Intraluminal administration of zingerol, a non-pungent analogue of zingerone, inhibits colonic motility in rats

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ABSTRACT

Zingerone, a pungent component of ginger, may exert beneficial therapeutic effects on hypermotility-induced diarrhea because it has the ability to inhibit contractions of colonic smooth muscles. However, the pungency is undesirable for possible therapeutic use. The purpose of this study was to examine effects of zingerol, a non-pungent analogue of zingerone, in rats. Colonic motility in vivo was evaluated by measuring intraluminal pressure changes and expelled fluid volume from the colon in anesthetized rats. Mechanical contractile activities of isolated colonic segments were also recorded. Intracolonic administration of zingerol attenuated colonic motility in vivo without affecting blood pressure and heart rate in a manner similar to that of zingerone. Zingerol also inhibited spontaneous contractile movements in isolated colonic segments, suggesting that zingerol directly acts on the colon. Zingerol had no effect on jejunal motility, although zingerone showed an inhibitory effect to the jejunum. These findings suggest that zingerol can inhibit colonic motility without adverse effects on small intestinal motility and the cardiovascular system. The non-pungent property of zingerol will be useful as an oral or suppository medicine for treating diarrhea and other gastrointestinal disorders.

Ginger (the rhizome of Zingiber officinale Roscoe) and its components have various pharmacological actions, including anti-inflammatory, anti-cancer, anti-emetic, anti-constipation and anti-diarrhea activities (1, 9, 17). Zingerone, as well as gingerol and shogaol, is one of the pungent components of ginger (21). We have recently demonstrated that zingerone can effectively suppress colonic motility not only in vitro but also in vivo in rats (5). In addition, it has been reported that zingerone inhibits enterotoxin-induced fluid secretion in the ileum in mice (2). Since abnormal facilitation of gastrointestinal motility and excessive fluid secretion of gastrointestinal tracts cause diarrhea (8), it is likely that zingerone is the active constituent responsible for the anti-diarrheal activity of ginger. Therefore, zingerone might exert beneficial therapeutic effects on diarrhea. However, the pungent property of the compound would be undesirable for possible therapeutic use.

Zingerol, which is not a natural component of ginger, is a reduced analogue of zingerone (6) (Fig. 1). The chemical lacks a pungent property and thus might be useful for oral and/or rectal administrations. On the basis of the chemical structure, it was expected that zingerol would have an inhibitory effect on colonic motility similar to that of zingerone. However, there are few reports in which pharmacological actions of zingerol are described. Hence, the purpose of the present study was to clarify the effects of zingerol on colonic motility in rats.

Male Wistar rats (12–15 weeks of age, 300–500 g)
were used in the present study. The experiments were approved by the Animal Care and Use Committee of Gifu University. Colonic or jejunal motility was evaluated in vivo by measuring intraluminal pressure changes and expelled fluid volume from the intestinal tracts in anesthetized rats as described previously (3, 5, 16). The colon was cannulated in the region of the colonic flexure and at the anus under anaesthesia by α-chloralose (Nakalai Tesque, Kyoto, Japan; 20–25 mg/kg/h) combined with ketamine hydrochloride (Daiichi-Sankyo, Tokyo, Japan; 5–7 mg/kg/h). Alpha-chloralose was dissolved in ethanol and solubilized with 10% 2-hydroxypropyl-β-cyclodextrin (Wako, Osaka, Japan) and then made up to an isotonic solution with NaCl for infusion. The oral cannula was connected to a Marriotte bottle filled with warm saline, and the distal cannula was connected to a pressure transducer, one-way valve and a fluid outlet, with insertion of two thin polyethylene tubes connected a push-pull pump from both ends for intraluminal application of drugs. Blood pressure was also measured from the femoral artery. The rat jejenum was cannulated in a way similar to that for the colon. In addition, we used isolated colonic segments from rats, in which mechanical contractile movements were recorded as described previously (5). Each segment of distal colon (3–4 cm in length) was mounted in longitudinal orientation in a Magnus apparatus (10 mL in capacity) filled with Tyrode’s solution (NaCl 135.9, KCl 2.68, CaCl2 1.8, MgCl2 1.0, NaHCO3 11.9, NaH2PO4 0.41 and glucose 5.55 mM). The distal end of each segment was tied to an opened grass tube fixed at the Magnus tube. A thin polyethylene tube was inserted from the proximal end, and then the proximal end was secured with a silk thread to an isometric force transducer (T7-30-240; Orientec, Tokyo, Japan). Mechanical contractile movements were filtered and amplified by an amplifier (AS1202; NEC, Tokyo, Japan) and recorded using a Power Lab system (model 2/25; AD Instruments, Bella Vista NSW, Australia). Zingerone and zingerol (kind gifts from Kanebo Institute, Tokyo, Japan) were dissolved in ethanol. Intraluminal applications of zingerone and zingerol were performed through the inserted polyethylene tubes by using the push-pull system to minimize artificial changes of the intraluminal pressure. Tetrodotoxin (Sigma, St. Louis, MO, USA) was dissolved in citrate solution and was applied to Tyrode’s solution in the Magnus tube. The concentrations of drugs given were final concentrations in Tyrode’s solution or saline. The highest concentration of vehicles and osmotic pressure had no effect on the basal tone and spontaneous activity in colonic preparations.

In our in vivo experiments, intraluminal pressure of the colon was maintained at 3–5 mmHg. Under the basal condition, the distal colon exhibited periodic rises in intraluminal pressure accompanied by fluid output through the anal cannula (Fig. 2A). Intraluminal administration of zingerol (20 mg/kg) decreased the amplitude of intraluminal pressure changes as did zingerone (Fig. 2A). The fluid output associated with colonic motility was also attenuated by application of zingerol (Fig. 2B). The inhibitory effects of zingerol on the colonic motility were sustained for at least 30 min. After washing out intraluminal contents, the colonic motility was recovered. Blood pressure and heart rate were not affected by intraluminal application of zingerol (data not shown). These results suggest that zingerol can inhibit colonic motility effectively in vivo without unexpected side effects, at least in the cardiovascular system.

To clarify whether zingerol acts on the colon directly or elicits its effects exclusively through activation of the central nervous system, we examined the effects of zingerol by using an isolated segment of the rat distal colon. Isolated colonic preparations fixed in a Magnus apparatus for tension recordings exhibited rhythmic and cyclic movements even in the absence of electrical or chemical stimulations (Fig. 3). The amplitude and frequency of the spontaneous contractions were sustained stably for at least 1 h after mounting the preparations. Intraluminal application of zingerol (30 mM) inhibited the colonic spontaneous motility in vitro efficiently, comparable to the effects of zingerone (Fig. 3). The results indicate that zingerol acts directly on the colon and inhibits its motility. In addition, tetrodotoxin, a blocker of voltage-dependent sodium channels on neurons,
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Thus, it is generally accepted that non-pungent chemicals do not effectively activate TRPV1. Accordingly, it seems unlikely that the inhibition of colonic motility by a non-pungent chemical zingerol is a consequence of an activation of TRPV1. In agreement with this, we have previously demonstrated that zingerone, a pungent analogue of zingerol, exerts an inhibitory effect on colonic motility in the presence of TRPV1 blockade (5). Taken together, the results indicate the inhibition of colonic motility by intraluminal application of zingerol is due to a TRPV1-independent mechanism.

We then investigated the effects of zingerol on jejunal motility in vivo to determine whether zingerol exclusively inhibits motility of the large intestine or whether it also inhibits motility of the small intestine. Under a condition in which intraluminal pressure of the jejunum was maintained at 3–5 mmHg, the rat jejunum exhibited periodic rises in intraluminal pressure accompanied by fluid output through the anal cannula. Intraluminal administration of zingerone (20 mg/kg) decreased both the amplitude of the pressure changes (Fig. 4) and the fluid output associated with colonic motility (data not shown). In contrast, zingerol (20 mg/kg) did not affect jejunal motility (Fig. 4). The distinct effects of zingerone and zingerol on the small intestine are interesting because these structurally related compounds commonly inhibit motility of the large intestine. We have previously demonstrated that the inhibition of colonic motility by zingerone largely slightly but significantly blocked the suppressive effects of zingerol on colonic movements (data not shown), suggesting that zingerol acts on both smooth muscles and neurons in the colon in contrast to zingerone (5). It should be noted, however, that these data do not necessarily rule out the possibility that a part of the inhibitory effects of zingerol on colonic motility is brought about by the autonomic nerves as a consequence of an activation of the central nervous system in vivo.

Some natural pungent compounds such as capsaicin can activate a non-selective cation channel termed transient receptor potential vanilloid-1 (TRPV1) (18, 19, 21). In accordance with this, it has been demonstrated in an experiment using the patch-clamp technique that zingerone evokes opening of TRPV1 (10). TRPV1 primarily localized on sensory nerves can evoke a pungent sensation (20). Thus, it is generally accepted that non-pungent chemicals do not effectively activate TRPV1. Accordingly, it seems unlikely that the inhibition of colonic motility by a non-pungent chemical zingerol is a consequence of an activation of TRPV1. In agreement with this, we have previously demonstrated that zingerone, a pungent analogue of zingerol, exerts an inhibitory effect on colonic motility in the presence of TRPV1 blockade (5). Taken together, the results indicate the inhibition of colonic motility by intraluminal application of zingerol is due to a TRPV1-independent mechanism.

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the present study, we showed that zingerol, a non-
12). These facts suggest that capsiate may be an
ment of energy expenditure and reduction of body
11, 12, 14, 15). It has been reported that most of the
13). Our results showing that zingerol inhibits colonic motility without affecting jejunal motility are reasonable if the target molecule of zingerol is the L-type calcium-channel. Further
zingerol, in contrast to zingerone, has no effect on motility of the small intestine. This also indicates the potential utility of zingerol as an orally-active anti-diarrhea drugs.
In summary, we characterized the pharmacological actions of zingerol on colonic motility in rats by comparing its actions with those of zingerone. Our findings suggest that zingerol can inhibit colonic motility without influencing small intestinal motility and the cardiovascular system. The non-pungent property of zingerol may contribute to its use for treating diarrhea and other gastrointestinal disorders as an oral or suppository medicine.

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