Allicin Induces Electrogenic Secretion of Chloride and Bicarbonate Ions in Rat Colon via the TRP A1 Receptor

Yo Tsuchiya¹ and Koichi Kawamata²

¹Department of Health and Nutrition, Faculty of Home Economics, Tohoku Women's College, 1–1–16 Kiyohara, Hirosaki, Aomori 036–8530, Japan
²Department of Domestic Science, Faculty of Domestic Science, Tohoku Seikatsu Bunka University, 1–18–2 Nijinooka, Izumi-ku, Sendai, Miyagi 981–8585, Japan
(Received February 2, 2019)

Summary Allicin, an antioxidant from garlic, is known to regulate intestinal contractions, but its effect on intestinal ion transport is unclear. The aim of this study was to examine the role of allicin in the regulation of electrogenic ion transport in rat intestine by measuring the transmural potential difference (ΔPD). Allicin induced significant positive ΔPD, when administered to the serosal side of the colonic mucosal-submucosal preparation. Allicin-induced colonic ΔPD was largely diminished by incubation in the chloride-free solution, although the transient peak of ΔPD after application of allicin remained. This transient peak of ΔPD was significantly diminished in both the chloride- and the bicarbonate-free incubation solution. Induction of ΔPD by allicin was greatly diminished by AP-18, an inhibitor of the transient receptor potential (TRP) cation channel subfamily A member 1, TRP A1. Both alliin and S-allylcysteine, the analogues of allicin, had no effect on ΔPD and did not affect allicin-induced ΔPD in the colon. These results suggest that allicin mainly evokes the electrogenic chloride secretion and only partially increases the electrogenic bicarbonate secretion via TRP A1.

Key Words anion transport, garlic, intestine, transmural potential difference, TRP channel

Garlic (Allium sativum) is used globally not only as a spice but also as a herbal medicine to treat many kinds of ailments. Allicin (diallylthiosulfinate) is a major component from garlic. Allicin is produced from alliin (S-allylcysteine sulfoxide) by the enzyme, allinase, upon tissue damage (1). Allicin has been shown to possess a variety of pharmacological and therapeutic effects, such as antimicrobial activity (2, 3), anti-tumor effects (4), and the selective reduction of plasma lipid (triacylglycerol and LDL-cholesterol) concentrations (5).

Allicin is an agonist of the transient receptor potential (TRP) cation channel subfamily A member 1, TRP A1, which is activated by noxious cold, mustard oil (TRP). It has been reported that the agonists of TRP A1, including allicin and allyl isothiocyanates, may have a regulatory effect on gastrointestinal motility (9, 10). It has been established that contraction of the intestine correlates with transport of mucosal ions, which contributes to the smooth movement of intestinal contents and efficient absorption of nutrients. See et al. reported that the submucosal myenteric plexus in the rat jejunum causes the reflex that couples ion transport to muscle contraction (11). Studies on rat colon have reported that endothelin-1 induces bowel contraction and epithelial chloride secretion (12) and that adrenomedullin modulates bowel contraction (13).

Although allicin has been shown to regulate contractions of the gastrointestinal parts, it is unclear whether these effects are related to its role in the gastrointestinal ion transport. The aim of this study was to investigate the effect of allicin on the electrogenic ion transport in rat intestine by measuring the transmural potential difference (ΔPD) and to elucidate any related factors that aid in regulation of this mechanism. Our results indicate that serosal allicin stimulates the secretion of electrogenic chloride and bicarbonate ions in the colon via TRP A1.

MATERIALS AND METHODS

Chemicals. Allicin (10 mg/mL in methanol) was purchased from LKT Laboratories, Inc. (Minnesota, USA). Capsazepine, AP-18 (TRP A1 antagonist), and bumetanide were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Bumetanide 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.

Experimental animals. 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: 2016-01, 2017-02). Rats were anesthetized with urethane (1.0 g/kg IP). A segment of the colon was isolated within 10 min of administering anesthesia, followed by the rats being euthanized by overdose of urethane.

**Measurement of transmural potential in a preparation of rat intestinal sacs.** As our previously reported method (14), a 5-cm section of the intestine comprising the jejunum, ileum, and colon was briefly removed and tied off with a thread. For measuring ΔPD, the intestinal sac was connected to a silicon tube (outer diameter, 4 mm) and filled with a standard buffer (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). The intestinal sacs were incubated at 37˚C in 40 mL of a standard buffer under an atmosphere of 95% O₂ and 5% CO₂ gas.

To measure ΔPD, 2% agar containing 1 M KCl bridges was placed on both the serosal and mucosal sides of the intestinal sacs preparation obtained from the procedure mentioned above. The ΔPD was continuously measured by connecting calomel half-cells to the mucosal and serosal solution using 2% agar bridges; data were recorded using a high-sensitivity DC chart recorder (PR8112, HIOKI, Nagano, Japan). The ΔPD value was considered positive, when cations were transported from the mucosal to the serosal side of the intestine.

**Measurement of transmural potential in the mucosal-submucosal preparation of rat colon.** The ΔPD in a mucosal-submucosal preparation of rat colon was measured in vitro in a Ussing chamber using our previous method (15). A segment of the rat colon was cut open longitudinally on a flat sheet. The serosal and muscular layers were removed with fine forceps to obtain a mucosal-submucosal preparation. The tissue was mounted vertically between the 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 standard buffer 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. The intestinal sacs were incubated at 37˚C in 40 mL of a standard buffer under an atmosphere of 95% O₂ and 5% CO₂ gas.

To measure ΔPD, 2% agar containing 1 M KCl bridges was placed on both the serosal and mucosal sides of the colon mucosal-submucosal preparation. The method of measuring ΔPD was similar to that used for the preparation of rat intestinal sacs.

**Statistical analyses.** Data are expressed as means±standard error (SE). Statistical comparison between two means was performed using the 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**

**Effect of the serosal application of allicin on transmural ion transport in rat intestinal sacs.**

To determine the effect of allicin on ion transport in the rat intestine, allicin was added to the serosal side of the intestinal sacs preparations of the rat colon, ileum, and jejunum. As shown in Fig. 1A, the administration of 10 and 30 μM allicin to the serosal side of the colon induced an increase in ΔPD, which reached a steady value within 3 min at both concentrations of allicin. Figure 1B shows that the allicin-dependent induction of transmural ΔPD was markedly higher in the colon than in the ileum and jejunum, thus, we investigated the effect of allicin on colonic ion transport only as discussed below.

**Effect of allicin application on transmural ion transport in the mucosal-submucosal preparation of rat colon.**

To determine the effect of allicin on the rat colonic ion transport in detail, we investigated the effect of allicin on the colonic mucosal-submucosal preparation using...
the Ussing chamber method. Figure 2A shows that the escalations in colonic transmural $D_{PD}$ by serosal administration by allicin were concentration-dependent. Mucosal administration of allicin also induced an increase in $D_{PD}$ but this value was significantly lower than in serosal administration (Fig. 2B). Chloride and bicarbonate ions are known to be the major anions transported across the colon wall (16). We investigated the involvement of the secretion of electrogenic chloride and bicarbonate in allicin-induced $D_{PD}$ in the rat colon. As shown in Fig. 3A, allicin-induced colonic $D_{PD}$ was largely diminished by incubation in the chloride-free solution, although the transient peak of $D_{PD}$ after application of allicin remained. This allicin-induced transient peak of $D_{PD}$ significantly diminished in both the chloride- and the bicarbonate-free incubation solution (Fig. 3B, C). On single administration of bumetanide, which is an inhibitor of the Na$^+$–K$^+$–2Cl$^-$ cotransporter as the standard solution, allicin-induced colonic $D_{PD}$ significantly diminished to 75% less than that in the conditions without the inhibitor (data not shown). These results suggest that allicin mainly evokes the electrogenic chloride secretion and only partially increases the electrogenic bicarbonate secretion.

Involvement of TRPA1 in allicin-induced electrogenic anion secretion in the colon

Allicin is an agonist of TRPA1; it has been reported previously that TRPA1 is expressed in the submucosa and muscle layer in the rat colon (17). We determine whether allicin stimulates anion secretion in the colon via interaction with TRPA1. In this experiment a TRPA1 antagonist, AP-18 (30 μM) was pretreated to the serosal side of the colon for 30 min prior to the addition of allicin. We observed that AP-18 largely blocked allicin-stimulated electrogenic anion secretion (Fig. 4A, C). On the other hand, pretreatment with capsazepine, which is an inhibitor of the capsaicin receptor, TRPV1, did not affect allicin-induced electrogenic anion secretion (Fig. 4B). These results suggest that allicin-induced transmural anion secretion occurs in the colon via interaction with TRPA1.
Allicin Induces Colonic Anion Secretion

Effect of alliin analogues on electrogenic anion secretion in the colon

Alliin is a precursor of allicin in fresh garlic and is produced from γ-glutamyl-S-allylcysteine (1). S-allylcysteine is an analogue of alliin and produced from γ-glutamyl-S-allylcysteine in aged garlic (18, 19). To determine the effects of alliin analogues on the electrogenic anion secretion in the colon, 30 μM allin and 30 μM S-allylcysteine were administered to the serosal and mucosal side of the rat colonic mucosal-submucosal preparation. As shown in Fig. 5A and B, neither serosal nor mucosal administration of alliin and S-allylcysteine in the colon changed the colonic basal ΔPD. In addition, pretreatment with these two analogues did not change alliin-induced ΔPD (Fig. 5C). These results suggest that both alliin and S-allylcysteine have no inducible effect on the electrogenic ion transport, and they do not affect allicin-induced electrogenic anion secretion in the colon.

DISCUSSION

Allicin has been demonstrated to have a variety of beneficial pharmacological and physiological effects. Although allicin plays a role in the regulation of intestinal contractions, it was unclear until now whether it has any significant effects on transepithelial ion transport in the intestine. In the present study, we found that serosal administration of allicin stimulates electrogenic anions secretion in the rat colon via TRPA1. This is the first report on the effect of allicin on transepithelial ion transport.
transport in the epithelial tissue, including in the kidneys and lungs.

Electrogenic chloride ion secretion in the colon takes place by entrance of ions via the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (NKCC1) in the basolateral membrane, followed by their excretion via the cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane (16, 20). This secretion plays an important role in water secretion to the luminal side and contributes to the smooth movement of intestinal contents. Bicarbonate ion is a weakly alkaline substance that is secreted after it enters through the basolateral Na\(^+\)/HCO\(_3\)\(^-\) exchanger and is excreted from the apical CFTR in the colon (16). Bicarbonate secretion is thought to limit acid damage on the mucosa due to the production of short-chain fatty acids in the colon. Although we did not investigate the direct effect of allicin on water transport and luminal alkalization in the colon, we hypothesize that allicin-induced colonic chloride secretion may contribute toward the smooth transport of mucus out of the crypts and that weak-alkaline bicarbonate secretion may contribute to protection of colonic lumen from acid damage.

Our results indicate that allicin increases electrogeneric anion secretion via TRP A1. It has been established that allicin is an agonist of TRP A1. Furthermore, it has been reported that TRP A1 is localized in the nerve fibers of the muscle layer (Auerbach’s plexus) and the basolateral side of submucosa (Meissner’s plexus) in the rat and mouse colon (17, 21). Our results show that allicin-induced colonic anion secretion occurs in both the intestinal sacs containing the muscle layer and mucosal-submucosal preparations without the muscle layer. We also observed that allicin-induced colonic anion secretion occurs via the activation of TRPA1 expressed in the Meissner’s plexus. However, we cannot exclude the possibility that activation of TRPA1 expressed in the colonic muscle layer might secondarily contribute toward the induction of colonic anion secretion, probably by mechanical and/or chemical stimulatory effects of the muscle layer. Further investigation is needed to elucidate this possibility.

The present study showed that alliin and S-allylcysteine, the two analogues of allicin, had no effect on the smooth muscle layer and intact isolated rat colon. Therefore, we suggest that the low binding affinity to TRP A1.

Allicin and other TRPA1 agonists, such as allyl isothiocyanate (AITC), are known to regulate intestinal peristalsis. Penuelas et al. reported that allicin induced the contraction of an isolated mouse colon (9). Doihara et al. reported that AITC stimulates gastric antrum and jejunal motility, as well as induces the giant migrating colonic contraction (10). Although we did not examine the effects of allicin on intestinal contractions in the present study, we can consider the possibility of a significant contraction of the colon may occur mechanical or chemical stimulatory effects on electrogeneric anion transport. Further studies are needed to elucidate the correlation between colonic contraction and electrogeneric anion transport in the colon.

Acknowledgments

This work was supported by The Salt Science Research Foundation (No. 1747) and Aomori Research, Art, and Culture Foundation. We would like to thank Editage (www.editage.jp) for their English language editing services.

REFERENCES

7) Jordt SE, Bautista DM, Chuang IH, McKemy D, Zyg-
Allicin Induces Colonic Anion Secretion


