Structure and activity of uroguanylin and guanylin from the intestine and urine of rats

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Fan, Xiaohui, F. Kent Hamra, Roslyn M. London, Sammy L. Eber, William J. Krause, Ronald H. Freeman, Christine E. Smith, Mark G. Currie, and Leonard R. Forte. Structure and activity of uroguanylin and guanylin from the intestine and urine of rats. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E957–E964, 1997.—Uroguanylin and guanylin are related peptides that activate common guanylate cyclase signaling molecules in the intestine and kidney. Uroguanylin was isolated from urine and duodenum but was not detected in extracts from the colon of rats. Guanylin was identified in extracts from small and large intestine but was not detected in urine. Uroguanylin and guanylin have distinct biochemical and chromatographic properties that facilitated the separation, purification, and identification of these peptides. Northern assays revealed that mRNA transcripts for uroguanylin were more abundant in small intestine compared with large intestine, whereas guanylin mRNA levels were greater in large intestine relative to small intestine. Synthetic rat uroguanylin and guanylin had similar potencies in the activation of receptors in T84 intestine cells. Production of uroguanylin and guanylin in the mucosa of duodenum is consistent with the postulate that both peptides influence the activity of an intracellular guanosine 3′,5′-cyclic monophosphate signaling pathway that regulates the transepithelial secretion of chloride and bicarbonate in the intestinal epithelium.

GUANYLIN AND UROGUANYLIN are small peptides that activate membrane receptor-guanylate cyclase signaling molecules in the intestine, kidney, and other epithelia (reviewed in Ref. 8). These receptors are localized to apical membranes of cells lining the gastrointestinal tract (6, 20, 23, 30) and renal proximal tubules (9, 10, 21). Heat-stable enterotoxin (ST) peptides secreted by enteric bacteria that cause traveler’s diarrhea act as molecular mimics of uroguanylin and guanylin (4, 6, 8, 14). Intracellular accumulation of the second messenger guanosine 3′,5′-cyclic monophosphate (cGMP) influences the phosphorylation state and putative chloride (Cl−) channel activity of the cystic fibrosis transmembrane conductance regulator protein, which may serve as an efflux pathway for Cl− secretion from the intestinal mucosa (8). The net effect of receptor activation in the intestine is to stimulate the transepithelial secretion of Cl− and HCO3−, thus increasing fluid secretion and modulating the intraluminal pH (4, 6, 11, 14, 17). Guanylin was first isolated from the intestine of rats as a 15-amino acid peptide containing four cysteines with two disulfide bonds that are required for bioactivity (4). Then, cDNAs encoding preproguanylin of 115–116 amino acids containing the COOH-terminal guanylin peptides were isolated (31, 32). Guanylin mRNA is highly expressed in the ileum and colon, with considerably lower amounts found in the duodenum and jejunum (31, 32). The cellular sites of guanylin production in the intestinal mucosa are reported to include goblet cells and absorptive cells (22, 23, 26). Uroguanylin was initially isolated from opossum urine (14). A search for the tissue source of urinary uroguanylin resulted in the purification of prouroguanylin and uroguanylin from large intestine (13, 15). Recently, cDNAs encoding preprouroguanylin were isolated from opossum, human, and rat intestinal cDNA libraries (1, 5, 16, 25, 28, 29). The bioactive uroguanylin peptides found in urine are located at the COOH terminus of prouroguanylin.

In the present study, we isolated uroguanylin from urine and duodenum of rats to investigate the structure and biological activity of uroguanylin in this species. Uroguanylin and guanylin were identified by their unique chromatographic properties, by NH2-terminal sequence analyses, and by the effects of medium pH on the relative potencies of the bioactive peptides. The bioactive peptide in the urine is uroguanylin, whereas guanylin and uroguanylin were both isolated from the duodenum. Only guanylin was purified from the large intestine.

MATERIALS AND METHODS

Purification of uroguanylin from urine. Three batches of urine (2–3 liters) pooled from 12 male Sprague-Dawley rats were used to isolate and identify uroguanylin with chromatographic methods that have been previously described (13–15). Briefly, urine was collected daily from rats housed in metabolic cages, pooled, and stored at −20°C. After thawing, the urine was centrifuged at 10,000 g for 20 min. Trifluoroacetic acid (TFA), 0.1%, was added to the supernatant, and the sample was then applied to C18 Sep-Pak cartridges and eluted with 40% acetonitrile and 0.1% TFA. The eluted polypeptides were dried and resuspended in 50 mM ammonium acetate and then chromatographed using a 2.5 × 90-cm column of Sephadex G-25 gel. Fractions eluted from the G-25 column and in subsequent purification steps were bioassayed using T84 cells by measurement of cGMP accumulation as previously described (8, 9). The active fractions were pooled, dried in a Speed-Vac, resuspended in 0.1% TFA, and loaded onto C18 Sep-Pak cartridges. The peptides were eluted with a gradient...
of 10%, followed by 30% and then 60% acetonitrile solutions containing 0.1% TFA. The bioactive peptides were eluted with the 30% acetonitrile-0.1% TFA solution, and this fraction was dried, resuspended in 10% acetonitrile and 0.1% TFA, and applied to a C<sub>18</sub> semipreparative high-performance liquid chromatography (HPLC) column (Waters semipreparative µBondapak, 7.8 mm × 30 cm). The peptides were eluted with a gradient of 10% acetonitrile-0.1% TFA to 30% acetonitrile-0.1% TFA over a period of 180 min. The peaks of bioactive peptides were pooled, dried, resuspended in H<sub>2</sub>O with 0.8% amphotelytes [pH range 3–10 (Bio-Rad)], and subjected to preparative isoelectric focusing (Rotorfor, Bio-Rad). The fractions containing bioactivity were combined, applied to a C<sub>18</sub> HPLC column (Waters analytic µBondapak, 3.9 mm × 30 cm), and eluted with a gradient of 5% acetonitrile-10 mM ammonium acetate (pH 6.2) to 25% acetonitrile-10 mM ammonium acetate (pH 6.2) over 180 min. The peak of bioactive peptides was subjected to a second purification procedure with the same C<sub>18</sub> analytic HPLC column, but with the acetonitrile gradient containing 0.1% TFA instead of ammonium acetate. The bioactive peptides were then applied to a C<sub>8</sub> microbore column and eluted with a gradient of 0.33% of acetonitrile and 0.1% TFA per minute as previously described (4, 14, 15). The purified peptides were subjected to automated Edman NH<sub>2</sub>-terminal sequencing, as previously described (4, 14, 15).

Purification of peptides from the mucosa of colon and duodenum. The mucosa (100 g wet weight) was scraped from colon by use of a glass microscope slide and then boiled in 10 volumes of 1 M acetic acid for 10 min, homogenized, and centrifuged at 10,000 g for 20 min. The supernatant was extracted with C<sub>18</sub> Sep-Pak cartridges followed by Sephadex G-25 column fractionation, as described above. The bioactive peptide fractions from the gel column were combined and fractionated a second time with C<sub>18</sub> Sep-Pak cartridges. The peptides were eluted using 5, 10, 15, 20, 25, 40, and 60% acetonitrile solutions containing 0.1% TFA. The bioactive peptide fractions (i.e., 25% acetonitrile) were pooled and subjected to isoelectric focusing as described above. The final purification of the active peptides was accomplished by HPLC by use of a series of C<sub>18</sub> columns as we have described. Fifty-five grams wet weight of mucosa were scraped from the duodenum (proximal one-third of the small intestine), and the bioactive peptides were purified using the same methods as described above for the peptides isolated from colonic mucosa, except that the isoelectric focusing step was not used.

Northern assays of uroguanylin and guanylin mRNA. Total RNA was prepared (RNeasy kit, Qiagen) from the mucosa of individual intestinal segments, and 20 µg of each RNA preparation were subjected to electrophoresis in formaldehyde-agarose gels and then transferred to nylon membranes (Bio-Rad). The blots were hybridized with rat uroguanylin and β-actin cDNAs or rat guanylin plus β-actin cDNAs (27). Prehybridization was for 1 h with QuickHyb (Stratagene, La Jolla, CA) at 68°C, which was followed by hybridization for 2 h at 68°C with each cDNA probe labeled by random priming (Boehringer Mannheim). The blots were washed twice with 2× standard sodium citrate (SSC) and 0.1% sodium dodecyl sulfate (SDS) for 15 min at room temperature and once with 0.2× SSC and 0.1% SDS for 15 min at 60°C. The exposure to film was for 24 h at ~80°C with intensifying screens. Rat uroguanylin cDNA (nucleotides 117–292) was produced by polymerase chain reaction (PCR) amplification from intestinal mRNA-cDNA (1, 27). This cDNA was isolated and sequenced to confirm that it matched the uroguanylin expressed sequence tag (EST) of rat uroguanylin with 100% identity. A rat guanylin cDNA (nucleotides 1–531) was generously provided by Dr. Roger Weigand (Monsanto, St. Louis, MO).

Cell culture. T84 cells were obtained from Dr. Jim McRoberts (Harbor-University of California, Los Angeles, CA) at passage 21. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F-12 medium (1:1), supplemented with 5% fetal bovine serum, 60 mg penicillin/ml, and 100 mg streptomycin/ml.

cGMP bio assay in T84 cells. T84 cells were cultured in 24-well plastic dishes, and cellular cGMP levels were measured in control and agonist-stimulated cells by radioimmunoassay (12–15). Briefly, column fractions of the synthetic peptides, uroguanylin and guanylin, were suspended in 200 µl of DMEM containing 20 mM N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (HEPES), pH 7.4 or 5.5 buffer, consisting of DMEM, 20 mM 2-(N-morpholino)-ethanesulfonic acid (MES, pH 5.5), and 1 mM isobutylmethylxanthine (IBMX). The solutions containing bioactive peptides were then added to cultured cells and incubated at 37°C for 40 min. After incubation, the reaction medium was aspirated and 200 µl of 3.3% perchloric acid were added per well to stop the reaction and extract cGMP. The extract was adjusted to pH 7.0 with KOH and centrifuged, and 50 µl of the supernatant were used to measure cGMP.

RESULTS

Purification of bioactive uroguanylin from urine. Uroguanylin was purified from rat urine using C<sub>18</sub> Sep-Pak and gel filtration chromatography, preparative isoelectric focusing, and a series of reverse phase (RP)-HPLC steps (4, 13–15).

After the isolation of bioactive peptides with C<sub>18</sub> cartridges, a second chromatographic step with a Sephadex G-25 column yielded a single peak of peptides that stimulated cGMP accumulation in T84 cells (data not shown). This peak of bioactive peptides eluted at a position identical to that previously found for opossum uroguanylin (14, 15). Preparative isoelectric focusing separates the more highly acidic uroguanylin from guanylin (14, 15). The bioactive peptides eluting from Sephadex G-25 columns were subjected to preparative isoelectric focusing, and the active peptides migrated to the most acidic region, eluting at pH values of 2.4–3.7 in fractions 1–3 (Fig. 1). This peptide fraction from urine stimulated cGMP accumulation in the T84 cells to a greater magnitude when the medium pH was 5.5 compared with the stimulation at pH 7.4. The profile of pH dependence for agonist activity in T84 cells is consistent with this urine peptide being uroguanylin (12). This peptide fraction was then combined and subjected to RP-HPLC by use of C<sub>18</sub> columns and a gradient of 5–25% acetonitrile containing 10 mM ammonium acetate, pH 6.2 (13–15). Under these RP-HPLC conditions, guanylin elutes at 16–18% acetonitrile, whereas uroguanylin elutes at 10–11% acetonitrile. Fractions 1–3 from the isoelectric focusing purification step (Fig. 1) were combined for RP-HPLC under these conditions. A single peak of bioactive peptides eluted at 10% acetonitrile and 10 mM ammonium acetate, an elution pattern consistent with this peptide being uroguanylin (Fig. 2). This peak of bioactive peptides was purified further using the same C<sub>18</sub> column by RP-HPLC with an acetonitrile gradient containing 0.1% TFA.
The bioactive peptides were eluted at 21% acetonitrile and were combined for microbore RP-HPLC (data not shown). After further purification with RP-HPLC with a C8 microbore column (Fig. 3), the bioactive peptides in the shaded portion of the ultraviolet absorbance tracing were combined and subjected to NH2-terminal sequence analysis (5, 20). A partial sequence of E/DXXELXINVAXTGX (X is unknown) was obtained because of the low quantity of peptides remaining at this stage of purification. The partial amino acid sequence obtained for the rat urine peptide is similar to the corresponding residues reported for opossum and human forms of bioactive uroguanylin isolated from urine (14, 18) and identical to the deduced sequence from a uroguanylin EST cDNA isolated from rat intestine (1). An acidic residue of either glutamate or aspartate was observed at the position where glutamate is found in opossum uroguanylin and where aspartate occurs in human uroguanylin (14, 18). The amino acids identified by sequence analysis consisting of ELXINVAXTGX are identical to the corresponding residues found in opossum uroguanylin. Taken together, these findings suggest that uroguanylin is the major bioactive peptide appearing in the urine of rats.

Purification of uroguanylin and guanylin from intestine. Isolation of uroguanylin and prouroguanylin from colon and small intestine and uroguanylin mRNA expression in the intestinal mucosa of other species suggests that the intestine of rats may be a source of uroguanylin in urine (5, 13, 15, 16). To investigate this possibility, we isolated bioactive peptides from the mucosa of colon and duodenum from rats. Extracts of colonic mucosa were prepared and purified by C18 chromatography, followed by Sephadex G-25 chromatography as described above. A single peak of bioactive peptides was observed eluting from the Sephadex G-25 column (data not shown). The active peptide peak was combined and subjected to preparative isoelectric focusing. The bioactive peptides eluted in fractions 1–3 with pH values of 2.6–3.5 (Fig. 4). At this stage of purification, the peptide components from rat colon exhibited a property similar to that of guanylin, because the colon peptides stimulated cGMP accumulation in T84 cells to a greater level at the medium pH of 7.4 compared with the cGMP responses at pH 5.5 (12). When the active
fractions were combined and subjected to RP-HPLC with a C_{18} analytic column, the bioactive peptides eluted at 15.5% acetonitrile (Fig. 5). This characteristic elution profile for rat guanylin (4) indicates that the active peptides isolated from the colonic mucosa of rats are predominantly guanylin. This peak of guanylin-like peptides was purified further by use of C_{18} RP-HPLC with an acetonitrile gradient containing 0.1% TFA and finally by microbore RP-HPLC with a C_{8} column as described above. This peptide fraction was then subjected to NH_{2}-terminal sequence analysis, and the 15-residue peptide PNTCEICAYAACTGC was obtained. This is the same amino acid sequence as that obtained when guanylin was originally isolated from the jejunum of rats (4).

The duodenum may produce uroguanylin, because the content of guanylin mRNA in duodenum of rats is considerably lower than the mRNA levels of colon, and the duodenum has substantial cGMP responses to these peptides (20, 21, 31). Bioactive peptides were isolated from the mucosa of rat duodenum, and two separate peaks of peptide bioactivity eluted at different positions within the internal volume of Sephadex G-25 columns (Fig. 6). When these fractions were bioassayed using T84 cells, we found that peak 1 stimulated cGMP accumulation greater at pH 5.0 than at pH 8.0 (uroguanylin-like) and that peak 2 stimulated cGMP accumulation greater at pH 8.0 than at pH 5.0 (guanylin-like). The very low stimulation of cGMP accumulation observed for the peak 1 aliquot at pH 8.0 and the correspondingly low stimulation for the peak 2 aliquot at pH 5.0 may be explained by the relatively low concentrations of these peptides in the aliquots from the columns that were bioassayed. Peak 1 (uroguanylin) was pooled and further purified by C_{18} RP-HPLC by use of a 5–25% acetonitrile gradient containing ammonium acetate. The bioactive peptides eluted at 11% acetonitrile, which is consistent with this peptide being uroguanylin (Fig. 7). Moreover, this peptide stimulated cGMP accumulation greater at the medium pH of 5.0 than at pH 8.0, which is also a property found in the uroguanylin peptides. To summarize, the chromatographic elution profile using RP-HPLC and the pH dependency for activation of receptor guanylate cyclases (GCs) of this peptide from the duodenum mucosa are characteristic properties of uroguanylin. An insufficient quantity of the purified uroguanylin-like peptide was available for NH_{2}-terminal sequence analysis; thus confirmation of these findings by elucidation of the peptide’s sequence was not possible.

A partial cDNA EST encoding the COOH-terminal portion of prouroguanylin was isolated from the duodenum of zinc-deficient rats (1). This information facilitated the production of a uroguanylin cDNA by use of reverse transcription of RNA from rat duodenum and the PCR to amplify this form of uroguanylin cDNA. The uroguanylin cDNA was cloned and sequenced to confirm its identity and then used as a cDNA probe in Northern assays to assess the relative abundance of uroguanylin mRNA compared with guanylin mRNA in the intestine. Uroguanylin transcripts of ~0.75 kilo-
base (kb) were detected throughout the intestinal tract, but the highest levels were found in the duodenum and jejunum of small intestine (Fig. 8). Lower levels of uroguanylin mRNA were observed in ileum and the cecum and colon compared with duodenum and jejunum. Guanylin mRNA transcripts of ~0.6 kb were detected throughout the intestinal tract, with the highest mRNA levels observed in cecum and colon compared with the levels in small intestine. The lowest guanylin mRNA levels were found in the duodenum relative to other segments of intestine. Progressively greater levels of guanylin mRNA were found along the longitudinal axis of the small intestine from duodenum to ileum, with the greatest mRNA levels observed in the cecum and colon.

Analysis of the EST for uroguanylin derived from rat intestine (1) confirmed that the partial amino acid sequence obtained for the urinary peptide was consistent with the sequence predicted by the uroguanylin EST. Thus a synthetic peptide was prepared on the basis of the amino acid sequence TDECEL CinVAC TGC, and the potency of this peptide was compared with the potencies of rat guanylin and a truncated form of uroguanylin, CEL CinVAC TGC, by use of the T84 cell bio assay (4, 14). Uroguanylin and guanylin had similar potencies in the activation of receptor GCs in T84 cells, but the truncated form of uroguanylin was substantially less potent (Fig. 9). These data indicate that the NH₂-terminal residues found in the bioactive uroguanylin peptide consisting of TDE increase the potencies of this peptide agonist for activation of receptor GCs on T84 cells compared with the potency of the truncated 12-residue form of uroguanylin. However, the 12 amino acids in the truncated uroguanylin analog containing the peptide domain between the first and last cysteine residues with two intramolecular disulfide bonds represent a core structure that is required for biological activity in this assay.

**DISCUSSION**

Uroguanylin was isolated from both urine and duodenum mucosa of rats and identified by its unique biochemical and pharmacological properties (12–15). Uroguanylin is present in the urine of rats, as it is in the urine of the opossum and human species (14, 18). Guanylin was isolated from mucosa of both the duodenum and large intestine, but active guanylin peptides were not detected in urine. Sequence analysis of uroguanylin from rat urine revealed that the eight residues obtained were identical to those found in opossum uroguanylin. One of the two NH₂-terminal acidic amino acids unique to uroguanylin was not clearly defined (Glu or Asp), and the other acidic amino acid was not

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**Fig. 8. Distribution of uroguanylin and guanylin mRNA in the intestine.** Total RNA of 20 µg from mucosa of rat proximal small intestine (Prox. SI), middle small intestine (Mid. SI), distal small intestine (Dist. SI), cecum, and colon were loaded on each lane. A: arrows mark single transcripts for β-actin mRNA of 1.9 kilobase (kb) and uroguanylin mRNA of 0.75 kb; B: arrows indicate single transcripts for β-actin mRNA of 1.9 kb and guanylin mRNA of 0.6 kb.
Published observations; 8–10). Recent evidence that low-pathway to regulate renal function in vivo (unpublished observations). Thus uroguanylin may serve in an endocrine role to gain access to these receptors. Activation of the renal receptor GCs in the perfused rat kidney elicits a natriuresis, kaliuresis, and diuresis (unpublished observations). Plasma for uroguanylin enters the tubules by glomerular filtration and the action of this peptide is inactivated by chymotrypsin, which cleaves the peptide bond COOH terminal to the aromatic residues for rat uroguanylin is TDECELCINVACTGC, which agrees with the partial sequence that we obtained in the present study. A synthetic peptide prepared according to this sequence activated the T84 cell receptor GC. Values are representative of 3 experiments conducted with cultured T84 cells and are means of duplicate assays at each peptide concentration.

**Fig. 9. Bioactivity of synthetic uroguanylin and guanylin in T84 cells.** Values are representative of 3 experiments conducted with cultured T84 cells and are means of duplicate assays at each peptide concentration. ■, Rat guanylin (PTCEICAYAACTGC); ▲, rat uroguanylin (TDECELCINVACTGC); ○, 12-residue portion of uroguanylin (CELCINVACTGC). Disulfide bonds in these synthetic peptides occur between 1st to 3rd and 2nd to 4th cysteine residues. Medium is DMEM at pH 7.4 for this assay.

The reason bioactive guanylin is not found in rat or human urine is unclear, but it may be the susceptibility of guanylin in the tubular filtrate to cleavage and inactivation by proteases within renal tubules. Guanylin is inactivated by chymotrypsin, which cleaves the peptide bond COOH terminal to the aromatic residues of guanylin peptides (13, 15). In contrast, uroguanylin and Escherichia coli ST are resistant to chymotrypsin because these peptides have asparagine residues instead of tyrosine or phenylalanine (13–15, 18). Plasma uroguanylin and guanylin may enter the tubular filtrate by glomerular filtration (5, 19). Also, recent evidence suggests that the kidney produces uroguanylin, because mRNAs encoding this peptide are expressed in the kidney (29). The presence of bioactive uroguanylin in urine is significant because renal receptors for uroguanylin are localized on the apical membranes of proximal tubular cells (9, 10, 21). Circulating uroguanylin enters the tubules by glomerular filtration to gain access to these receptors. Activation of the receptor GCs in the perfused rat kidney elicits a natriuresis, kaliuresis, and diuresis (unpublished observations). Thus uroguanylin may serve in an endocrine pathway to regulate renal function in vivo (unpublished observations; 8–10). Recent evidence that low-sodium diets downregulate the guanylin receptor-GC signaling pathway in the rat colon suggests that the guanylin signaling pathway may participate in the maintenance of salt and water homeostasis (24).

In rats, guanylin mRNA levels appear to be most abundant in colon and ileum, with intermediate mRNA levels in jejunum and the lowest mRNA levels in duodenum (25, 27, 31). In the present experiments, bioactive guanylin was isolated from colonic mucosa but bioactive uroguanylin was not detected. This finding is consistent with the high levels of guanylin mRNA that were detected using Northern assays with total RNA from colon and cecum in this study compared with the lower uroguanylin mRNA levels of large intestine. The lower abundance of uroguanylin mRNAs in the colon and cecum provides one explanation for our inability to detect bioactive uroguanylin in extracts of large intestine. Isolation of uroguanylin and guanylin from duodenum in this study suggests that both peptides are present and may regulate the activity of receptor-GC signaling molecules in this segment. Whereas Northern assays suggest that uroguanylin mRNA expression is greater than guanylin mRNA expression in the duodenum, both peptides are present in the mucosa of the duodenum in concentrations sufficient for purification and identification of this bioactive peptide (25, 27, 29). Other studies have also found a similar pattern of expression of guanylin and uroguanylin mRNA levels along the longitudinal axis of the intestinal tract of rats (25, 27, 29).

Uroguanylin and guanylin markedly stimulate the transepithelial secretion of both Cl and HCO₃⁻ anions in the duodenum (11, 17). Exposure of the apical surface of duodenum to these peptides elicits a stimulation of short-circuit current consisting of both Cl and HCO₃⁻ transport components. Both peptides may be released from enterocytes into the luminal microdomain at the surface of this epithelium where binding of the peptides to receptor-GCs occurs, thus activating these signaling molecules and regulating anion secretion via intracellular cGMP. Because the potency of uroguanylin is markedly enhanced when the intraluminal pH is acidic and an acidic pH markedly decreases the potency of guanylin, it may be postulated that the secretion of uroguanylin is increased when acidic chyme is delivered from the stomach to the duodenum (12, 13, 15). The relative potencies of guanylin and uroguanylin for activation of intestinal receptor GCs and the stimulation of transepithelial Cl⁻ secretion are markedly influenced by mucosal acidity (12). In the present study, uroguanylin isolated from rat duodenum (or urine) stimulates cGMP accumulation in T84 cells to a greater magnitude at a medium pH of 5.5 than at pH 7.5. Guanylin isolated from the intestine increased cGMP to a greater level in T84 cells at pH 7.5 than at pH 5.5. Thus uroguanylin and guanylin isolated from rat intestine exhibit properties similar to those previously defined for the homologous peptides derived from human subjects and opossums (12, 13, 15). Evolution of the unique primary structures for uroguanylin and guanylin may have occurred to allow different peptide...
hormones that function cooperatively to regulate intestinal Cl− and HCO3− secretion during digestion. The lumen of the intestine and the mucosal (microclimate) surface is acidified when chyme containing HCl enters the duodenum. Under this condition, uroguanylin may be a more effective agonist for regulating receptor-GC activity than is guanylin. When the mucosal surface of the duodenum becomes alkalinized through enhanced HCO3− secretion, the affinity of guanylin for binding to receptor GCs would be increased, thus facilitating the binding of guanylin to receptors and activation of these signaling molecules (12, 13, 15). Thus uroguanylin and guanylin may participate in a cGMP signaling pathway controlling intestinal Cl− and HCO3− secretion (4, 6, 11, 14, 17).

In summary, uroguanylin was isolated from urine and the duodenum of rats. Bioactive guanylin was not detected in urine, but this peptide was isolated from colon and duodenum. Uroguanylin mRNA levels were greater in small than in large intestine, whereas the levels of guanylin mRNA transcripts were greater in large than in small intestine. Synthetic rat uroguanylin was approximately equipotent to rat guanylin in the activation of cGMP production in T84 intestinal cells when assessed at the medium pH of 7.4. Uroguanylin and guanylin may both participate in the regulation of Cl− and HCO3− secretion via the intracellular second messenger cGMP.

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