Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum

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Submitted 29 September 2004; accepted in final form 14 December 2004

Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum. Am J Physiol Gastrointest Liver Physiol 288: G956–G963, 2005. First published December 23, 2004; doi:10.1152/ajpgi.00441.2004.—Zn, an essential micronutrient and second most abundant trace element in cell and tissues, reduces stool output when administered to children with acute diarrhea. The mechanism by which Zn improves diarrhea is not known but could result from stimulating Na absorption and/or inhibiting anion secretion. The aim of this study was to investigate the direct effect of Zn on intestinal epithelial ion absorption and secretion. Rat ileum was partially stripped of serosal and muscle layers, and the mucosa was mounted in lumbic chambers. Potential difference and short-circuit current were measured by conventional current-voltage clamp method. 86Rb efflux and uptake were assessed for serosal K channel blockers in the presence of ouabain and bumetanide, whereas uptake experiments were performed in low-Cl isotonic buffer containing Ba and ouabain. Neither mucosal nor serosal Zn affected glucose-stimulated Na absorption. In contrast, forskolin-induced Cl secretion was markedly reduced by serosal but not mucosal addition of Zn. Zn also substantially reversed the increase in Cl secretion induced by 8-bromoadenosine 3’,5’-cyclic monophosphate (8-BrcAMP) with half-maximal inhibitory concentration of 0.43 mM. In contrast, serosal Zn did not alter Cl secretion stimulated by carbachol, a Ca-dependent agonist. Zn inhibited 8-BrcAMP-stimulated 86Rb efflux but not carbachol-stimulated 86Rb efflux. Zn had no effect on bumetanide-sensitive 86Rb uptake, Na-K-ATPase, or CFTR. We conclude from these studies that Zn inhibits cAMP-induced Cl secretion by blocking basolateral membrane K channels.

enterocyte; 86Rb efflux; 86Rb uptake; K-channel inhibitors

diarrheal diseases remain a major public health problem especially in children in developing countries throughout the world, with ~1.5 billion episodes per year. It is estimated that diarrheal diseases cause 1.5–2.5 million deaths each year among children younger than five years old and contribute substantially to malnutrition in surviving children (9, 22, 30). After the introduction of oral rehydration solution (ORS) more than 30 years ago, the treatment of acute diarrhea has dramatically changed and ORS use has become widespread. ORS corrects dehydration and metabolic acidosis due to fluid and bicarbonate losses and reduces mortality. However, ORS does not substantially decrease stool output nor does it decrease the duration of episodes or their consequences such as malnutrition (33, 51). As a result, improved or “super ORSs” as well as other approaches for enhancing treatment of acute diarrhea have continually been sought and evaluated.

Recently, Zn supplementation to ORS has been shown to reduce substantially the duration and severity of diarrhea in children with both acute and persistent diarrhea (6, 7, 36, 38, 50). The initial suggestion of the mechanism by which Zn was effective to improve diarrhea was that Zn administration corrected an underlying micronutrient deficiency that had contributed, in some way, to the child’s diarrhea. However, the demonstration that Zn is effective in both Zn-deficient and nondeficient children (O. Fontaine, personal communication) suggested that the action of Zn might be related to a direct effect on intestinal absorptive and/or secretory transport processes.

Diarrheal disorders are almost always associated with changes in fluid and electrolyte movement so that a decrease in absorption and/or an increase in secretion have been invariably found in most diarrheal disorders. An effective anti diarrheal agent should either enhance fluid and electrolyte absorption and/or reduce fluid and electrolyte secretion. ORS is effective by virtue of its stimulation of small intestinal fluid absorption as a result of glucose stimulation of Na absorption via SGLT1. Over the past 25 years, multiple detailed studies of active Cl secretion, which has long been considered the driving force for fluid secretion, have been performed. The present model of Cl secretion includes the coordinated action of an apical membrane Cl channel (e.g., CFTR) and on the basolateral membrane Na-K-ATPase, Na-K-2Cl cotransport (NKCC), and one or more K channels (3, 24). As the effect of Zn on intestinal ion transport has not been previously evaluated, the aim of this present study was to investigate the effect of Zn on Na absorption and Cl secretion in rat ileal mucosa to determine whether Zn enhances Na absorption and/or inhibits Cl secretion. These experiments demonstrated that Zn selectively inhibited cAMP-induced Cl secretion, whereas additional studies established that Zn was a potential inhibitor of cAMP-activated K channels on basolateral membrane of ileal epithelial cells.

MATERIALS AND METHODS
Preparation of Epithelia

Nonfasting male Sprague-Dawley rats (200–250 g) were used in all experiments. Rats were anesthetized and killed by inhalation of ether. All the experimental protocol performed in this study had been approved by the Institutional Animal Care and Use Committee of Yale University.

The distal ileum removed from anesthetized rats was placed in HCO3+-free Ringer solution that contained (in mM) 140 NaCl, 5 KCl, 1.5 billion episodes per year. It is estimated that diarrheal diseases cause 1.5–2.5 million deaths each year among children younger than five years old and contribute substantially to malnutrition in surviving children (9, 22, 30). After the introduction of oral rehydration solution (ORS) more than 30 years ago, the treatment of acute diarrhea has dramatically changed and ORS use has become widespread. ORS corrects dehydration and metabolic acidosis due to fluid and bicarbonate losses and reduces mortality. However, ORS does not substantially decrease stool output nor does it decrease the duration of episodes or their consequences such as malnutrition (33, 51). As a result, improved or “super ORSs” as well as other approaches for enhancing treatment of acute diarrhea have continually been sought and evaluated.

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The ileum was partially stripped of serosal and muscle layers, and the mucosa was mounted in Lucite chambers and bathed at 37°C on both sides with HCO₃⁻-free Ringer solution, which was continually circulated with a gas lift oxygenated with 100% O₂. An HCO₃⁻/CO₂ buffer system was not used due to Zn insolubility in such a solution. Zn was used as the chloride salt (ZnCl₂), which was prepared in HCO₃⁻-free Ringer just before its addition to the chamber. Potential difference was measured; tissues were studied under voltage-clamp conditions (DVC 1000 dual voltage clamp, WPI, Sarasota, FL), and conductance was calculated.

Isolation of Intestinal Crypt Enterocytes

Epithelial cells were isolated from rat ileum according to previously described methods (44, 49) with modification. Briefly, the distal portion of intestine was removed and washed free of fecal material with ice-cold 0.9% NaCl containing 1 mM DTT. The intestine was then filled with a solution containing (in mM) 96 NaCl, 27 sodium citrate, 1.5 KCl, 0.8 KH₂PO₄, 5.6 K₂HPO₄, and 4 glucose, pH 7.3. After being emptied, the intestinal lumen was again filled with this solution and incubated for 15 min at 37°C. After drainage of the fluid, the intestine was then filled with a buffer containing (in mM) 115 NaCl, 25 NaHCO₃, 2.4 K₂HPO₄, 0.4 KH₂PO₄, 4 glucose, 0.5 EDTA, and 0.5 DTT. The intestine was drained, and after being filled with this solution a second time, it was incubated for 6 min with frequent finger tapping to facilitate cell dispersion with collection of the contents. Five additional cell fractions were collected and passed through two to three layers of cheese cloth. The cell fractions were centrifuged at 1,000 g for 5 min, and the pellet was then washed with Hanks’ balanced salt solution (HBSS; Invitrogen, Carlsbad, California) and suspended in HBSS at room temperature (pH 7.4). Crypt cell-enriched fractions isolated in fractions 4–6 were combined and used for the uptake and efflux studies. Cell numbers were determined with a hemocytometer, and a viability test was performed with trypan blue.

Radioisotopic Efflux and Influx Studies

86Rb efflux in isolated crypt enterocytes. According to the previously described methods (12, 20, 28), these isolated cells were suspended in HBSS containing 2 mM RbCl and then loaded with 2 μCi/ml 86Rb by incubation at 37°C for 40–60 min, by which time the incorporation of 86Rb into cells had achieved a steady-state concentration (data not shown); 85 ± 5% cells were viable. Ouabain (0.5 mM) and bumetanide (0.2 mM) were added for the final 15–20 min to inhibit 86Rb movement via Na-K-ATPase and NKCC, respectively.

86Rb uptake studies in isolated cells. Uptake studies were performed according to methods previously described (13, 29, 31). Isolated rat enterocytes were washed with HBSS (pH 7.4) and were equilibrated for 20–30 min in a low-Cl isotonic medium. The low-Cl isotonic medium contained (in mM) 135 Na-glucolate, 5 K-gluconate, 1 CaCl₂, 1 MgCl₂, 1 NaH₂PO₄, 2 Na₂SO₄, 15 HEPES, and 4 glucose (pH 7.4). Cells were then incubated for 15–20 min in the same solution in presence of ouabain (0.5 mM), an Na-K-ATPase inhibitor, and Ba (3 mM), a K-channel blocker, with or without Zn (1 mM). 8-BrcAMP was used for an additional 5-min incubation. Uptake was initiated by transferring 100-μl cell aliquots (4 × 10⁶ cells/ml cell suspension) to the low-Cl isotonic medium containing 2 μCi/ml 86Rb. Influx was terminated after 3 min by transferring the suspension of whole cells to the 0.45-μm filter paper, which was rapidly washed three times with ice-cold 100 mM MgCl₂ and 10 mM HEPES-Tris (pH 7.4). 86Rb content was measured. cAMP-stimulated, ouabain-insensitive, Ba-insensitive, and bumetanide-sensitive uptake was considered to represent NKCC activity.

Materials

86Rb was purchased from Amersham (Piscataway, NJ). All other chemicals of analytical grade were obtained from Sigma (St. Louis, MO) or from J. T. Baker (Phillipsburg, NJ).

Statistics

Results are presented as means ± SE. Statistical analyses were performed using paired and unpaired t-test by the statistical software Origin (version 6.0). In the 86Rb efflux and uptake studies ANOVA with post hoc Bonferroni’s test was used to assess statistical significance. P < 0.05 was considered significant.

RESULTS

Effect of Zn on glucose-stimulated Iₛₑ

To determine whether Zn affected glucose-stimulated Na absorption via SGLT1, cotransport experiments were performed in which 1 mM Zn was added either to the mucosal or to the serosal solutions 10 min before the addition of 10 mM glucose to the mucosal solution. The increase in Iₛₑ following the addition of mucosal glucose reflects an increase in Na absorption and was identical in the tissues pretreated with mucosal Zn compared with the control group. Serosal addition of 1 mM Zn also did not have any effect on glucose-stimulated Iₛₑ (Fig. 1). In addition, the addition of 1 mM Zn either to the mucosal or to the serosal solution did not significantly alter basal Iₛₑ over 20 min (data not shown). These observations suggest that mucosal pretreatment of Zn did not have any stimulatory effect on glucose-stimulated Na absorption.

Effect of Zn on Forskolin-Stimulated Cl Secretion

To assess whether Zn modified cAMP-stimulated Cl secretion, experiments were performed to evaluate the effect of Zn on FSK-stimulated Iₛₑ. FSK activates adenylyl cyclase resulting in an increase in mucosal cAMP levels and stimulation of active Cl secretion in many tissues (41). Similar to earlier observations (15, 44), serosal addition of 10 μM FSK resulted in an immediate and sustained increase in Iₛₑ (ΔIₛₑ: 54.6 ± 1.1
not mucosal addition of Zn reduced FSK-stimulated $I_{sc}$ (Fig. 2B).

Effect of Zn on 8-BrcAMP-Stimulated Cl Secretion

To distinguish between the effects of Zn at one or more sites of the cAMP-stimulated Cl secretory process vs. an inhibitory effect on adenylate cyclase, experiments were performed in which the effect of Zn on 8-BrcAMP-stimulated $I_{sc}$ was determined. The addition of 1 mM 8-BrcAMP to the serosal solution caused an immediate and sustained increase in $I_{sc}$. This sustained increase of $I_{sc}$ stimulated by 8-BrcAMP was also reversed by serosal addition of 1 mM Zn. In contrast, mucosal addition of 1 mM Zn did not alter 8-BrcAMP-stimulated $I_{sc}$ (Fig. 3).

The inhibition of cAMP-stimulated $I_{sc}$ by Zn was also evaluated by experiments in which Zn was added before the stimulation of Cl secretion. The change in $I_{sc}$ following the addition of 8-BrcAMP in tissues that had been pretreated with Zn was also significantly reduced compared with that of control tissues (7.7 ± 3.1 vs. 53.5 ± 7.1 μA/cm²; $P < 0.01$). In contrast, the mucosal addition of Zn did not alter the subsequent increase in $I_{sc}$ produced by 8-BrcAMP (56.2 ± 5.7 vs. 53.5 ± 7.1 μA/cm²; not significantly different). All subsequent experiments that assessed the effect of Zn on cAMP-mediated changes in $I_{sc}$ were thus performed with 1 mM 8-BrcAMP.

To establish the half-maximal inhibitory concentration (IC$_{50}$) of Zn on 8-BrcAMP-stimulated Cl secretion, experiments were performed in which varying concentrations of Zn were added before stimulation of $I_{sc}$ by 8-BrcAMP. The inhibition of 8-BrcAMP-stimulated $I_{sc}$ by Zn was dose dependent (Fig. 4), with maximal inhibition observed with 1 mM Zn. IC$_{50}$ for inhibition of 8-BrcAMP-induced $I_{sc}$ was derived from a nonlinear fit of the dose-response data according to the Boltzman equation (42). The determined IC$_{50}$ value was 0.43 ± 0.04 mM Zn. These observations indicate that serosal Zn prevents cAMP stimulation of active Cl secretion, possibly by inhibiting one or more basolateral transport sites that are critical for cAMP-mediated active Cl secretion but not by affecting either adenylate cyclase or apical membrane CFTR.
Effect of Zn on NKCC

The results presented in Figs. 2 and 3 indicate that Zn inhibited cAMP-induced $I_{sc}$ by an effect on a basolateral but not an apical membrane transport mechanism. Basolateral transport mechanisms associated with cAMP-induced Cl secretion include NKCC, Na-K-ATPase, and K channels. Basolateral NKCC represents the major ion-entry pathway required for transepithelial Cl secretion, and its activity has been determined by the bumetanide-sensitive component of $86$Rb uptake, as previously reported (19, 27). $8$-BrcAMP-induced $86$Rb uptake (as a surrogate of K) was determined in the presence of 0.5 mM ouabain and 3 mM Ba. Figure 5 shows that neither bumetanide-sensitive nor -insensitive $86$Rb fluxes were affected by Zn in the presence of 8-BrcAMP.

Effect of Zn on K Channels

To determine whether Zn acts as a K-channel blocker, $86$Rb efflux experiments were performed using enterocytes isolated from rat ileum. To inhibit cAMP-stimulated $86$Rb efflux via non-K-channel pathways, e.g., NKCC and Na-K-ATPase, these experiments were performed in the presence of bumetanide and ouabain, respectively. Figure 6A demonstrates the effect of Zn and Ba on 8-BrcAMP-induced $86$Rb efflux. The sensitivity of 8-BrcAMP-induced $86$Rb efflux to Zn was potent (65%) and greater than that of 3 mM Ba (52.2%). The combined effect of Zn plus Ba on $86$Rb efflux was also determined. Inhibition of $86$Rb efflux by the presence of both Zn plus Ba was significantly less than that affected by Zn alone. Inhibition of $86$Rb efflux by Ba did not significantly differ from that of both Zn and Ba together (Fig. 6A). The effect of 293B, a specific inhibitor of cAMP-activated K channels (25), on $86$Rb efflux was also examined. Figure 6B demonstrates that 293B alone and Zn alone both inhibited cAMP stimulation by ~70%. CAM-activated $86$Rb efflux was also inhibited by ~70% in the presence of 293B and Zn together, suggesting that Zn was blocking the same K channel as does 293B.

To establish that Zn inhibits 8-BrcAMP-induced Cl secretion by blocking K channels, IC$_{50}$ for Zn on 8-BrcAMP-stimulated $86$Rb efflux was also determined (Fig. 7). Increasing Zn concentrations progressively inhibit 8-BrcAMP-stimulated $86$Rb efflux with an IC$_{50}$ for Zn of 0.53 ± 0.06 mM Zn (Fig. 7). These observations suggest that Zn inhibits cAMP-stimulated Cl secretion by blocking basolateral membrane K channels that provide a driving force for Cl secretion.

Effect of Barium on 8-BrcAMP-Stimulated Cl Secretion

Previous studies (2, 21, 26, 34) have shown that two pharmacologically distinct K channels are responsible for providing the driving force for transepithelial Cl secretion in a number of...
epithelia and T84 cells, one that is sensitive to inhibition by Ba and associated with increased mucosal cAMP levels and a second Ba-insensitive pathway that is activated by elevated intracellular Ca. Therefore, experiments were designed to establish whether Cl secretion induced by 8-BrcAMP was also inhibited by Ba. The addition of 3 mM Ba to the serosal bath significantly inhibited the increase in I_sc induced by 8-BrcAMP (Fig. 8). Because mucosal Ba has also been shown to inhibit aldosterone-induced K secretion in rat distal colon (45), the effect of Ba added to the mucosal bath on 8-BrcAMP-induced Cl secretion was also examined. In these present studies, both serosal and mucosal Ba also inhibited Cl secretion (Fig. 8).

**Studies with Carbachol**

To establish the specificity of the effect of Zn on cAMP-stimulated I_sc, experiments were designed to assess the effect of Zn on the increase in I_sc induced by agonists that stimulate Cl secretion as a result of an increase in intracellular Ca. In these experiments the addition of 200 μM carbachol to the serosal bath resulted in a prompt increase in I_sc, similar to that previously observed in T84 cells and rabbit distal colon (34, 35). In contrast to that observed with 8-BrcAMP, the effect of carbachol on I_sc was not altered in tissue preincubated with either 1 mM serosal Zn or 3 mM serosal Ba (Fig. 9A). Therefore, 86Rb efflux was also determined in carbachol-stimulated cells to evaluate whether Zn would modify calcium-mediated efflux. Figure 9B shows that carbachol-stimulated 86Rb efflux was not affected either by Zn or by Ba. In contrast, clotrimazole, a known inhibitor of Ca-mediated Cl secretion, significantly inhibited 86Rb efflux induced by carbachol. These observations establish that Zn selectively inhibits basolateral K channels that are activated by cAMP but not by Ca.

**DISCUSSION**

The primary treatment of acute diarrhea especially in children has been oral rehydration solution. In controlled studies, oral rehydration therapy is very effective in correcting dehydration and reducing mortality (48). However, oral rehydration solution neither dramatically decreases stool volume nor reduces the duration of the disease course (47). Therefore, several different approaches have been evaluated to improve...
the therapy of acute diarrhea by modifying oral rehydration solution with the primary aim to reduce stool fluid losses and shorten the duration of diarrhea. Most efforts have focused on the enhancement of small intestinal fluid and Na absorption with the use of meal- or cereal-based and/or hypo-osmolar solutions (46). Recently, the use of amylase-resistant starch added to ORS was evaluated based on the premise that increased delivery of nonabsorbed carbohydrate to the colon will result in enhanced production of short-chain fatty acids, which, in turn, will stimulate Na and fluid absorption (8, 32).

Several prior studies have demonstrated the efficacy of Zn in the treatment of acute diarrhea (6, 7, 36, 38, 50) but have not provided any insight into the mechanism by which Zn improves fluid and electrolyte transport. Dietary deficiency of some micronutrients has been shown to increase the susceptibility of infants and children to gastrointestinal infection and to adversely affect gastrointestinal tract structure and function (5, 38). In particular, Zn deficiency can be associated with chronic diarrhea (1). It has been reported that in Zn deficiency-associated diarrhea, Zn may help maintain the integrity of the gut mucosa to reduce or prevent fluid loss (4). However, studies of Zn treatment of acute and persistent diarrhea did not demonstrate that nutritional status affected the beneficial effects of Zn, suggesting that Zn was effective in the treatment of diarrhea in both Zn-deficient and -replete children (O. Fontaine, personal communication). To date, there are no reports of the pharmacological and/or pathophysiological effect of Zn on intestinal ion transport, although in one recent study (11), the effect of both theophylline, a phosphodiesterase inhibitor, and 5-HT, a serotonin agonist, on I_{sc} across ileal mucosa was reduced in piglets fed a relatively high Zn diet compared with that in piglets fed a “normal” diet. These observations are consistent with the present experimental results.

Symptomatic antidiarrheal therapy should either enhance fluid and Na absorption and/or inhibit fluid and anion secretion (10). For example, oral rehydration solution is effective by virtue of its stimulation of Na absorption via SGLT1, whereas the effect of clonidine on diabetic enterocytes (Figs. 5–7). In intact tissue, Zn inhibited cAMP-stimulated I_{sc} also indicated that Zn did not induce an increase in glucose-stimulated I_{sc} as a result of increasing Na-K-ATPase, and the population of K channels inhibited by Zn is not active during glucose-stimulated increase in I_{sc}. The effect of Zn on electroneutral Na-Cl absorption was not examined in these present studies.

In contrast to the absence of an effect of Zn on glucose-stimulated Na absorption, these present results demonstrated that Zn inhibited cAMP-stimulated Cl secretion. Because Zn inhibited both FSK- and cAMP-stimulated Cl secretion, the action of Zn on cAMP-stimulated Cl secretion is at a site distal to the generation of cAMP and is not due to its inhibition of adenylate cyclase. A model of cAMP stimulation of Cl secretion has been well established in experiments with both intact intestinal tissue and T84 cells during the past two decades. cAMP-stimulated Cl secretion requires the coordinated interaction of apical membrane CFTR with Na-K-ATPase, NKCC, and K channel on the basolateral membrane (3, 24). These present studies in which mucosal Zn did not alter cAMP-stimulated Cl secretion suggest that Zn did not inhibit effect on CFTR. It is unlikely that Zn inhibited Na-K-ATPase, because serosal Zn did not inhibit glucose-stimulated I_{sc}. Furthermore, Zn did not affect NKCC activity, which was estimated by the bumetanide-sensitive component of 86Rb uptake (Fig. 5). Furthermore, it is unlikely that Zn affects NKCC function, because NKCC is critical to both cAMP- and Ca-mediated Cl secretion and Zn inhibited cAMP-mediated but not Ca-mediated Cl secretion.

The present experiments systematically examined the effect of Zn on electrogenic absorptive and secretory processes in the rat ileum to assess whether Zn enhanced Na absorption and/or inhibited Cl secretion. These results established that mucosal pretreatment of isolated rat ileal tissue with Zn did not have any stimulatory effect on glucose-stimulated Na absorption. The failure of basolateral addition of Zn to modify glucose-stimulated I_{sc} also indicated that Zn did not induce an increase in glucose-stimulated I_{sc} as a result of increasing Na-K-ATPase, and the population of K channels inhibited by Zn is not active during glucose-stimulated increase in I_{sc}. The effect of Zn on electroneutral Na-Cl absorption was not examined in these present studies.

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Several experiments provided compelling evidence that Zn inhibited cAMP-stimulated Cl secretion as a result of its inhibition of cAMP-mediated basolateral membrane K channels. First, Zn blocked cAMP-induced 86Rb efflux from isolated ileal enterocytes (Figs. 5–7). In intact tissue, Zn inhibited 8-Br-cAMP-stimulated Cl secretion with an identical IC_{50} (0.46 mM; Fig. 4) to that which inhibited 8-Br-cAMP-stimulated 86Rb efflux in isolated cells (0.53 mM; Fig. 7). Second, Ba, a known K-channel blocker, also inhibited cAMP-induced Cl secretion, and Ba prevented the inhibition of 86Rb efflux by Zn (Fig. 6). Third, carbachol, an agonist that also induces Cl...
secretion via an increase in intracellular Ca, increased $^{86}$Rb efflux, which was not inhibited by Zn (Fig. 9). Fourth, Zn did not affect either CFTR, Na-K-ATPase function, or NKCC activity. These several observations suggest that Zn selectively acts to block cAMP-mediated but not Ca-dependent basolateral K channels.

The effects of Zn and Ba on 8-BrcAMP-stimulated Cl secretion were not identical. Although both mucosal and serosal Ba affected 8-BrcAMP Cl secretion, only serosal Zn inhibited 8-BrcAMP-stimulated Cl secretion. cAMP induces active K secretion by virtue of its activation of one or more apical membrane K channels. These observations indicate that distinct K channels are activated by cAMP on the apical and basolateral membranes and suggest that the apical membrane K channel is Ba but not Zn sensitive, whereas basolateral K channels are sensitive to both Ba and Zn.

The protocol for the $^{86}$Rb efflux studies required the presence of ouabain to inhibit Rb efflux via the Na pump. Such inhibition can induce cell swelling, resulting in activation of both K channels and K-Cl cotransport. However, it is unlikely that the inhibition of $^{86}$Rb efflux by Zn solely represents inhibition of volume-sensitive K channels; as such, K channels are cAMP independent. In contrast, Zn most likely selectively blocks cAMP-activated K channels, because both Zn and 293B, a specific inhibitor of cAMP-activated channels (49), appear to block the same K channel (Fig. 6B).

Future studies are required to delineate the specific basolateral K channel that is inhibited by Zn. Because both cAMP-activated (KCQ1) and Ca-activated K channels have been identified in basolateral membranes of enterocytes (49–51), it is likely that Zn inhibits KCQ1. The recent demonstration that barium affects the gating of KCQ1 (17) is consistent with the speculation that Zn acts via KCQ1, because the present results indicate that barium and Zn block the same K channel (Fig. 6A). However, the site of the action of Zn must also be established, because Zn may initially enter the cell and bind to the cytosolic side of a basolateral K channel. Prior studies (51) have established that Zn interacts with large-conductance (BK) K channels in neurons. However, these studies demonstrated that Zn prevented the opening of the BK channel. Such an effect differs from the present observations in that Zn reversed the effect of cAMP-stimulated Cl secretion (Fig. 3).

In conclusion, the data presented in this study provide definitive evidence that in intact tissue and in isolated enterocytes of rat ileum, Zn inhibits cAMP-stimulated Cl secretion by selectively inhibiting a cAMP-activated basolateral K channel. These observations suggest that Zn may be effective in other cAMP-mediated secretory diarrheal disorders and that other K-channel blockers may have the potential for development as anti-diarrheal agents.

ACKNOWLEDGMENTS

This study was supported in part by a United States Public Health Service research grant (National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-60069) and by a grant from the Wellcome Trust.

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