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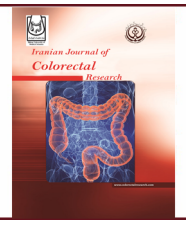

Allicin-induced Activation of Peristalsis in Rat Ileum Depends on Bicarbonate Ion Transport

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Abstract

Background: Allicin, a main component of garlic, possesses various beneficial pharmacological and therapeutic properties, including anticarcinogenic, bactericidal, and intestinal regulatory effects. Although it is known to regulate intestinal contraction and ion transport, the direct correlation between these two activities remains unclear. Hence, this study investigated the correlation between the allicin-induced activation of peristalsis and ion transport in the rat intestine.

Methods: In this work, we used ileal segments of nine-week-old Sprague-Dawley rats, which have more active spontaneous contractions than colonic segments. To examine the role of allicin in regulating electrogenic ion transport in the rat ileum, we measured the transmural potential difference (ΔPD) with an Ussing chamber system. To study intestinal peristalsis, we performed an experiment to measure the velocity of the movement of an artificial pellet during intestinal peristalsis by recording videos with an overhead camera. SPSS software was used for data analysis and P-values<0.05 was considered as significant.

Results: A dose of 100 µM allicin induced a significant positive ΔPD when administered to the serosal side of the ileum (P-value for 100 µM vs. 30 µM=0.038). Removing chloride ions from the incubation solution did not significantly change the allicin-induced positive ΔPD. Removing bicarbonate ions from the incubating solution completely suppressed the allicin-induced increase in ΔPD. Allicin-induced pellet movement in the ileum significantly diminished when bicarbonate ions were removed from the incubation solution. Allicin-induced ileal spontaneous peristalsis was completely suppressed in the presence of an inhibitor of a bicarbonate ion transporter (30 µM 5-nitro-2-(3-phenylpropylamine) benzoic acid).

Conclusion: The present study on ion transport suggests that allicin mainly induces the ileal electrogenic secretion of bicarbonate ions in the rat ileum. In addition, a study of intestinal peristalsis suggested that allicin-induced ileal peristalsis depends on the extracellular bicarbonate ions electrogenically secreted in the ileum.

Keywords: Allicin, Bicarbonate ion, Ileum, Peristalsis

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Introduction

Garlic (*Allium sativum*) is widely used not only as a spice but also as an herbal medicine to treat many diseases. Allicin (diallyl thiosulfinate) is a major component of garlic. Allicin is produced from alliin (S-allyl cysteine sulfoxide) by the enzyme alliinase in response to tissue damage (1). Allicin possesses various beneficial pharmacological and therapeutic effects such as antimicrobial activity (2, 3), antitumor effects (4), and 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 (TRPA1), which is activated by noxious cold mustard oil (6-8). In the gastrointestinal tract, the agonists of TRPA1, including allicin and allyl isothiocyanates, regulate gastrointestinal motility (9, 10). Our previous study showed that allicin induces the electrogenic secretion of chloride and bicarbonate ions in the rat colon via the TRPA1 receptor (11).

Intestinal contractions correlate with the transport of mucosal ions. Chloride is one of the major anions secreted in the intestine (12). Chloride secretion accompanies water secretion, contributing to the smooth movement of intestinal contents and efficient absorption of nutrients. Studies on the rat colon have reported that endothelin-1 induces bowel contraction and epithelial chloride secretion (13) and that adrenomedullin modulates water and chloride ion transport, which correlates with bowel contractions (14). Although allicin regulates contractions in the gastrointestinal tract, it is unclear whether these effects directly relate to its role in gastrointestinal ion transport. This study aimed to investigate the correlation between allicin-induced activation of peristalsis and ion transport in the intestine.

Several researchers have used isotonic transducers to measure intestinal peristalsis (9, 15). Isotonic contractions can be evaluated by measuring changes in intestinal length. In this study, we measured the movement velocity of artificial pellets during intestinal peristalsis. As the intestines transport nutritional contents and feces, we considered that measuring pellet movement would replicate physiological conditions better than measuring isotonic contractions. In our previous study, we could not elucidate whether allicin-induced intestinal anion secretion was directly related to the activation of intestinal peristalsis (11). In this study, we investigated the effect of allicin on ion transport and peristalsis in the rat ileum, which has more active spontaneous contractions than the colon, and the correlation between activation of peristalsis and ion transport.

Materials and Methods

Chemicals

Allicin (10 mg/mL in methanol) was purchased from LKT Laboratories, Inc. (Minnesota, USA). We purchased 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and dissolved it in dimethyl sulfoxide (DMSO).

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 according to the Animal Usage Ethics Committee of Tohoku Women's College (approval number: 2016-01, 2017-02). The rats were anesthetized with urethane (1.0 g/kg IP). A segment of the ileum was isolated within 10 min of anesthesia administration, and the rats were euthanized with an overdose of urethane.

Measurement of Transmural Potential in the Mucosal-submucosal Preparation of Rat Ileum

The transmural potential difference (ΔPD) in a mucosal-submucosal preparation of rat ileum was measured in vitro in a Ussing chamber using our previously reported method (16). A segment of rat ileum was cut open longitudinally onto a flat sheet. The serosal layers were removed using fine forceps to obtain the musculo-mucosal preparations. The tissue was mounted vertically between Ussing chambers made of acrylic resin with an internal surface area of 0.5 cm^2. 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 $NaHCO_3$, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 10 mM glucose in an atmosphere of 95% O_2 and 5% CO_2 (pH 7.4). Chloride ion substitution was achieved by introducing gluconate. Bicarbonate ion substitution was achieved using 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES). Substitution of both chloride and bicarbonate ions was achieved by the following composition: 135 mM Na-gluconate, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 1.2mM Mg-gluconate, 1.2 mM Ca-gluconate, 10 mM HEPES, and 10 mm glucose, in an atmosphere of 100% O_2 (pH 7.4). To measure ΔPD, 2% agar containing 1 M KCl bridges was placed on both the serosal and mucosal sides of the ileal muscular-mucosal-preparation.

Data were recorded using a high-sensitivity DC chart recorder (PR8112; HIOKI, Nagano, Japan). The PD value was considered positive when the anions were transported from the serosal side to the mucosal side of the intestine.

Evaluation of the Effect of Allicin on Ileal Peristalsis

A 5 cm section of the intestine comprising the ileum was briefly removed and incubated in a buffer solution for 10–20 min to allow spontaneous evacuation of the ileal contents. The mesentery

of the isolated ileum was fixed using two pins in silicone rubber to prevent secondary extension by an external force. The ileum was placed linearly. Under these conditions, spontaneous peristalsis of the ileum was not restricted. In this experiment, the components of the standard buffer solution were 118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 10 mM glucose in an atmosphere of 95% O_2 and 5% CO_2 (pH 7.4). Chloride ion substitution was achieved by introducing gluconate. Bicarbonate ion substitution was achieved using HEPES. Substitution of both chloride and bicarbonate ions was achieved by the following composition: 113 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM Na-gluconate, 10 mM HEPES, and 10 mM glucose, in an atmosphere of 100% O_2 (pH 7.4).

Artificial plastic pellets (diameter: 4 mm) were used to measure peristalsis velocity by modifying the method of Jin et al. (17). The control velocity was measured by inserting a pellet into the proximal end of the ileal preparation and allowing it to exit spontaneously toward the distal end. The second and third pellets were sequentially inserted into the proximal end to repeat the velocity measurements. The control velocity was calculated as the mean velocity of peristalsis for three successive pellets. After equilibration, allicin was added to the incubation solution, and the velocity of peristalsis of three successive pellets was measured. The movement of the pellets was recorded using an overhead camera (Ziggi-HD, IPEVO, Tokyo, Japan) coupled with AG-web camera recorder software for analysis, as described in Figure 1. The peristalsis velocity was calculated from the time taken by a pellet to move through a marked 3-cm segment.

Statistical Analyses

Data are expressed as means±standard errors (SEs). The Shapiro-Wilk test was performed to assess the normality of the data and evaluate whether the data were parametric. Subsequently, the two-tailed Student's t-test was used to determine significant differences between two paired experimental groups. Differences were considered significant at P-values<0.05.

Results

The Effect of Allicin on Electrogenic Bicarbonate Ion Transport in the Rat Ileum

To determine the effect of allicin on bicarbonate ion transport in the rat ileum, we investigated the effect of allicin on ileal musculo-mucosal preparations using the Ussing chamber method. As shown in Figure 2A, the administration of 100 µM allicin to the serosal side of the ileum induced an increase in ΔPD, which reached a steady value within 3 min. The 100 µM allicin-induced ΔPD increase was significantly larger than when 30 µM was used (P=0.038). On the other hand, the administration of 100 µM allicin to the mucosal side of the ileum did not change the ΔPD.

Chloride and bicarbonate ions are the major anions transported across the intestinal wall. We investigated the involvement of the secretion of electrogenic chloride and bicarbonate ions in allicin-induced ΔPD in the rat ileum. As shown in Figure 2B, the allicin-induced ileal ΔPD did not change significantly with incubation in the chloride-free solution, and the ΔPD after adding allicin remained constant. The allicin-induced ileal ΔPD completely diminished in the bicarbonate-free (HEPES) solution (Figure 2C). These results suggest that allicin induces electrogenic bicarbonate secretion in the rat ileum.

The Correlation between Allicin-induced Activation of Peristalsis and Ion Transport in the Ileum

To determine the correlation between allicin-induced activation of peristalsis and ion transport in the ileum, we measured the velocity of ileal peristalsis using artificial pellets in the presence or absence of bicarbonate ions. As shown in Table 1,

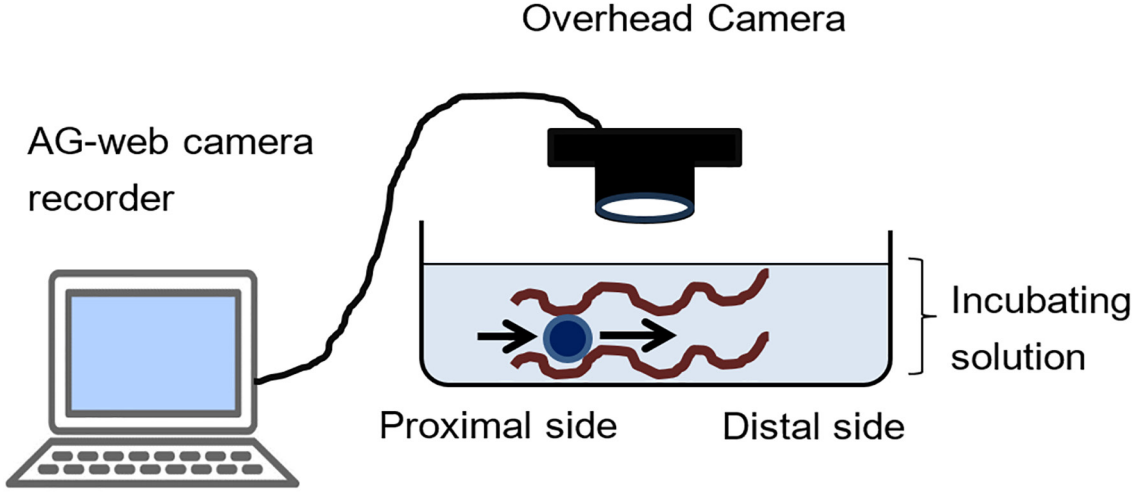

Figure 1: Measurement of the velocity of movement of an artificial pellet during the ileal peristalsis. The movement of a pellet from the proximal to the distal side of the ileum was captured using the overhead camera and recorded using the AG-web camera recorder.

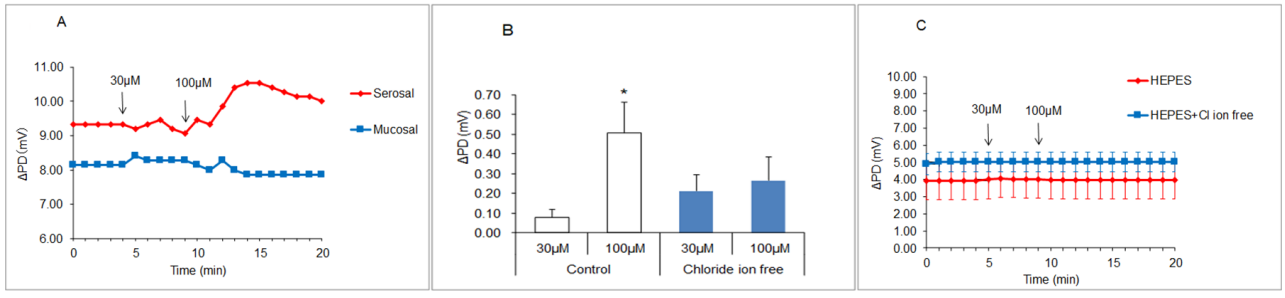

Figure 2: The effect of allicin on electrogenic bicarbonate ion transport in the rat ileum. (A) Plot of a typical tracing of changes in transmural potential difference (ΔPD) after serosal and mucosal administration of 30 and 100 μM allicin in the musculo-mucosal preparation of the rat ileum. (B) Plot of the allicin-induced peak of ΔPD in the normal incubation and Cl^--free solution. (C) Plot of the average tracing of changes in ΔPD after serosal administration of 30 and 100 μM allicin in the musculo-mucosal preparation that had been incubated in bicarbonate-free solution (HEPES) and in both bicarbonate- and Cl^--free solution (HEPES+Cl^--free) in rat ileum. In (B) and (C), values are presented as means ± standard errors (SEs) (n=4). *P<0.05 vs. 30 μM of allicin in the control group.

Table 1: The effects of extracellular bicarbonate ions on allicin-induced ileal peristalsis

	Control	Bicarbonate free	NPPB 30 μM
Starting time(s)	75±7.6	240±12.7*	No movement
Velocity(mm/s)	2.1±0.13	0.7±0.11*	0

The starting time is when the plastic pellet started its movement after administration of 100 μM allicin in the normal incubating solution (control), in bicarbonate-free solution, and in pretreatment with 30 μM NPPB. Values are presented as means ± standard errors (SEs) (n=4). *P<0.05 as compared to the control group.

the pellet started to move at 75±7.6 s (starting time) after adding 100 μM of allicin to the incubating solution, and the velocity was 2.1±0.13 mm/s. In the bicarbonate-free solution, the starting time of pellet movement was delayed, and the velocity decreased significantly (P=0.032 for the starting time and P=0.043 for the velocity). In the presence of a cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor, 30 μM NPPB, allicin-induced movement of the pellet did not occur. These results suggest that allicin-induced ileal peristalsis depends on extracellular bicarbonate ions and probably on transepithelial bicarbonate transport via CFTR.

Discussion

Although allicin plays a role in regulating intestinal contractions, it was unclear whether these effects are directly related to allicin-induced intestinal ion transport. The present study found that allicin induces bicarbonate ion transport in the rat ileum and that allicin-induced ileal peristalsis depends on bicarbonate transport.

Our previous study concluded that allicin mainly induces electrogenic chloride ion transport in rat colons (11). The present study suggests that allicin mainly induces electrogenic bicarbonate transport in the rat ileum. Our results indicate that the effect of allicin on anion transport differs among the intestinal segments (Figure 3). Bicarbonate ions are secreted after entering the basolateral Na^+/HCO_3^- exchanger and are excreted from the apical CFTR in the intestine (18). Although the reason for the different effects of allicin on the ileum and colon is unclear, we consider the possibility that the expression level of CFRT, the intracellular regulatory system regulating cation transport, or the molecular mechanisms regulating cation transport may be different in these two parts of the intestine. Further investigation is required to explore these explanations.

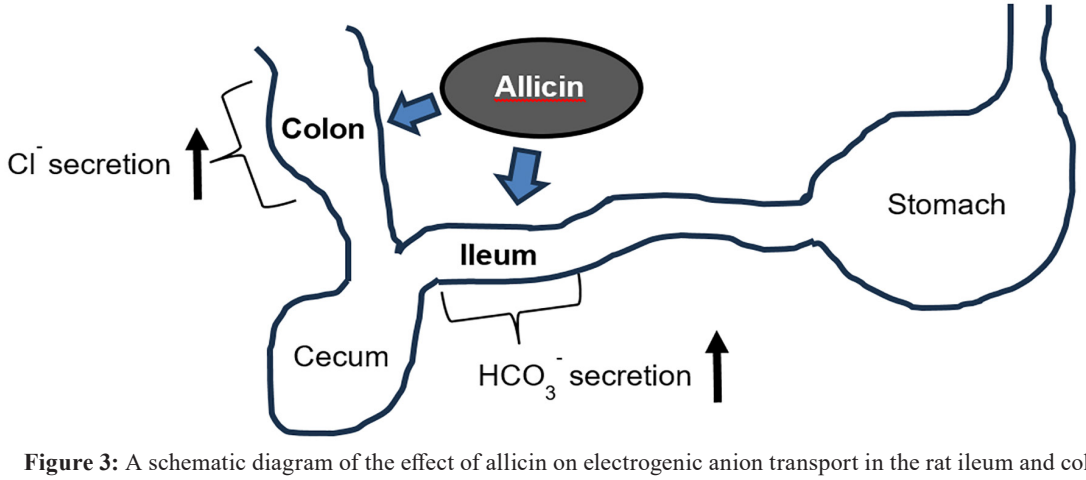

Figure 3: A schematic diagram of the effect of allicin on electrogenic anion transport in the rat ileum and colon.

Bicarbonate ions are weakly alkaline. Bicarbonate secretion is thought to limit acid damage to the mucosa owing to metabolites derived from nutritional production. Although we did not investigate the direct effects of allicin on luminal alkalization in the ileum, we hypothesized that allicin-induced ileal bicarbonate secretion protects the ileal lumen from acid damage. In this study, allicin-induced ileal peristalsis depended on bicarbonate ion transport. However, the detailed mechanism by which allicin-induced bicarbonate secretion activates ileal peristalsis remains unclear. Allicin is an agonist of TRPA1, which is localized in the nerve fibers of the muscle layer (Auerbach's plexus) and the basolateral side of the submucosa (Meissner's plexus) in the rat colon and the mouse ileum (19, 20). Our results show that allicin-induced ileal bicarbonate secretion occurs in rat muscular-mucosal preparations. We consider that the activation of TRPA1 expressed in the ileal muscle layer might increase ileal muscle contraction, depending on transepithelial bicarbonate ion transport. However, we cannot exclude the possibility that activation of TRPA1 expressed on the basolateral side of the submucosa increases bicarbonate ion transport and secondarily induces ileal muscle contraction. Further studies are required to elucidate this.

Intestinal contractions and peristalsis were previously measured using an isotonic transducer (9, 15). Penuelas et al. found that allicin induces contractions in the mouse colon (9). They used intestines that had no contents on their luminal side. In our study, we evaluated intestinal motility by measuring the velocity of the movement of artificial pellets in the ileum. The ileum contains and transports digested and prefecal products. Therefore, we believe that our experiment was performed under more physiological conditions than in an isotonic transducer. In our experimental system, allicin significantly increased the velocity of pellet movement in the ileum. These results indicate that our experimental system was appropriate for measuring intestinal contractions and peristalsis.

In the present study, allicin-induced ileal peristalsis was completely suppressed by adding NPPB, which inhibits CFTR (21). CFTR is expressed in the rat ileum (22), and bicarbonate ions are secreted via CFTR in the rat duodenum and colon (18). We considered the possibility that allicin-induced bicarbonate ion secretion via CFTR and bicarbonate transport contributes to the activation of peristalsis in the rat ileum.

In the rat colon and mouse ileum, bicarbonate ion transport is regulated by several intracellular secondary messengers, such as cAMP and Ca_2^+ (12, 23, 24). Allicin and AITC evoked an increase in intracellular Ca_2^+ influx in rat TRPA1-expressing HEK293, which are human embryonic kidney cell lines (10, 25). These results suggest that an increase in intracellular Ca_2^+ mediates the effect of allicin on intestinal bicarbonate ion transport observed via TRPA1.

The main limitation of our experimental study was that we could not elucidate the regulatory mechanisms at the intracellular and molecular levels. Additional experiments at the molecular levels are required to explain the direct involvement of intracellular Ca_2^+ and other secondary messengers in the effects of allicin on electrogenic bicarbonate secretion in the ileum.

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Authors' Contribution

Writing: Yo Tsuchiya; Experimentation and Data Analysis: Yo Tsuchiya, Nozomi Sasaki, Sae Satou, Kana Tozawa; All authors have read and agreed to the published version of the manuscript.

Conclusion

We found that allicin-induced ileal peristalsis directly depends on its effect on electrogenic bicarbonate secretion in the ileum.

Conflict of interest: None declared.

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