory, and anti-emetic activities. Although [6]-gingerol is known to regulate the contraction of the intestine, its effect on intestinal ion transport is unclear. The aim of this study was to examine the role of [6]-gingerol in the regulation of electrogenic ion transport in the rat intestine by measuring the transmural potential difference (ΔPD). [6]-Gingerol induced significant positive ΔPD when administered to the serosal but not mucosal side of the colon, ileum, and jejunum; the highest effect was detected in the colon at a concentration of 10 μM. [6]-Gingerol-induced increase in ΔPD was suppressed by ouabain, an inhibitor of Na+/K+-ATPase, whereas no effect was observed in response to bumetanide, an inhibitor of the Na+/K+2Cl−-cotransporter. In addition, ΔPD induction by [6]-gingerol was greatly diminished by capsazepine, an inhibitor of the capsaicin receptor TRPV1. These results suggest that [6]-gingerol induced the electrogenic absorption of sodium in the rat colon via TRPV1.

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

The ginger plant (Zingiber officinale) is used globally not only as a spice but also as a herbal medicine to treat a wide array of ailments. Gingerols, the bioactive components of ginger, have been shown to exert a variety of beneficial pharmacological and therapeutic effects. In particular, [6]-gingerol possesses anti-carcinogenic (1, 2), anti-inflammatory (3–5) and anti-emetic properties (6, 7).

With respect to the anti-emetic activity, it has been found that [6]-gingerol inhibits contractions of the ileum induced by the activation of the serotonin receptor 5-HT3 (8). In contrast, a single treatment of [6]-gingerol has been shown to induce contractions of the ileum (9). In addition, [6]-gingerol has been shown to affect gastric motility and secretion, and Okumi et al. found that the oral administration of [6]-gingerol inhibited gastric acid secretion in mice (10).

It has been established that contraction of the intestines correlates with mucosal ion transport. See et al. found that in the rat jejunum the submucosal myenteric plexus alone integrates the reflex that couples ion transport to muscle contraction (11). Studies in the rat colon have reported that endothelin-1 induced bowel contraction and epithelial chloride secretion (12) and that adrenomedullin modulated water and chloride ion transport correlated with the bowel contraction (13). These contribute the smooth movement of intestinal contents and efficient absorption of nutrients. Although [6]-gingerol has been shown to regulate contractions of the gastrointestinal parts, it is unclear whether these effects are related to its activity in gastrointestinal ion transport. The aims of this study were to investigate the effect of [6]-gingerol on the electrogenic ion transport in rat intestines by measuring the transmural potential difference (ΔPD) and to elucidate the related factor to regulate that mechanism. Our results indicate that [6]-gingerol stimulates electrogenic sodium absorption in the colon via TRPV1.

MATERIALS AND METHODS

Experimental animals. The animals used in this study were treated in accordance with the institutional and national guidelines for the care and use of laboratory animals and the study was approved by the Animal Usage Ethics Committee of Tohoku Women’s College. Everted and non-everted intestinal sacs (length, 4.0 cm) were prepared from the jejunum, ileum, and colon of male Sprague Dawley rats (CLEA Japan, Inc., Shizuoka), 9 wk old and weighing approximately 320 g; the animals were fasted for 12 h prior to the experiments. Rats were anesthetized with urethane (1.0 g/kg ip). Briefly, a 5-cm section of the intestine comprising the jejunum, ileum, and colon was removed and tied off with a thread. The everted sacs were prepared by slowly evertting the intestine using a glass bar. For measuring ΔPD, the intestinal sac was connected to a silicon tube (outer diameter, 4 mm) and filled with a standard buffer (137 mM NaCl, 3 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 0.4 mM NaH2PO4, 12 mM NaHCO3, 5 mM glucose, pH 7.4). In all experiments, the intestinal sacs were incubated at 37°C in 40 mL of Tyrode’s solution oxygenated with 95% O2/5% CO2 gas.

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Measurement of transmural potential in the rat intestines.

The intestinal sacs connected to a silicon tube were incubated in Tyrode’s solution and 2% agar containing 1 mM KCl bridges were kept both on the inside and outside of the intestinal sacs. $\Delta PD$ was continuously measured by connecting calomel half-cells to the mucosal and serosal solution by means of 2% agar bridges, and was recorded using a high-sensitivity DC chart recorder (056-1001, Hitachi, Tokyo, Japan) (14). The $\Delta PD$ value was expressed as positive when cations were transported from the mucosal side to the serosal side of the intestine. In all experiments, [6]-gingerol and each inhibitor were applied to 40 mL of Tyrode’s solution.

Chemicals. [6]-Gingerol, bumetanide, and capsazepine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). [6]-Gingerol and bumetanide were dissolved in DMSO and capsazepine was dissolved in 100% ethanol. Ouabain octahydrate was purchased from Sigma Aldrich (St. Louis, MO) as a concentrated stock solution in water.

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

RESULTS

Effect of [6]-gingerol serosal and mucosal application on transmural ion transport

To determine the effect of [6]-gingerol on the ion transport in the rat intestines, 10 $\mu$M [6]-gingerol was added to the serosal or mucosal side of the rat colon, ileum, and jejunum. As shown in Fig. 1, the administration of [6]-gingerol to the mucosal side did not induce changes in PD in any of the intestinal sections. On the other hand, [6]-gingerol application to the serosa induced an increase in $\Delta PD$, which reached its maximum value within 3 min. In the colon and ileum, $\Delta PD$ gradually returned to the basal level within 20–40 min. Figure 2 shows that the [6]-gingerol-dependent induction of transmural $\Delta PD$ was markedly higher in the colon than in the ileum and jejunum and that the $\Delta PD$ induced by serosal administration of [6]-gingerol was insignificant and comparable to that observed after mucosal addition in the ileum and jejunum. The increases in colonic transmural $\Delta PD$ stimulated by [6]-gingerol were concentration-dependent (Fig. 3).

Effect of [6]-gingerol on electrogenic sodium absorption in the rat colon

Figures 1–3 show that [6]-gingerol induced an increase of transmural electrogenic ion transport in the colon. [6]-Gingerol was cumulatively administered to the serosal side of the colon to reach 1, 3, 10, and 30 $\mu$M levels. Values are presented as mean $\pm$ SE ($n$=4). *$p<0.05$ compared to mucosal side of the same intestinal section; # $p<0.05$ compared to serosal side of the ileum and jejunum.

Statistical comparisons were performed using the paired Student’s t-test. More than three mean values were compared by ANOVA followed by the Bonferroni-Dunn post hoc test using StatView software (SAS Institute, Cary, NC). Differences with $p$ values less than 0.05 were considered significant.

Effect of [6]-gingerol on electrogenic sodium absorption in the rat colon

Figures 1–3 show that [6]-gingerol induced an increase of transmural electrogenic ion transport in the colon. Given that sodium is the major cation transported across the colon wall, we sought to determine the involvement of electrogenic sodium absorption in [6]-gingerol-stimulated $\Delta PD$. The Na$^+$/K$^+$-ATPase inhibitor ouabain (1 mM) was added to the serosal side of the colon for 90 min and the [6]-gingerol-dependent stimulation of transmural $\Delta PD$ was assessed.
Effect of Ginger on Na Absorption

Fig. 4. The effect of [6]-gingerol on electrogenic sodium absorption in the colon. The experiment was performed with (hatched bar) or without (white bar) 1 mM ouabain in the serosal side of the colon. After ouabain pretreatment for 90 min, 10 μM [6]-gingerol was administered to the serosal side of the colon. Values are presented as mean±SE (n=4) of maximal ΔPD. *p<0.05 compared to the control group.

Fig. 5. The effect of [6]-gingerol on electrogenic chloride secretion and potassium absorption in the colon. After pretreatment with 0.1 mM bumetanide (hatched bar) or the same volume of DMSO (white bar) for 90 min, 10 μM [6]-gingerol was administered to the serosal side of the colon. Values are presented as mean±SE (n=4) of maximal ΔPD.

Fig. 6. Involvement of the capsaicin receptor in the increase of electrogenic sodium absorption induced by [6]-gingerol. After pretreatment with 30 μM capsa zepine (hatched bar) or the same volume of 100% ethanol (white bar) for 30 min, 10 μM [6]-gingerol was administered to the serosal side of the colon. Values are presented as mean±SE (n=4) of maximal ΔPD. *p<0.05 compared to the control group.

The impact of ouabain suppressed the stimulatory effects of 10 μM [6]-gingerol on ΔPD in the colon (Fig. 4), suggesting that [6]-gingerol increased colonic transmural ΔPD mostly through the upregulation of sodium absorption. Effect of [6]-gingerol on electrogenic chloride and potassium transport in the rat colon

The electrogenic secretion of chloride and potassium also constitutes major ion transport pathways in the colon (15). To determine the involvement of electrogenic chloride and potassium absorption in [6]-gingerol-stimulated ΔPD increase, we added the Na+/K+/2Cl− co-transporter inhibitor bumetanide (0.1 mM) to the serosal side of the colon for 90 min and recorded [6]-gingerol-mediated effects on ΔPD. Bumetanide did not significantly affect [6]-gingerol-dependent stimulation of ΔPD (Fig. 5), suggesting that [6]-gingerol does not influence electrogenic chloride or potassium secretion in the rat colon.

Involvement of the capsaicin receptor in [6]-gingerol-stimulated electrogenic sodium absorption

A significant stimulation of electrogenic sodium absorption in the rat colon by the serosal administration of [6]-gingerol suggests the involvement of serosal receptors. [6]-Gingerol possesses a vanillyl group, which is considered important for the activation of the capsaicin receptor TRPV1, transient receptor potential (TRP) cation channel subfamily V member 1. TRPV1 is a non-selective cation channel expressed mainly in nociceptive sensory neurons and is responsible for the detection and regulation of body temperature (16). TRPV1 has been shown to be efficiently activated by [6]-gingerol in many physiological functions (17–19).

To determine whether [6]-gingerol stimulated sodium absorption in the colon via an interaction with the capsaicin receptor, we pretreated the serosal side of the 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. 6), suggesting that [6]-gingerol induced transmural sodium transport via an interaction with TRPV1.

DISCUSSION

[6]-Gingerol has been shown to have a variety of beneficial pharmacological and physiological effects. Although [6]-gingerol has a role in the regulation of intestinal contraction, it was unclear whether it has a significant effect on the transepithelial ion transport in the intestine. In the present study, we found that the serosal administration of [6]-gingerol stimulated electrogenic sodium absorption in the rat colon via TRPV1. This is the first report to show the effect of [6]-gingerol on the transepithelial ion transport in epithelial tissue, including the kidneys and lungs.

Sodium absorption in the colon plays an important role in salt and water homeostasis. Two transport mechanisms involved in sodium absorption have been described: an electroneutral pathway mediated by the apical Na+/H+ exchanger functionally coupled to the Cl−/HCO3− exchanger, and an electrogenic pathway based on the generation of transmural potential difference (ΔPD) and mediated by the apical amiloride-
sensitive epithelial type sodium channel (ENaC). The electrogenic sodium absorption in the colon is similar to that in frog skin, the distal nephron, and the respiratory epithelium (20, 21) and is under the control of extracellular neurotransmitters, hormones, and paracrine substances (15). Although we did not investigate a 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 have anti-diuretic effects.

Our data show that [6]-gingerol stimulated electrogenic sodium absorption when applied to the serosal but not the mucosal side of the colon. Under physiological conditions, orally administered [6]-gingerol is absorbed through the intestinal mucosa and transferred to the serosal side. Jiang et al. reported that the maximal plasma concentration of [6]-gingerol was reached 10 min after oral administration (22), suggesting that [6]-gingerol is mostly absorbed in the small intestine rather than in the colon. The serosal side of the colon possesses the enteric nervous system and blood vessel. It has been reported that colonic electrogenic sodium absorption is able to be controlled by the enteric nervous system and mediated by extracellular neurotransmitters, hormones, and paracrine regulators in the serosal side (15). We haven’t elucidated yet whether [6]-gingerol stimulates sodium absorption via a neuronal and/or humoral regulator. A more detailed study is needed to elucidate direct involvement of those regulators.

Electrogenic sodium absorption by colonic epithelial cells occurs via apical sodium channels and is regulated by intracellular Na⁺ concentrations established by the basolateral Na⁺/K⁺-ATPase. The results of the present study suggest that [6]-gingerol may activate one or both of these Na⁺ transport components. To examine this hypothesis, the activity of sodium channels and Na⁺/K⁺-ATPase should be measured in the mucosa-submucosa preparation of the colon.

Our results indicate that [6]-gingerol increases electrogenic sodium absorption via TRPV1. It has been well established that [6]-gingerol is an agonist of TRPV1. TRPV1 is mainly localized to the nerve fibers in the basolateral side of the mucosa and submucosa in the rat colon, (23) and in the mouse colon (24). TRPV1 has also been detected in nerve fibers of the muscle layer (24). The intestinal sacs used in this study contained the mucosal, submucosal, and muscle layers. Thus, the stimulation of electrogenic sodium absorption may occur either via [6]-gingerol binding directly to TRPV1 in the nerve fibers of the mucosa or via binding to the neuronal receptors in the muscle layer and then stimulation of the electrogenic sodium absorption in the mucosal cells. To clarify these possibilities, experiments will need to be performed using the intestinal preparation with and without the muscle layer. [6]-Gingerol is also an agonist of TRPA1, one of the TRP cation channel subfamily (25). It has been reported that rat colonic epithelia express TRPA1 (26). We cannot exclude the possibility that in our study [6]-gingerol induced electrogenic sodium absorption via TRPA1. More study is need to elucidate this possibility using a selective inhibitor of TRPA1, for example, AP18.

[6]-Gingerol is known to regulate intestinal peristalsis, and was shown to induce contraction of the isolated guinea-pig ileum while suppressing 5-HT₁ receptor-mediated intestinal contraction (8, 9). Although in the present study we did not examine the effects of [6]-gingerol on intestinal contraction, we can consider the possibility that significant contraction of the colon may exert mechanical or chemical stimulatory effects on electrogenic sodium absorption. Further studies are needed to elucidate the correlation between contraction and electrogenic sodium absorption in the colon.

Ion transport in the colon is regulated by several secondary messengers. Our earlier results showed that intracellular cAMP mediated β-adrenergic stimulation of electrogenic sodium transport in the rat colon (27, 28). An increase in intracellular Ca²⁺ was also demonstrated to be involved in the upregulation of electrogenic sodium absorption by hypotonicity in renal epithelial cells (29). [6]-Gingerol was shown to induce intracellular Ca²⁺ via TRPV1 (19), suggesting the possibility that an increase in intracellular Ca²⁺ may mediate the effect of [6]-gingerol on intestinal sodium transport observed in the present study. Additional experiments are needed to elucidate the direct involvement of intracellular Ca²⁺ and other secondary messengers in the effect of [6]-gingerol on electrogenic sodium absorption in the colon.

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