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Regulation of intestinal uroguanylin/guanylin receptor-mediated responses by mucosal acidity

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ABSTRACT **Guanylin and uroguanylin are intestinal peptides that stimulate chloride secretion by activating a common set of receptor–guanylate cyclase signaling molecules located on the mucosal surface of enterocytes. High mucosal acidity, similar to the pH occurring within the fluid microclimate domain at the mucosal surface of the intestine, markedly enhances the cGMP accumulation responses of T84 human intestinal cells to uroguanylin. In contrast, a mucosal acidity of pH 5.0 renders guanylin essentially inactive. T84 cells were used as a model epithelium to further explore the concept that mucosal acidity imposes agonist selectivity for activation of the intestinal receptors for uroguanylin and guanylin, thus providing a rationale for the evolution of these related peptides. At an acidic mucosal pH of 5.0, uroguanylin is 100-fold more potent than guanylin, but at an alkaline pH of 8.0 guanylin is more potent than uroguanylin in stimulating intracellular cGMP accumulation and transepithelial chloride secretion. The relative affinities of uroguanylin and guanylin for binding to receptors on the mucosal surface of T84 cells is influenced dramatically by mucosal acidity, which explains the strong pH dependency of the cGMP and chloride secretion responses to these peptides. The guanylin-binding affinities for peptide–receptor interaction were reduced by 100-fold at pH 5 versus pH 8, whereas the affinities of uroguanylin for these receptors were increased 10-fold by acidic pH conditions. Deletion of the N-terminal acidic amino acids in uroguanylin demonstrated that these residues are responsible for the increase in binding affinities that are observed for uroguanylin at acidic pH. We conclude that guanylin and uroguanylin evolved distinctly different structures, which enables both peptides to regulate, in a pH-dependent fashion, the activity of receptors that control intestinal salt and water transport via cGMP.**

Guanylin and uroguanylin are structurally related peptides that were isolated from intestinal mucosa and urine (1–5). A receptor for guanylin and uroguanylin that has been identified at the molecular level is a transmembrane form of guanylate cyclase, termed GC-C (6). This membrane protein was originally discovered as an intestinal receptor for the heat-stable toxin (ST) peptides, which are secreted intraluminally by enteric bacteria that cause traveler's diarrhea (7). Bacterial ST peptides are related in primary structure to uroguanylin and guanylin, thus acting as molecular mimics of the enteric peptide hormones (reviewed in refs. 8 and 9). Membrane receptor–guanylate cyclases are found on the luminal surface of enterocytes throughout the small and large intestine and in other epithelia (10–13). Binding of peptide agonists to an extracellular domain of the receptor activates the intracellular catalytic domain producing the second messenger cGMP within target enterocytes (1–6). Intracellular cGMP stimulates transepithelial chloride secretion by regulating the phosphorylation state and chloride channel activity of the cystic fibrosis transmembrane conductance regulator, an apical protein that is located with the receptors for uroguanylin, guanylin, and ST peptides (14–16).

Isolation of uroguanylin from opossum urine (2) followed by the cloning of a colon cDNA that encodes opossum prepouroguanylin (17) revealed that the uroguanylin and guanylin genes are evolutionarily related (18–20). Furthermore, the mRNAs and precursor proteins for both uroguanylin and guanylin are expressed together throughout the mucosa of small and large intestine along with their receptors (5, 11, 17–20). This raised a question of whether the differences in primary structure between guanylin and uroguanylin evolved to regulate intestinal salt and water transport through a cooperative mechanism using common receptor–guanylate cyclase signaling molecules located on the mucosal surface of the intestine.

During the isolation of uroguanylin, guanylin, and their prohormone precursors, we observed that acidic column reagents markedly attenuated the cGMP responses of T84 cells to guanylin, but enhanced the responses to uroguanylin (4, 5). This pH dependency for activation of guanylate cyclase was successfully used to detect guanylin and uroguanylin during their separation and purification from intestinal mucosa. The possibility was then considered that the primary structures of guanylin and uroguanylin could have evolved to regulate the enzymatic activity of a common set of receptors over the wide range of mucosal acidity that occurs within the intestinal lumen during digestion (21–24). We report here that high mucosal acidity rendered guanylin ineffective as a cGMP agonist and chloride secretogogue, whereas an acid pH markedly enhanced the potency of uroguanylin. A mucosal pH of 8.0 substantially increased the potency of guanylin but decreased the potency of uroguanylin. These changes in agonist potencies were explained by corresponding directional shifts in the affinities of guanylin and uroguanylin for binding to receptors at pH 5.0 versus 8.0. Uroguanylin and guanylin cooperatively regulate the guanylate cyclase activity of a common set of mucosal receptors in a pH-dependent fashion, thus providing an enteric signaling pathway for the intrinsic, paracrine regulation of intestinal salt and water transport.

MATERIALS AND METHODS

cGMP Accumulation Assay in T84 Cells. T84 cells were cultured in 24-well plastic dishes, and the cGMP levels were

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Abbreviation: ST, heat-stable toxin.

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measured in control and agonist-stimulated cells by radioimmunoassay (2). Synthetic peptides were suspended in 200 μl of each of two assay buffers; pH 8.0 buffer [Dulbecco's modified Eagle's medium (DMEM)/20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (Hepes)/50 mM sodium bicarbonate, pH 8.0/1 mM isobutylmethylxanthine (IBMX)] and pH 5.0 buffer [DMEM/20 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), pH 5.0/1 mM IBMX]. T84 cells were washed twice with 200 μl of the respective pH 8.0 and pH 5.0 buffers before addition of the peptides. These solutions containing the bioactive peptides were then added to T84 cells and incubated at 37°C for 40 min. Following incubation, the reaction medium was aspirated, and 200 μl of 3.3% perchloric acid was added per well to stop the reaction and extract cGMP. The extract was adjusted to pH 7.0 with KOH, centrifuged, and 50 μl of the supernatant was used to measure cGMP.

Measurement of Short Circuit Current in T84 Cell Monolayers. T84 cells were raised on collagen-coated permeable filters and mounted in custom-made Ussing chambers for measurement of transepithelial chloride secretion as described (15, 25). The buffer in the basolateral reservoir was a Krebs–Ringer solution (pH 7.4) (25 mM sodium bicarbonate), containing 10 mM glucose. Buffers in the apical reservoir were Krebs–Ringer solutions containing 10 mM glucose and adjusted to either pH 5.5 (25 mM Mes, minus sodium bicarbonate) or pH 7.8 (60 mM sodium bicarbonate). The pH of basolateral and apical reservoir buffer solutions was maintained by bubbling 95% O_2/5% CO_2 through the medium (except for the apical reservoir at pH 5.5, which did not use a bicarbonate/CO_2 buffering system). The signals for short circuit current (Isc) and potential difference across the epithelium were measured every 20 sec, digitized with an ADC-1 data recording system (Remote Measurement Systems, Seattle) and stored for later analysis. The Isc observed with T84 cells cultured on permeable filters has been shown to be caused by the net secretion of chloride across T84 cell monolayers when cells were treated with various chloride secretogogues, including guanylin, uroguanylin, and *Escherichia coli* ST (1, 2, 15, 26).

Competitive Radioligand-Binding Assay in T84 Cells. Competitive radioligand-binding experiments were performed with intact, T84 cells cultured in 24-well plastic dishes using methods that were previously described (15), but at medium pH values of 5.0 and 8.0. Identical buffer conditions at pH 5.0 and pH 8.0 were used in the competitive radioligand-binding assays as those used in the cGMP accumulation bioassays. ^{125}I-ST-(1–19) was used as the radioligand (2, 15). Concentration-response curves for cGMP accumulation and competitive radioligand-binding curves performed with each agonist were analyzed with the computer program PRISM (Graphpad, San Diego). A better fit of the binding data was consistently obtained with a two-site model as compared with a single-site model for all agonists at either pH 5 or pH 8 (15). The concentrations at which specific binding of the radioligand at each binding site was inhibited by 50% IC_{50}, were obtained by nonlinear regression of the untransformed competition binding data. The apparent equilibrium dissociation constants, K_i, for the competing ligands were calculated from the computed IC_{50} values using the previously reported estimates of the affinity of the radioligand in these cells, $K_d \approx 15$ nM (27): $K_i = IC_{50}/1 + (L/K_d)$, where L equals the radioligand concentration. It should be noted that the calculated IC_{50} and K_i values are essentially identical because the concentration of the radioligand used in these studies (≈120 pM) was a small fraction of the reported binding affinity of the radioligand.

Synthesis of Uroguanylin, Guanylin, and ST Peptides. Human uroguanylin (NDDCELCVNVACTGCL) and human guanylin (PGTCEICAYAACTGC), and the opossum forms of uroguanylin$^{95–109}$ (QEDCELCINVACTGC), uroguanylin$^{98–109}$ (CELCINVACTGC), and *E. coli* ST-(5–17) (CCELCCNPACAGC) were synthesized by the solid-phase method on an Applied Biosystems model 431A peptide synthesizer and purified by reverse-phase C_{18} chromatography as previously (1–3). The structure and mass of synthetic peptides were verified by electrospray mass spectrometry, gas-phase sequence analysis, and amino acid composition analysis.

Cell Culture. T84 cells (passage 21 obtained from Jim McRoberts, Harbor–University of California Los Angeles Medical Center, Torrance, CA) were cultured in DMEM and Ham's F-12 medium (1:1) containing 5% fetal bovine serum and 60 μg of penicillin plus 100 μg of streptomycin per ml as described (2, 15).

RESULTS

The relative potencies of the synthetic forms of human uroguanylin and guanylin for stimulation of cGMP accumulation in intact T84 intestinal cells were assessed at medium pH values of 5.0 and 8.0, which represent the extremes of microclimate pH found at the mucosal surface of the intestine. Experiments using opossum uroguanylin and guanylin provided additional insights into the optimal medium pH values used in this study (4). The potency of guanylin for eliciting cGMP accumulation responses in T84 cells was 10-fold greater when tested at pH 8.0 compared with its potency at pH 5.0 (Fig. 1*A*). In contrast, the potency of uroguanylin was reduced by 10-fold at pH 8.0 compared with its potency at pH 5.0 (Fig. 1*B*). We previously reported that *E. coli* ST-(5–17) was 2- to 3-fold more potent at acidic pH compared with alkaline pH (4). Under acidic conditions, uroguanylin was 100-fold more potent than guanylin. At pH 5.0, 3000 nM guanylin was required to stimulate ≈200-fold increases in cellular cGMP, whereas 30 nM uroguanylin elicited this magnitude of cGMP response. However, this rank order of potency was reversed at pH 8.0 with guanylin becoming 3-fold more potent than uroguanylin. For example, 30 nM guanylin caused ≈50-fold increases in cGMP levels, whereas 100 nM uroguanylin was required at pH 8.0. Threshold stimulation of cGMP levels was observed with ≈0.1 nM uroguanylin and ≈10 nM guanylin at pH 5.0, whereas at pH 8, ≈0.3 nM guanylin and ≈3 nM uroguanylin were required to stimulate cGMP increases by at least 2-fold over the basal cGMP levels. Thus, variations in mucosal pH similar to those that may occur within the intestinal lumen during digestion markedly and differentially influenced the cGMP responses of T84 cells to guanylin and uroguanylin.

We further tested the effects of mucosal acidity on the relative potencies of guanylin, uroguanylin, and ST-(5–17) for stimulation of chloride secretion across monolayers of T84 cells cultured on permeable filters and mounted in Ussing chambers. T84 cells secrete chloride in the serosal to mucosal direction and the magnitude of chloride transport can be measured as the input current required to maintain a transepithelial potential difference equal to zero (short circuit current, Isc). Guanylin, uroguanylin, and *E. coli* ST stimulate the Isc of T84 cells when added to the mucosal bath of Ussing chambers (1–3, 15, 26). In these experiments, the basolateral (serosal) surfaces of T84 cells were maintained at pH 7.4, whereas the apical (mucosal) surface was maintained at either pH 5.5 or pH 7.8. The potency of guanylin for stimulation of chloride secretion was markedly increased at an apical pH of 7.8 compared with pH 5.5 (Fig. 2 *Upper*). In contrast, uroguanylin was considerably more potent in the stimulation of chloride secretion when the apical pH was 5.5 compared with its potency at pH 7.8 (Fig. 2 *Lower*). Fig. 3 compares the relative potencies of guanylin, uroguanylin, and ST-(5–17) for stimulating chloride secretion at a mucosal pH of 5.5 compared with pH 7.8 over a wide range of agonist concentrations. ST-(5–17) and uroguanylin act similarly by stimulating greater increases in chloride secretion at a mucosal pH of 5.5 compared with the stimulation observed at pH 7.8 (Fig. 3). The

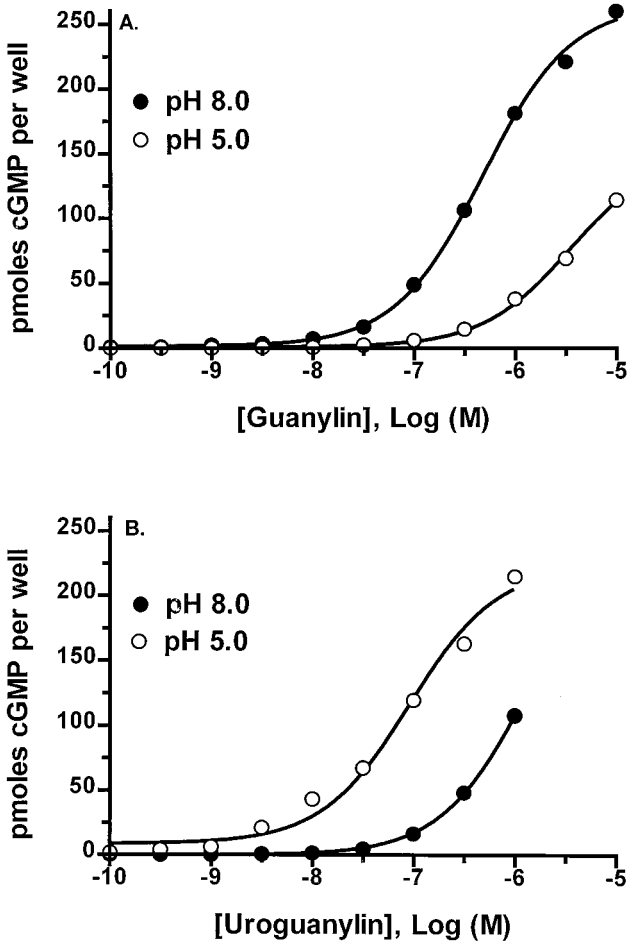

FIG. 1. Comparison of cGMP accumulation responses to the synthetic forms of human guanylin and uroguanylin in T84 cells under acidic and alkaline conditions. T84 cells were treated with the indicated concentrations of guanylin (*A*) or uroguanylin (*B*) for 40 min at a mucosal pH of 5.0 (○) or pH 8.0 (●). Data are the mean of duplicate experiments performed with each agonist at each pH value. Concentrations of guanylin and uroguanylin greater than 10 μM and 1 μM, respectively, were not used because it consumed excessive amounts of the peptides.

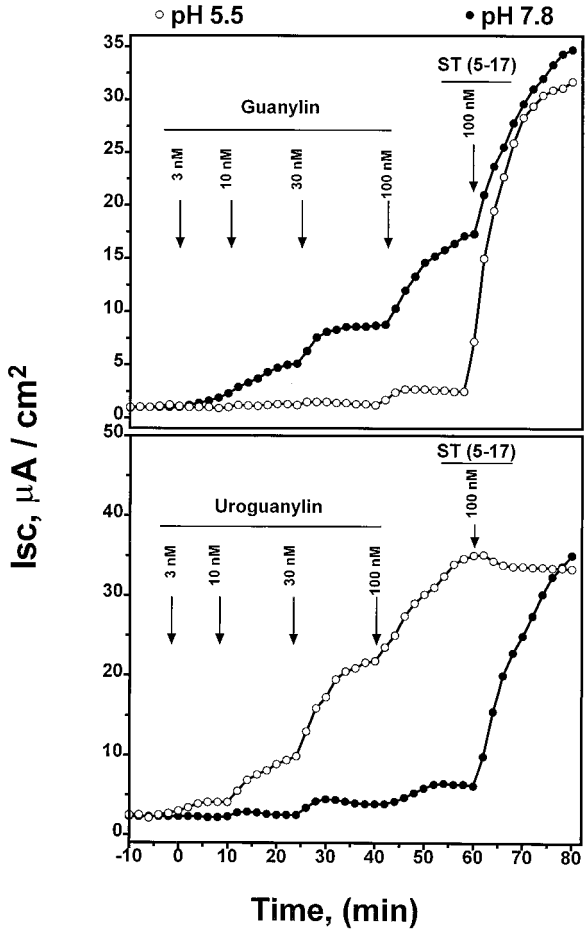

FIG. 2. Effects of mucosal pH on the stimulation of Isc in T84 cells by guanylin and uroguanylin. T84 cells were cultured on collagen-coated membranes and mounted in Ussing chambers as described. At the arrows, the indicated concentrations of guanylin (*Upper*), uroguanylin (*Lower*), and *E. coli* ST-(5–17) were added to the apical reservoirs containing medium at either pH 5.5 (○) or pH 7.8 (●). For all experiments, the basolateral reservoir contained medium at pH 7.4. Data are representative experiments of at least five separate experiments performed with each peptide at the mucosal pH values of 5.5 and pH 7.8. Concentrations of guanylin and uroguanylin greater than 100 nM were not used in these experiments because they consumed excessive amounts of the peptides.

rank order of potencies for agonist-mediated stimulation of chloride secretion was ST > uroguanylin > guanylin at acidic pH and ST > guanylin > uroguanylin at an alkaline pH (Fig. 3). The relative potencies of uroguanylin, guanylin, and ST-(5–17) in the stimulation of transepithelial chloride secretion across monolayers of T84 cells at acidic versus alkaline pH matched their relative potencies for stimulation of cGMP levels under these conditions.

Modulation by mucosal acidity of the relative affinities of uroguanylin and guanylin for binding to a common set of receptors (1, 2, 15) could account for the effects of medium pH on the cGMP and chloride secretion responses elicited by these peptides in T84 cells. This hypothesis was tested using competitive radioligand-binding assays in cultured T84 cells with ^{125}I-ST-(1–19) as the radioligand (2, 15). Uroguanylin, guanylin, and ST-(5–17) fully inhibited the binding of ^{125}I-ST-(1–19) to apical receptors on T84 cells when tested at medium pH values of 5.0 and 8.0. Examination of the radioligand-binding data using computer-assisted fitting of curves to a two-site model (15) confirms that T84 cells have both high- and low-affinity binding sites for each ligand. The K_i values for each peptide at medium pH values of 5.0 versus 8.0 are found in the legend to Fig. 4. Guanylin had a 100-fold increase in affinity for binding to the high affinity site and a 30-fold increase in affinity for binding to the low affinity site when tested at pH 8.0 compared with pH 5.0 (Fig. 4 *Top*). In contrast, parallel 9- to 10-fold increases in affinities for the high and low affinity sites were observed when uroguanylin was tested at pH 5.0 versus pH 8.0, indicating that mucosal acidity increased the affinity of uroguanylin for binding to receptors (Fig. 4 *Middle*). In agreement with a previous report (27), mucosal acidity had little influence on the binding affinities for ST-(5–17) interaction with the receptors on T84 cells (Fig. 4 *Bottom*). The remarkable effects of mucosal pH on cGMP accumulation and chloride secretion responses to these peptides may be explained by pH-dependent shifts in the affinities of uroguanylin and guanylin for binding to guanylate cyclase effector molecules on the apical surface of this model intestinal epithelium.

The unique acidic residues at the N terminus of uroguanylin were postulated to be involved in the increased potencies and binding affinities for uroguanylin in the interaction of this peptide with T84 cell receptors at acidic versus alkaline pH. A truncated form of opossum uroguanylin$^{95–109}$ was synthesized without the N-terminal Gln^{95}–Glu^{96}–Asp^{97} amino acids to test the hypothesis that conformational changes in these residues within the uroguanylin$^{95–109}$ molecule contribute to the in-

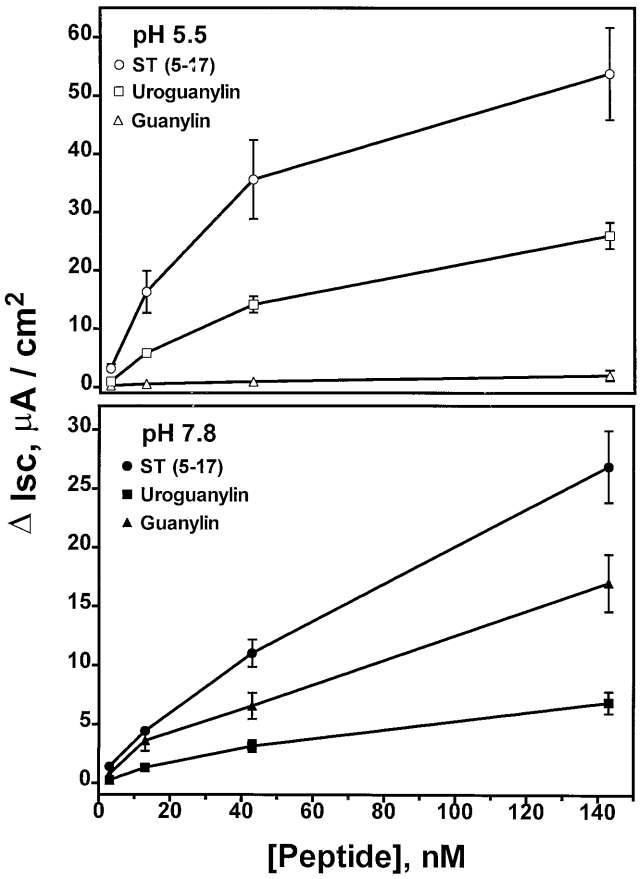

FIG. 3. Comparison of the relative potencies of uroguanylin, guanylin, and *E. coli* ST-(5–17) in the stimulation of Isc in T84 cells at the mucosal pH values of 5.5 (*Upper*) and pH 7.8 (*Lower*). Peptides were added to the apical reservior at 10–15 min intervals starting with the lowest concentrations shown, followed by successive additions of the peptide. The basolateral reservior was maintained at pH 7.4. Data are the mean of five experiments performed with each agonist. Horizontal bars indicate the SEM for each point.

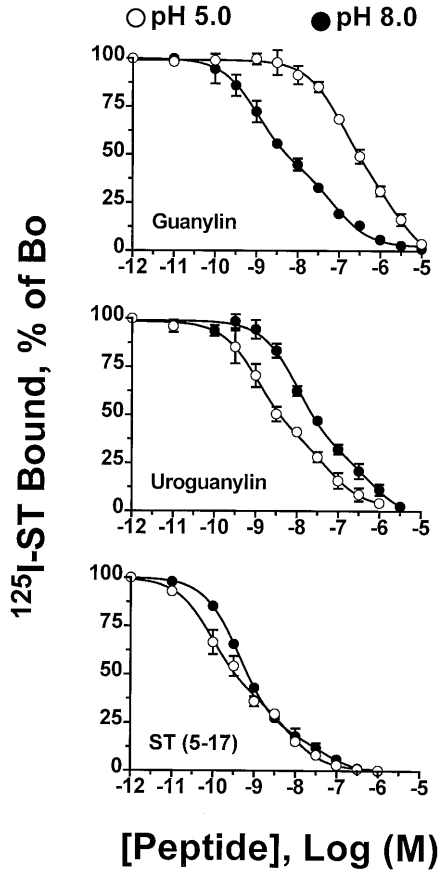

FIG. 4. Effects of medium pH on the relative affinities of guanylin, uroguanylin, and *E. coli* ST-(5–17) for binding to receptors on T84 cells. Binding of ^{125}I-ST-(1–19) to intact T84 cells was determined in the presence of the indicated concentrations of guanylin (*Top*), uroguanylin (*Middle*), and ST-(5–17) (*Bottom*) as described. The values shown are the composite data (mean ±SEM) from three experiments performed in duplicate with each peptide at pH 5 and pH 8 and are expressed as the total binding of ^{125}I-ST-(1–19) in the absence of a competing ligand. Nonspecific binding was measured using 1 μM ST-(5–17). Competitive radioligand binding curves are computer-derived best fits of the binding data to a two-site model (15). K_i values obtained for the high and low affinity sites were: guanylin, pH 5 ≈102 nM and 2.3 μM, pH 8 ≈1 nM and 77 nM; uroguanylin, pH 5 ≈1 nM and 70 nM, pH 8 ≈10 nM and 615 nM; ST-(5–17), pH 5 ≈94 pM and 7 nM, pH 8 ≈440 pM and 17 nM.

creased potency of uroguanylin at acidic versus alkaline pH (4, 17). The truncated peptide, uroguanylin$^{98-109}$, stimulated cGMP accumulation in T84 cells similarly at pH 5.0 compared with pH 8.0 (Fig. 5). The potency of this peptide was actually increased at alkaline pH, thus demonstrating that the truncated uroguanylin$^{98-109}$ peptide possessed this characteristic pharmacological property found with the guanylin peptides (Fig. 1, ref. 4). The 15 amino acid form of opossum uroguanylin$^{95-109}$, containing the N-terminal gln^{95} and the two acidic residues, glu^{96} and asp^{97}, was substantially more potent at pH 5.0 compared with pH 8.0. The opossum uroguanylin$^{95-109}$ peptide and human uroguanylin share this pH dependency for agonist potency (Fig. 1). We previously reported similar effects of acid pH on the potency of opossum uroguanylin$^{96-109}$ that did not have the N-terminal gln^{95} residue, but retained the two acidic amino acids (4). The presence or absence of the N-terminal glutamine in the opossum form of uroguanylin did not influence the characteristic enhancement of agonist potency elicited by the mucosal pH of 5.0.

In competitive radioligand-binding experiments, uroguanylin$^{98-109}$ bound to cell–surface receptors on T84 cells with similar affinities at pH 5.0 and pH 8.0 (Fig. 5). In this radioligand-binding assay, the K_i values for uroguanylin$^{98-109}$ binding to the high affinity site were 0.14 nM at pH 8.0 compared with 0.19 nM at pH 5.0 and the K_i values for uroguanylin$^{98-109}$ interaction with the low affinity site were 345 nM at pH 8.0 versus 404 nM at pH 5.0. Thus, uroguanylin$^{98-109}$ did not exhibit an increase in the affinity of this peptide for binding to receptors on T84 cells at acidic versus alkaline pH. This observation is consistent with the similar potencies measured at pH 5.0 compared with pH 8.0 for the cGMP accumulation response to uroguanylin$^{98-109}$. We conclude that the unique acidic amino acids at the N terminus of uroguanylin are required for the increased binding affinities, and accordingly, the enhanced potencies of uroguanylin in the stimulation of target cell responses under the acidic conditions of pH 5.0–5.5 maintained at the mucosal surface of T84 cells in this model epithelium.

DISCUSSION

At the surface of the intestinal mucosa, between the apical plasma membranes of enterocytes and a protective layer of hydrated mucin, the ligand-binding domains of a common set of receptors for uroguanylin and guanylin extend into an aqueous (microclimate) zone that has a variable pH (6, 10–13). It is in this microdomain of changing mucosal acidity where the luminally secreted agonists uroguanylin and guanylin (28) bind to and activate the intestinal receptor–guanylate cyclase sig-

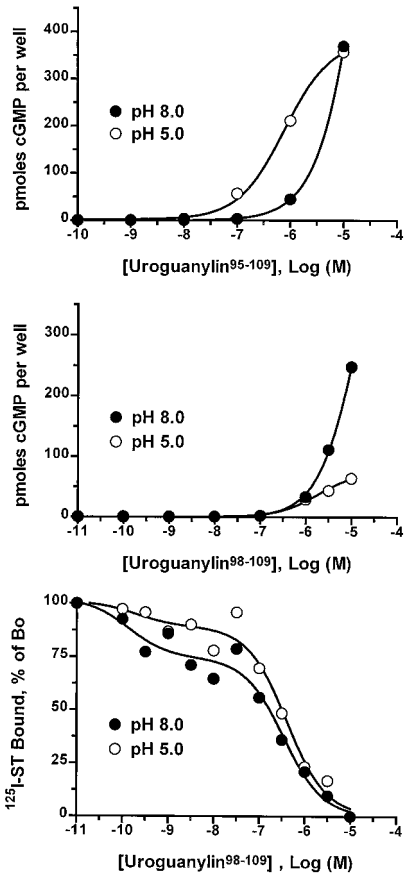

FIG. 5. Effects of uroguanylin$^{95–109}$ and uroguanylin$^{98–109}$ on cGMP accumulation and the affinities of uroguanylin$^{98–109}$ for binding to receptors on T84 cells at pH 5.0 versus pH 8.0. The data are representative experiments with duplicate assays. Each experiment was performed at least three times with similar results. The conditions are the same as those given in the *Materials and Methods* and the legends to Figs. 1 and 4. (*Top*) Stimulation of T84 cell cGMP accumulation by opossum uroguanylin$^{95–109}$. (*Middle*) Stimulation of cGMP accumulation by opossum uroguanylin$^{98–109}$. (*Bottom*) Inhibition of ^{125}I-ST binding to receptors on T84 cells by opossum uroguanylin$^{98–109}$.

naling molecules. Using a model intestinal epithelium, we demonstrated that potential changes in mucosal acidity can differentially influence the relative potencies of uroguanylin and guanylin for activation of these receptors located on the apical surface of intestinal cells (1, 2, 15, 26). Mucosal acidity markedly increases the potency of uroguanylin, while rendering guanylin ineffective in the stimulation of cGMP accumulation and transepithelial chloride secretion. In sharp contrast, a mucosal pH of 8.0 substantially increases the potency of guanylin, while diminishing the potency of uroguanylin. This striking effect of mucosal pH on agonist potencies was explained by the corresponding shifts in affinities of guanylin and uroguanylin for binding to receptors on T84 cells at the mucosal pH values of 5.0 versus pH 8.0. As a result, the affinities of guanylin and uroguanylin for binding to these receptors undergo pH-dependent shifts by as much as 100-fold. Thus, variation in mucosal acidity within the physiological limits observed at the surface of the intestinal mucosa (21–24) influences the activation of receptors by uroguanylin and guanylin.

Modulation of receptor–guanylate cyclase activity and chloride secretion by uroguanylin would be most effective in regions of the intestine where the luminal microclimate domain is acidic, whereas the actions of guanylin would be augmented when alkaline pH occurs at the mucosal surface. Acidic conditions occur intraluminally in the proximal small intestine and proximal colon during digestion (21–24). Gastric emptying introduces into the lumen of the duodenum a highly acidic chyme (22), thus increasing mucosal acidity and potentially enhancing the cGMP accumulation and chloride secretion responses of the intestine to uroguanylin, while rendering guanylin ineffective. The observation that uroguanylin mRNA is relatively abundant in the opossum duodenum compared with guanylin mRNA is consistent with a physiological role for uroguanylin in the proximal small intestine (17). Guanylin mRNA levels are also lower in the duodenum of other mammals compared with the mRNA levels in the ileum and colon (18). Luminal pH within the colon can become acidic due to the production of short chain fatty acids by enteric microorganisms (23, 24). High levels of uroguanylin and guanylin and their mRNAs are expressed in the mucosa lining the cecum and colon (4, 5, 11, 17, 18). Increased acidity in the lumen of the large intestine due to microbial metabolism could lower the microclimate pH, thus increasing the affinity of uroguanylin and reducing the affinity of guanylin for binding to and activation of receptors. In addition, alkalinization of the microclimate domain at the mucosal surface is achieved through bicarbonate secretion from the pancreas into the duodenum, and/or from epithelial cells lining the small and large intestine (22, 24). Alkalinization would enhance the potency of guanylin, while attenuating responses to uroguanylin. The intraluminal secretion of guanylin and uroguanylin provides an intrinsic mechanism for control of salt and water transport under the variable acidity conditions occurring in the microclimate domain that bathes the mucosal surface of enterocytes lining the intestinal tract (5, 11, 17–20).

A striking difference in the primary structure of uroguanylin compared with guanylin is the appearance of two acidic amino acids at the N terminus of uroguanylin (Fig. 6). All uroguanylin peptides have aspartate or glutamate residues at these positions (8, 9). Deletion of the N-terminal residues (Gln^{95}–Glu^{96}–Asp^{97}) of opossum uroguanylin$^{95–109}$ converted the truncated uroguanylin$^{98–109}$ into a uroguanylin analogue that possessed the pharmacological property that is characteristically observed in the guanylin subfamily of peptide agonists. The truncated uroguanylin$^{98–109}$ was actually somewhat more potent at pH 8.0 than at pH 5.0. We conclude that the N-terminal acidic residues of uroguanylin are required for the increased binding affinities, and therefore, the enhanced potency of uroguanylin for activation of receptors under acidic conditions. It is likely that acidic conditions influence the ionization and/or conformational state of the uroguanylin molecule as a molecular mechanism for the increased biological activity of uroguanylin in this circumstance. Presently, we have no infor-

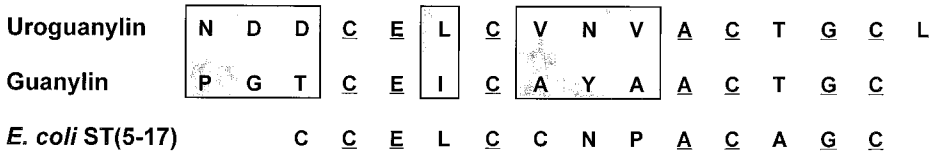

FIG. 6. Comparison of the primary structures of uroguanylin, guanylin, and *E. coli* ST. Underlined amino acids indicate the identical residues shared between uroguanylin, guanylin, and ST-(5–17). Shaded boxes highlight the differences in primary structure between uroguanylin and guanylin.

mation concerning the residues in guanylin that contribute to the remarkable increase in affinities for interaction with receptors at alkaline compared with acidic pH. Although these properties of uroguanylin and guanylin were discovered using the T84 cell line as a model epithelium, current studies have demonstrated that the short circuit current responses of mouse duodenum and cecum to uroguanylin *in vitro* are markedly increased when the luminal pH is acidic compared with the responses observed at a mucosal pH of 7.4 (29). This preliminary observation with uroguanylin using a native epithelium mounted into Ussing chambers is consistent with the increased affinity of uroguanylin for binding to and subsequent activation of the apical membrane receptor–guanylate cyclases of T84 cells revealed by experiments presented in this communication.

E. coli ST-(5–17) binds with extraordinarily high affinities to the uroguanylin/guanylin receptors on the apical surface of T84 cells and potently stimulates cGMP production and chloride secretion at both alkaline and acidic pH. The interactions of ST peptides with these receptors is little affected by mucosal pH in this model epithelium. Enteric bacteria have evolved a single peptide toxin that serves as a molecular mimic for both of the intestinal hormones, uroguanylin and guanylin. The remarkable potencies of ST peptides compared with the potencies of the enteric hormones is caused by higher affinities for ST binding to the intestinal receptors for uroguanylin and guanylin. Bacteria have created superagonist peptide toxins and this pharmacological property contributes to the remarkable toxicities of ST peptides in the molecular and cellular mechanism underlying Travelers diarrhea (8, 9, 30, 31).

We conclude that uroguanylin and guanylin cooperatively regulate a signaling pathway that modulates intestinal salt and water transport via an intrinsic, paracrine mechanism involving cGMP as a second messenger. Uroguanylin is a highly potent agonist under high mucosal acidity, a condition that renders guanylin ineffective. Conversely, guanylin is highly potent under low mucosal acidity, conditions that reduce the potency of uroguanylin. An influence of intraluminal pH on uroguanylin and guanylin actions may also occur in other epithelia such as the renal tubule. The filtrate bathing tubular cells lining the nephron also becomes acidic under normal conditions, thus potentially modulating the interaction of uroguanylin with renal receptors (13, 32, 33), which may influence the urinary excretion of sodium chloride (34). Finally, this study emphasizes that potentially novel regulation mechanisms may exist whereby normal constituents (such as the H^+ concentration) of fluids bathing the mucosal surface of epithelia may substantially influence the physiological responses of target cells to intraluminal peptide hormones.

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