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Review

Guanylin family: new intestinal peptides regulating electrolyte and water homeostasis

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The regulation of intestinal salt and water transport is critical to the maintenance of fluid volume. Control of this life-sustaining activity is mediated by the concerted actions of hormones, neurotransmitters, and locally acting factors. Guanylin and uroguanylin are novel peptides that were first isolated from rat jejunum and opossum urine, respectively. They bind to and activate guanylyl cyclase-C (GC-C) receptors to regulate intestinal and renal fluid and electrolyte transport through the second messenger, cyclic guanosine 3′,5′-monophosphate (GMP). Heat-stable enterotoxins produced by pathogenic bacteria have close structural similarities to guanylin and uroguanylin, and they use this mimicry to act on GC-C, causing life-threatening secretory diarrhea. Guanylin primarily is restricted to the intestine, whereas uroguanylin is present in the stomach, kidney, lung, and pancreas, in addition to intestine. Guanylin and uroguanylin are secreted into the intestinal lumen and blood in response to sodium chloride administration. These peptides function in salt and water transport in the intestine and kidney by luminocrine and endocrine actions. The guanylin family is involved in the pathophysiology of some gastrointestinal, renal, and heart diseases.

Key words: heat-stable enterotoxin, guanylin, uroguanylin, guanylyl cyclase, salt homeostasis, intestinal natriuretic factor

Introduction

Diarrheal diseases are a leading cause of morbidity and mortality in humans, causing up to 50% of infant deaths in developing countries.[1] Heat-stable enterotoxins (STs) elaborated by various pathogenic bacteria, including enterotoxigenic *Escherichia coli* and *Yersinia enterocolitica*, bring about acute diarrhea, and also cause ‘travelers diarrhea’ or ‘turista’. STs are 15- to 30-amino acid peptides. In 1990, STs were shown to bind to intestinal receptor-guanylyl cyclase (GC-C) in the brush border membrane,[2] which subsequently leads to the activation of guanylyl cyclase.[3] Human GC-C, a 1050-amino acid protein and rat GC-C, a 1053-amino acid protein, have an extracellular domain, a transmembrane domain, and intracellular protein kinase-like and guanylyl cyclase catalytic domains. Included in this type of plasma membrane form of guanylyl cyclase are GC-A and GC-B, which are receptors of the natriuretic peptide family that functions in the regulation of body fluid balance. The paradox of a bacterial toxin acting on a mammalian receptor remained unclear until the following recent discoveries were made.

Discoveries of guanylin and uroguanylin

Just as opiates derived from the poppy predicted the existence of endogenous opium-like regulatory peptides, the isolation of bacterial ST peptides and elucidation of their cyclic guanosine 3′,5′-monophosphate (cGMP)-regulating activity foreshadowed the discovery of endogenous ligands for GC-C in mammals. STs act on GC-C, produce a second messenger, cGMP, and elicit the stimulation of Cl^- secretion and the inhibition of Na^+ and H_2O absorption, thereby causing secretory diarrhea.[4] Cells transfected with GC-C cDNA were proposed as a means to search for the endogenous ligand by monitoring cGMP production, but cells that had a high sensitivity and a maximum response to STs were unavailable. Currie et al.[5] found that T_{84} cells, a human colon cancer cell line, had the desired ability for the monitoring of cGMP production by STs, and an

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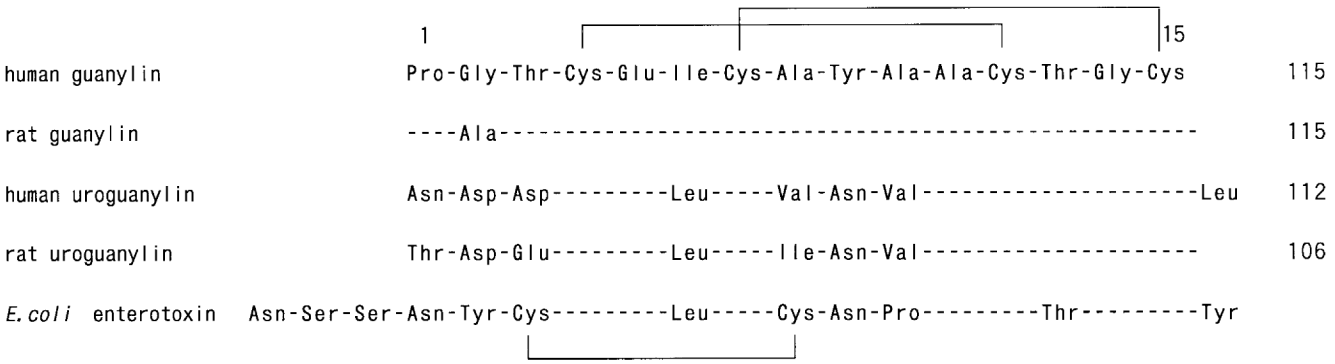

Fig. 1. Primary structures of guanylin and uroguanylin. *Dotted lines* indicate the same amino acids as those in human guanlyin, and *brackets* indicate disulfide bond linkage. Guanylin and rat uroguanylin are 15-amino acid peptides, and human uroguanylin is a 16-amino acid peptide. The numbers of amino acid residues of precursor proteins are shown on the *right*

unidentified endogenous ligand. Indeed, T_{84} cells possessed the sensitivity to detect 10 pM ST, a maximum 30 000-fold increase in cGMP to ST, and specificity for ST-like agonists. Using this assay combined with multistep chromatography, Currie's group isolated a cognate endogenous ligand for GC-C from rat jejunum.[5] This peptide was designated guanylin after its binding to GC-C. One year after the discovery of guanylin, another guanylin-like peptide, called uroguanylin, was isolated from the urine and intestinal mucosa of an opossum (*Didelphis virniana*, an American marsupial), using the same methodology.[6] Uroguanylin then was identified in the intestines and urine of humans and rats.[6–8] Guanylin and uroguanylin are 15- to 16-amino acid peptides (Fig. 1). These two are novel peptides that have no sequence identities to other known peptides. Human and rat uroguanylin has two additional acidic residues that are not found in guanylin.

As expected, the primary structures of STs are quite similar to those of the guanylin family (Fig. 1). Human guanylin shares 7 of 15 amino acids with *Escherichia coli* ST, and human uroguanylin shares 9 of 16 residues with it (47% and 56% similarity, respectively). STs are thought to use this mimicry to act on GC-C. A major structural difference between the guanlyin family peptides and STs is the number of cysteines and disulfide bonds: guanylin and uroguanylin have four cysteines and two disulfide bridges compared with STs, with six cysteines and three disulfides. These disulfides are indispensable for optimal peptide potencies in the stimulation of cGMP production in vitro. The potency of the stimulation of guanylyl cyclase activity, in descending order, is STs, uroguanylin, and guanylin.[6,7] An additional pair of cysteine residues in STs may contribute to their apparently higher potencies.[4,9] Guanylin and uroguanylin are small molecules and have two intramolecular disulfide bonds. This structural characteristic produces two topological isomers, a right-handed spiral form and a left-handed spiral form.[10,11] One form is bioactive to stimulate cGMP production, but the other is not. These isomers are interconvertible; however, the biological significance and mechanism of the interconversion are still unclear.

Gene sequence and tissue distribution

Based on the primary structure of guanylin and uroguanylin, cDNAs encoding their precursors were isolated.[12–20] Guanylin and uroguanylin mRNAs in humans and rats have approximately 600 bases. Mature guanylin and uroguanylin peptides are located at the carboxy terminal ends of their precursor proteins. The sequence identity between human guanylin and uroguanylin is 20%, that between human and rat guanylin is 64%, and that between human and rat uroguanylin is 63%. Ten-kilodalton proguanylin is a major guanylin molecule in the intestinal mucosa and plasma of humans and rats, but it has no biological activity until cleaved by proteolytic enzymes to release the biologically active 15-amino acid peptide.[21–23] Ten-kilodalton prouroguanylin is also a major molecular form in the intestine of humans and rats, whereas mature 16-amino acid uroguanyin is a major form in the urine of both species.[8,22]

Uroguanylin and guanylin genes are arranged in a tail-to-tail array on the short arm of human chromosome 1p33–p34 and on mouse chromosome 4.[20,24,25] The respective intergenic distances between the two genes in the human and mouse genomes are approximately 6.5 and 8 kb. The intergenic region between the human uroguanylin and guanylin genes has an Alu sequence and a $(CA/TG)_{19}$ microsatellite sequence, suggesting that these two genes are produced by gene duplication. The genes of both human guanylin and uroguanylin consist of three exons and two introns

within an overall length of 2.5 kb. The 5′ flanking region has TATA and CAAT boxes. The genes also have multiple binding sites for promoter-specific transcription factor, activator protein-1, activator protein-2, and a cyclic adenosine 3′,5′-monophosphate (cAMP)-regulated enhancer element.

Northern blot and reverse transcription-polymerase chain reaction (RT-PCR) analyses have revealed that guanylin mRNA is most abundant in the intestine, and there are small amounts in the kidney, adrenal gland, uterus, and oviduct.[12–15] The distribution of guanylin mRNA in the intestine is characterized by relatively low levels in the duodenum and jejunum and much higher levels in the ileum and colon. Two radioimmunoassays (RIAs) for guanylin and uroguanylin, respectively, were developed.[8,21,26] Guanylin peptide is distributed widely from the duodenum to colon in both rats and humans, the highest contents being in the ileum and proximal colon. The plasma concentration of immunoreactive guanylin was 31.2 ± 3.0 fmol/ml (mean ± SE) in normal individuals and was markedly higher in patients with renal insufficiency, which may be due to impaired catabolism of guanylin. Patients with carcinoid tumors that cause secretory diarrhea had elevated levels of circulating guanylin.[27] The tumors contained guanylin, as detected by immunohistochemical study. Although serotonin is considered to be a major cause of diarrhea in patients with carcinoid tumors, guanylin released from the tumors may also be responsible for the stimulation of intestinal fluid secretion. Northern blot analysis and RIA showed that uroguanylin mRNA and peptide were expressed in the stomach, small and large intestines, kidney, heart, and lung of humans and rats.[8,16–20] The highest values were found in the upper small intestine. The plasma concentration of bioactive uroguanylin was 5.0 ± 0.3 fmol/ml in normal individuals, and it was higher in patients with renal insufficiency.[8] Plasma uroguanylin concentration in patients with heart failure was also significantly higher than that in normal controls, and increased with the severity of heart failure. Plasma uroguanylin levels in the coronary sinus and anterior interventricular vein were higher than that in the aorta, indicating that uroguanylin is secreted from the heart in heart failure. Uroguanylin has natriuretic activity, as described below. The possible function of uroguanylin in the regulation of body fluid balance in heart failure needs further investigation.

Cellular localization

The specific cellular sites of guanylin mRNA production in the intestine are a source of considerable debate, with conflicting reports.[15,28–30] A recent immunohistochemical finding indicates that the enterochromaffin (EC) cells of guinea pig stomach and small intestine are a potential source of guanylin.[30] On the other hand, uroguanylin-producing cells have been well defined. Uroguanylin-immunoreactive cells in the rat intestine are round, basket-shaped or tall flask-shaped cells with a dense accumulation of uroguanylin in the luminal cytoplasm and a long, thin basal process immunoreactive for uroguanylin.[26,31] Uroguanylin-positive cells in the rat intestine were identified as EC cells because they reacted with serotonin antibody. The EC cell, the most abundant type of enteroendocrine cell, is widely distributed in the intestine. When stimulated, the EC cell releases serotonin and substance P both apically (into the lumen) and basolaterally (into the circulation).[32] Uroguanylin also is released from the intestine bidirectionally and is thought to function in a luminocrine (luminal secretion), endocrine, and/or paracrine fashion. The EC cell appears to have a feasible mechanism for delivering uroguanylin to luminally oriented GC-C, as well as to remote tissues such as the kidney via the circulation.

Uroguanylin-producing cells in the rat stomach were identified as enterochromaffin-like (ECL) by immunocytochemical methods and in situ hybridization cytochemistry using gastric mucosal cells isolated by counterflow elutriation.[33] ECL cells release histamine, leading to the stimulation of gastric acid secretion from parietal cells. Seven distinct endocrine cells: EC, ECL, D, D1, P, G, and X cells, have been identified ultrastructurally and immunohistochemically in rat and human gastric mucosa.[34–36] Very recently, X cells have been clarified to produce ghrelin, a novel peptide that stimulates the release of growth hormone from the pituitary.[37,38] X cells thus can be abbreviated as Gr cells. ECL cells are small cells (8- to 10-nm in diameter) that contain cytoplasmic vesicles with eccentric electron-dense cores. ECL cells are scattered in the oxyntic glands, often in direct contact with parietal cells. Patients with Zollinger-Ellison syndrome, presenting with gastrinoma, peptic ulcer, and ECL hyperplasia, had markedly high plasma concentrations of uroguanylin. Further investigation of the cGMP-mediated gastric ion transport mechanism is a fascinating topic that could lead to better understanding of the regulation of gastric acid secretion.

Uroguanylin and GC-C mRNA are present in B cells of the pancreatic islets, but the involvement of uroguanylin in the regulation of glucose metabolism has not been determined. The peptides produced in the "gastro-entero-pancreatic (GEP) endocrine system" function to control or modulate all the processes linked to the digestion and absorption of nutrients and water.[39] Uroguanylin may be a constituent of the GEP endocrine system because of its cellular source and biological activity.

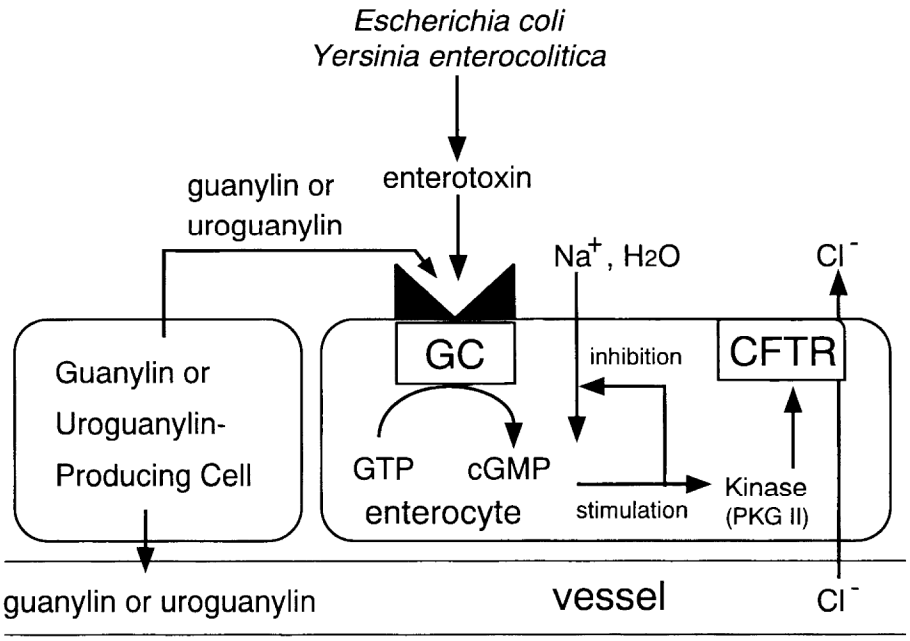

Fig. 2. A model for the guanylyl cyclase-C (*GC-C*) signaling pathway in intestinal Cl^--secreting cells. Luminally secreted guanylin and uroguanylin activate GC-C in nearby cells. These peptides are also secreted into the bloodstream to function in an endocrine fashion. Cl^- enters across the basolateral (serosal) membrane. Cyclic guanosine 3′,5′-monophosphate (*cGMP*) activates cGMP-dependent protein kinase II (*PKG II*). A key substrate for phosphorylation by PKG II is the apical membrane-localized CFTR protein. CFTR serves as a channel for Cl^- and HCO_3^- secretion across the apical (mucosal) membrane of epithelial cells. *GTP*, Guanosine 5′-triphosphate

Molecular mechanism of guanylin and uroguanylin action

A model for the GC-C signaling pathway in intestinal Cl^--secreting cells is shown in Fig. 2. GC-C is more abundant in the small intestine than in the large intestine. Moreover, a gradient of receptor levels was observed in small intestine, with the highest amounts in the upper portions of crypts and in the lower parts of villi adjacent to the crypts.[40] A truncated, GC-C-like mRNA that has a 159-nucleotide deletion was found in the mucosa of rat stomach and intestine.[41] The physiological implications of this truncated GC-C remain to be elucidated. Intestinal cells produce preproguanylin or preprouroguanylin. Luminal secretion of active peptides can activate GC-C in nearby cells of this epithelium in a luminocrine fashion. Proguanylin and prouroguanylin are secreted into the bloodstream to function in an endocrine fashion. cGMP activates cGMP-dependent protein kinase II (PKG II), which phosphorylates the apical membrane-localized CFTR protein. CFTR is present immunohistochemically in the epithelia of the intestine, stomach, airway, small pancreatic ducts, renal tubules, and the sweat duct. CFTR is one member of a large family of ABC proteins that transport small molecules across the cell membrane in an ATP-dependent fashion. Guanylin and uroguanylin were verified to stimulate Cl^- secretion in T_{84} cells, as measured by increases in the short circuit current, using an Ussing chamber.[6,42] Cl^- is secreted through the CFTR channel pathway across the apical (mucosal) membrane.

The signal transduction system and physiological implications of guanylin and uroguanylin were also investigated by using transgenic and knockout mice. The mutation of *CFTR* genes, resulting in either loss of the protein or modification of its activity, underlies the genetic disease of cystic fibrosis. CFTR knockout mice had marked reductions of intestinal Cl^- and HCO_3^- secretion responses to guanylin and uroguanylin.[43,44] Mice deficient in PKG II were resistant to *E. coli*.[45] PKG II knockout mice also developed dwarfism that was caused by a severe defect in endochondral ossification at the growth plates. GC-C-null mice had no STa-stimulable guanylyl cyclase activity.[46] Gavage with STa resulted in marked fluid accumulation within the intestine of wild-type and heterozygous suckling mice, but GC-C-null animals were resistant. Infection with enterotoxigenic bacteria that produce STa led to diarrhea and death in wild-type and heterozygous mice, whereas the null mice were protected. Diets including high carbohydrate, fat, or protein, or drinking water including high K^+ or Na^+ did not severely affect the GC-C-null animals.

A wide range of mucosal acidity occurs within the intestinal lumen during digestion. At an acidic mucosal pH of 5.0, uroguanylin was 100-fold more potent than guanylin, but at an alkaline pH of 8.0, guanylin was more potent than uroguanylin in stimulating intracellular cGMP production and transepithelial chloride secretion.[47] Uroguanylin and guanylin appear to cooperatively regulate the guanylyl cyclase activity of a common set of mucosal receptors in a pH-dependent fashion.

Uroguanylin as a candidate for intestinal natriuretic factor

When dietary sodium chloride is low, the regulation of salt homeostasis is maintained by mineralcorticoids. However, contemporary diets commonly have excess sodium; thus, mechanisms are required to achieve salt homeostasis during sodium surfeit. An intestinal natriuretic factor has been sought, because the oral ingestion of sodium chloride causes a dramatic increase in urine salt excretion, whereas the same amount administered intravenously has little effect on renal salt excretion.[48]

The intravenous administration of uroguanylin to mice induced diuresis, natriuresis, and kaliuresis.[49] Guanylin was less potent than uroguanylin and STa. When uroguanylin was administered into the renal artery, it was filtered through the glomerulus and then activated the GC-C/CFTR system localized in the tubules, thereby causing natriuresis and an increase in cGMP excretion.[50] Urinary uroguanylin excretion in persons who took a high-salt diet was significantly higher than that in persons with a low-salt diet.[51] The magnitude of uroguanylin excretion was proportional to increases in urinary Na^+ and cGMP excretion in subjects receiving high-salt diets. The oral administration of salt to rats augmented uroguanylin mRNA levels in the intestine and kidney. Uroguanylin secretion from the intestine in response to salt was studied in vitro. In isolated vascularly and luminally perfused rat intestine, uroguanylin produced in the intestine was secreted mainly in the lumen, but in part in the blood, in response to high-concentration sodium chloride administration. These findings taken together indicate that uroguanylin is a prime candidate for a substance that could link the intestine and kidney in an endocrine pathway that regulates renal salt metabolism. Uroguanylin secretion from the gastrointestinal tract or other organs such as the heart could be increased secondary to renal Na^+ retention as a compensatory mechanism involving enhanced circulating levels of uroguanylin functioning as a natriuretic and diuretic hormone. Thus, plasma uroguanylin may be elevated to help maintain body salt and water balance in both normal physiological circumstances and in diseases such as congestive heart failure and nephrosis,[50] in which Na^+ and water retention cause edema. In contrast to uroguanylin, guanylin gene expression was not affected by a high-salt diet.[52] A low-salt diet reduced the expression of guanylin to 30%–40% of the level found in control animals. The guanylin pathway is thought to be down-regulated as an adaptive response to salt restriction.

In conclusion, membrane guanylyl cyclase-C, GC-C, is an intestinal receptor for guanylin and uroguanylin that is responsible for the stimulation of Cl^- and HCO_3^- secretion into the intestinal lumen. Guanylin and uroguanylin are produced mainly in the gastrointestinal epithelial cells to serve in a luminocrine mechanism for the regulation of gastrointestinal fluid and electrolyte secretion. Uroguanylin also serves in an endocrine axis linking the intestine and kidney, where its natriuretic and diuretic actions contribute to the maintenance of Na^+ balance after the oral ingestion of NaCl. Enteric bacteria secrete peptide toxin that mimics guanylin peptides, activating GC-C, to produce secretory diarrhea. Guanylin peptides are involved in the pathophysiology of such gastrointestinal diseases as diarrhea and peptic ulcer, and in chronic renal diseases or congestive heart failure, in which guanylin and/or uroguanylin levels in the circulation and/or urine are elevated. Guanylin peptides function in the regulation of salt and water homeostasis in the gastrointestinal tract and kidney.

References

1. Field M, Rao MC, Chang EB. Intestinal electrolyte transport and diarrheal disease. N Engl J Med 1989;321:879–83.
2. Schulz S, Green CK, Yuen PS, Garbers DL. Guanylyl cyclase is a heat-stable enterotoxin receptor. Cell 1990;63:941–8.
3. Garbers DL, Lowe DG. Guanylyl cyclase receptors. J Biol Chem 1994;269:30741–4.
4. Giannella RA, Luttrell M, Thompson M. Binding of *Escherichia coli* heat-stable enterotoxin to receptors on rat intestinal cells. Am J Physiol 1983;245:G492–8.
5. Currie MG, Fok KF, Kato J, Moore RJ, Hamra FK, Duffin KL, et al. Guanylin: an endogenous activator of intestinal guanylate cyclase. Proc Natl Acad Sci USA 1992;89:947–51.
6. Hamra FK, Forte LR, Eber SL, Pidhorodeckyj NV, Krause WJ, Freeman RH, et al. Uroguanylin: structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. Proc Natl Acad Sci USA 1993;90:10464–8.
7. Kita T, Smith CE, Fok KF, Duffin KL, Moore WM, Karabatsos P, et al. Characterization of human uroguanylin: a member of the guanylin peptide family. Am J Physiol 1994;266:F342–8.
8. Nakazato M, Yamaguchi H, Kinoshita H, Kangawa K, Matsuo H, Chino N, et al. Identification of biologically active and inactive human uroguanylins in plasma and urine and their increases in renal insufficiency. Biochem Biophys Res Commun 1996;220:586–93.
9. Fan X, Hamra FK, London RM, Eber SL, Krause WJ, Freeman RH, et al. Structure and activity of uroguanylin and guanylin from the intestine and urine of rats. Am J Physiol 1997;273:E957–64.
10. Chino N, Kubo S, Miyazato M, Nakazato M, Kangawa K, Sakakibara S. Generation of two isomers with the same disulfide connectivity during disulfide bond formation of human uroguanylin. Lett Pept Sci 1996;3:45–52.
11. Chino N, Kubo S, Kitani T, Yoshida T, Tanabe R, Kobayashi Y, et al. Topological isomers of human uroguanylin: interconversion between biologically active and inactive isomers. FEBS Lett 1998;421:27–31.
12. Wiegand RC, Kato J, Currie MG. Rat guanylin cDNA: characterization of the precursor of an endogenous activator of intestinal guanylate cyclase. Biochem Biophys Res Commun 1992;185:812–7.

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