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Short communication

Effect of zaldaride maleate, an antidiarrheal compound, on intracellular cyclic nucleotide-mediated intestinal ion secretion in rats

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Abstract

The purpose of this study is to clarify the mechanisms of action of zaldaride, a calmodulin inhibitor. 16,16-Dimethyl prostaglandin E_2, forskolin, 8-bromo cAMP, nitroprusside, 8-bromo cGMP and *Escherichia coli* heat-stable enterotoxin STa increased the short-circuit current in rat colonic mucosa. Zaldaride at ≥ 10 μM significantly attenuated the 16,16-dimethyl prostaglandin E_2 and *Escherichia coli* heat-stable enterotoxin STa-induced increase in short-circuit current; whereas it did not affect other secretagogues-induced effects. These results suggest that zaldaride inhibits the activation of Ca^{2+}/calmodulin-sensitive adenylate cyclase or guanylate cyclase linked to a receptor.

Keywords: Zaldaride maleate; Calmodulin; Adenylate cyclase; Guanylate cyclase; Ion secretion

1. Introduction

Most currently used antidiarrheal drugs, such as loperamide and opium alkaloids, have an antipropulsive motility and antisecretory effect on intestinal tract. If the diarrhea is caused by microbiologic organisms, the antipropulsive motility action of drugs may enhance the bacterial invasion and also reduce the clearance of the bacteria from the intestinal tract. As a result, the duration of the diarrhea may be prolonged.

Zaldaride maleate can ameliorate secretory diarrhea without reducing gastrointestinal propulsive motility (Shook et al., 1989; Aikawa and Karasawa, 1998) and has no sedative effects at the doses used in reported studies. This compound is a more selective and potent inhibitor of calmodulin (Norman et al., 1987). However, the mechanisms of action of its antidiarrheal effects have not been sufficiently elucidated.

Calmodulin, a Ca^{2+}-binding protein, plays an important role in the secretion of electrolytes and water in the mammalian intestinal tract (Ilundain and Naftalin, 1979), and also regulates the activity of adenylate cyclase (Amiranoff et al., 1983; Neil et al., 1985) or guanylate cyclase (Dreyfus et al., 1984) and the metabolism of cyclic nucleotides (Cheung, 1970). cAMP and cGMP are produced by the activation of adenylate cyclase and guanylate cyclase, respectively, and are considered fundamental intracellular messengers in intestinal ion secretion. An increase in the level of cAMP or cGMP within intestinal epithelial cells evokes an intraluminal Cl^- secretion and inhibits Na^+, Cl^- absorption in the intestinal epithelium (Hardcastle et al., 1992; Volant et al., 1997).

In the present study, we investigated the effect of zaldaride on the intracellular cyclic nucleotide-mediated short-circuit current (I_{sc}) response indicating altered electrolyte transport in the rat colonic mucosa using in vitro Ussing chamber experiments. From these results, we hypothesize the mechanism of action of zaldaride with respect to its antisecretory effect.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (Charles River, Kanagawa, Japan) used in this study were housed in a controlled environment. Commercial rat chow and water were provided ad libitum. These experiments were conducted in

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compliance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and the experimental protocols approved by the Ethical Committee of the Pharmaceutical Research Institute, Kyowa Hakko Kogyo.

2.2. Materials

Zaldaride maleate was a gift from Novartis Consumer Health (Nyon, Switzerland). Forskolin, 8-bromo cAMP, nitroprusside sodium, 8-bromo cGMP, tetrodotoxin and trifluoperazine dimaleate were purchased from Wako (Osaka, Japan). *Escherichia coli* heat-stable STa (*E. coli* STa) and 16,16-dimethyl prostaglandin E_2 were purchased from Sigma (St. Louis, MO, USA). All other reagents were analytical grade.

2.3. Ussing chamber experiments

Rats weighing 275–420 g were killed, and the distal colon was carefully removed without distention. Mucosal preparations were obtained by stripping away the smooth muscle of the distal colon. Those preparations were mounted in Ussing chambers (surface area, 0.693 cm^2) and bathed on each side of the preparation with 10 ml of Krebs–Henseleit solution containing (mM): NaCl, 119.0; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $CaCl_2 \cdot 2H_2O$, 1.8; $NaHCO_3$, 24.9; and glucose, 11.1 (pH 7.4). This solution was maintained at 37°C and gassed with carbogen (5% CO_2 in 95% O_2). It also contained 0.05 mM ascorbic acid to prevent oxidation of compounds in the perfusion solution.

The change in I_{sc} was measured continuously, and the data were recorded on a chart-recorder. Zaldaride, trifluoperazine, tetrodotoxin or vehicle was added to the bath solution for 10 min before the addition of any secretagogues. Each secretagogue was used at a submaximal concentration. Because of its low solubility, zaldaride could not be tested at 100 μM and higher. Zaldaride, trifluoperazine, nitroprusside and forskolin were dissolved in dimethylsulfoxide, and other compounds were dissolved or diluted in distilled water. *E. coli* STa was applied to the apical (mucosal) side of the colonic preparations, and other compounds were added to the basolateral (serosal) side.

2.4. Statistical analysis

Each value is indicated as the mean ± S.E.M. for each group, and the data were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett's test or were analyzed by the Student's *t*-test or the Aspine–Welch test. A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Basal Isc

Tetrodotoxin at 1 μM has been shown to have no affect on the basal I_{sc} in the rat colonic mucosa and hardly affects I_{sc} responses to any secretagogues. At 30 μM, zaldaride increased the basal I_{sc} 5.0 ± 1.1 $\mu A/cm^2$ ($n = 38$); whereas the vehicle at a final concentration of 0.1% did not change the basal I_{sc} (-0.5 ± 0.8 $\mu A/cm^2$; $n = 38$) (data not shown).

Table 1
Effect of zaldaride on the cAMP-mediated I_{sc} response in the rat colonic mucosa treated with tetrodotoxin

Secretagogues	Compounds	Concentration (μM)	*n*	Increase in I_{sc} ($\mu A/cm^2$)
16,16-Dimethyl prostaglandin E_2 (1 μM)	Control		7	24.9 ± 3.7
	Zaldaride	3	7	21.1 ± 3.5
		10	7	10.5 ± 3.1[a]
		30	7	6.6 ± 2.2[b]
	Trifluoperazine	30	7	12.9 ± 3.3[a]
Forskolin (1 μM)	Control		7	60.9 ± 8.4
	Zaldaride	3	7	50.4 ± 9.0
		10	7	48.5 ± 2.6
		30	7	58.4 ± 5.1
	Trifluoperazine	30	7	53.6 ± 2.8
8-Bromo cAMP (1 mM)	Control		6	47.7 ± 7.4
	Zaldaride	3	6	48.7 ± 7.5
		10	6	40.6 ± 6.8
		30	6	39.2 ± 4.4
	Trifluoperazine	30	6	39.8 ± 5.9

Each value is indicated as the mean ± S.E.M.
I_{sc}: short circuit current; *n*: number of experiments.
[a] $P < 0.05$, statistically significant vs. the value of each control group.
[b] $P < 0.01$, statistically significant vs. the value of each control group.

Table 2
Effect of zaldaride on the cGMP-mediated I_{sc} response in the rat colonic mucosa treated with tetrodotoxin

Secretagogues	Compounds	Concentration (μM)	*n*	Increase in I_{sc} (μA/cm^2)
E. coli STa (100 μg/l)	Control		12	5.9 ± 0.8
	Zaldaride	3	12	4.6 ± 0.8
		10	12	2.5 ± 0.9[a]
		30	12	2.1 ± 0.8[b]
	Trifluoperazine	30	12	2.3 ± 0.8[b]
Nitroprusside (100 μM)	Control		6	9.3 ± 1.4
	Zaldaride	3	6	11.7 ± 1.4
		10	6	7.1 ± 2.1
		30	6	8.3 ± 1.1
	Trifluoperazine	30	6	7.5 ± 1.6
8-Bromo cGMP (100 μM)	Control		6	19.7 ± 3.4
	Zaldaride	3	6	10.5 ± 4.5
		10	6	12.2 ± 1.4
		30	6	15.6 ± 2.1
	Trifluoperazine	30	6	22.5 ± 4.9

Each value is indicated as the mean ± S.E.M.
E. coli STa: *Escherichia coli* heat-stable STa; I_{sc}: short circuit current; *n*: number of experiments.
[a] $P < 0.05$, statistically significant vs. the value of each control group.
[b] $P < 0.01$, statistically significant vs. the value of each control group.

3.2. Effect of zaldaride on the intracellular cAMP-mediated increase in Isc

In the presence of tetrodotoxin, zaldaride at 10 μM and higher significantly decreased the 16,16-dimethyl prostaglandin E_2 (1 μM)-induced increase in I_{sc}, whereas this compound did not affect the forskolin (1 μM) — or 8-bromo cAMP (1 mM)-induced effect (Table 1). At 30 μM, trifluoperazine also significantly reduced the I_{sc} response to 16,16-dimethyl prostaglandin E_2 but not to forskolin or 8-bromo cAMP in the presence of tetrodotoxin (Table 1).

3.3. Effect of zaldaride on the intracellular cGMP-mediated increase in Isc

Zaldaride at 10 μM and higher significantly decreased the I_{sc} response to *E. coli* STa (100 μg/l) in the presence of tetrodotoxin. However, this compound up to 30 μM did not affect the I_{sc} response to nitroprusside (100 μM) or 8-bromo cGMP (100 μM) in the presence of tetrodotoxin (Table 2). Trifluoperazine at 30 μM also significantly reduced the I_{sc} response to *E. coli* STa, but did not affect the 8-bromo cGMP — or *E. coli* STa-induced increase in I_{sc} in the rat colonic mucosa treated with tetrodotoxin (Table 2).

4. Discussion

In this study, zaldaride inhibited the I_{sc} responses to 16,16-dimethyl prostaglandin E_2, a stable analogue of prostaglandin E_2, which is known to stimulate Cl^- secretion in the rat colonic mucosa via the activation of adenylate cyclase–cAMP system mediated by the type-4 subtype of prostaglandin receptors (Ding et al., 1997). Moreover, zaldaride at 10 μM and higher inhibited vasoactive intestinal polypeptide-induced increases in I_{sc} (Aikawa et al., unpublished observation), which is also evoked by the receptor-mediated activation of adenylate cyclase–cAMP system (Sayadi et al., 1988; Rouyer-Fessard et al., 1989). In contrast, zaldaride did not attenuate the I_{sc} response to forskolin, which stimulates Cl^- secretion in the rat colonic mucosa by the direct activation of adenylate cyclase and elevation of cAMP levels within the epithelial cells (Bohme et al., 1991). Moreover, zaldaride did not inhibit the I_{sc} response to 8-bromo cAMP, a stable analogue of cAMP, to stimulate Cl^- secretion in the intestinal epithelium (Hardcastle et al., 1992). These findings demonstrate that zaldaride inhibits the I_{sc} response induced by receptor-mediated elevation of cAMP without affecting the response induced by direct activation of adenylate cyclase or cAMP itself. We could thus conclude that zaldaride reduces cAMP-mediated intestinal ion secretion by inhibiting the activation of Ca^{2+}/calmodulin-sensitive adenylate cyclase linked to a receptor.

The I_{sc} response to 16,16-dimethyl prostaglandin E_2 was around half as much as that to forskolin. Adenylate cyclase is known to consist of more than six subtypes. We suppose that 16,16-dimethyl prostaglandin E_2 stimulates only the type-4 receptor-linked adenylate cyclase, resulting in the I_{sc} response smaller than that induced by forskolin. Alternatively, the intrinsic activity of 16,16-dimethyl prostaglandin E_2 to induce activation of adenylate cyclase may be a partial one, not activating the enzyme to the maximal level. However, the precise mechanism for the smaller I_{sc} response to 16,16-dimethyl prostaglandin E_2 needs further investigation.

E. coli STa stimulates intraluminal Cl^- secretion via the activation of a particulate guanylate cyclase by activating a receptor in the apical membranes of enterocytes (Gyles, 1992). *E. coli* STa induces the activation of guanylate cyclase in rat intestinal epithelial cells, which is inhibited by calmodulin inhibitors (Dreyfus et al., 1984). In the present study, the I_{sc} response to *E. coli* STa was attenuated by zaldaride and trifluoperazine. These findings suggest that zaldaride reduces the I_{sc} response to *E. coli* STa by inhibiting the activation of a particulate guanylate cyclase that was linked to receptors and whose activity may be modulated by calmodulin.

Nitroprusside, a nitric oxide-donating compound, stimulates intestinal Cl^- secretion by the activation of soluble guanylate cyclase (Waldman and Murad, 1987). Nitroprusside also causes increases in cGMP production in the rat colonic mucosa (Wilson et al., 1996). In the present study, the I_{sc} response to nitroprusside was not attenuated by zaldaride or trifluoperazine. On the other hand, zaldaride is able to improve castor-oil-induced experimental diarrhea possibly by inhibiting constitutive nitric oxide synthase activity in rodents (Shook et al., 1989; Aikawa and Karasawa, 1998). In fact, castor-oil-induced diarrhea is evoked, at least in part, by the activation of a constitutive nitric oxide synthase (Uchida et al., 1997). It is thus possible that zaldaride, in vivo, is able to reduce intestinal ion transport by the inhibition of nitric oxide production.

The I_{sc} responses to cGMP-mediated secretagogues were smaller than those of the cAMP-mediated ones. On the other hand, *E. coli* STa is known to induce diarrhea, in vivo, presumably activating the guanylate cyclase–cGMP system. The ion transport in the distal site of rat colon is more sensitive to cAMP-mediated secretagogues; in contrast, cGMP-mediated secretagogues are more effective in the proximal site (Nobles et al., 1991). We suppose that in this study, the distal segments of the rat colon were used, and thus cGMP-mediated I_{sc} responses were smaller than cAMP-mediated effects. Accordingly, although the effect of zaldaride could not be sufficiently evaluated in this in vitro study, it is likely that zaldaride would ameliorate cGMP-mediated secretory diarrhea in vivo.

Zaldaride at 30 μM increased the basal I_{sc}. cAMP and cGMP are inactivated by cyclic nucleotide phosphodiesterases. At present, the five known isoforms of phosphodiesterase show a species-and organ-specific distribution. The type-I phosphodiesterase is a calmodulin-sensitive enzyme. An increase from the basal I_{sc} induced by 30 μM zaldaride may be due to accumulation of cAMP or cGMP in colonic epithelial cells by inhibition of the basal activity of this calmodulin-dependent phosphodiesterase.

In conclusion, zaldaride reduces colonic ion secretion by inhibiting the activation of Ca^{2+}/calmodulin-sensitive adenylate cyclase or guanylate cyclase linked to a receptor.

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