Guanylin peptides: renal actions mediated by cyclic GMP

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Forte, Leonard R., Roslyn M. London, Ronald H. Freeman, and William J. Krause. Guanylin peptides: renal actions mediated by cyclic GMP. Am. J. Physiol. Renal Physiol. 278: F180–F191, 2000.—The guanylin family of cGMP-regulating peptides has three subclasses of peptides containing either three intramolecular disulfides found in bacterial heat-stable enterotoxins (ST), or two disulfides observed in guanylin and uroguanylin, or a single disulfide exemplified by lymphoguanylin. These small, heat-stable peptides bind to and activate cell-surface receptors that have intrinsic guanylate cyclase (GC) activity. Two receptor GC signaling molecules have been identified that are highly expressed in the intestine (GC-C) and/or the kidney (OK-GC) and are selectively activated by the guanylin peptides. Stimulation of cGMP production in renal target cells by guanylin peptides in vivo or ex vivo elicits a long-lived diuresis, natriuresis, and kaliuresis. Activation of GC-C receptors in target cells of intestinal mucosa markedly stimulates the transepithelial secretion of Cl and HCO3, causing enhanced secretion of fluid and electrolytes into the intestinal lumen. Bacterial ST peptides act as mimics of guanylin and uroguanylin in the intestine, which provide a cellular mechanism underlying the diarrhea caused by ST-secreting strains of Escherichia coli. Uroguanylin and guanylin may participate in a novel endocrine axis linking the digestive system and kidney as a physiological mechanism that influences Na+ homeostasis. Guanylin, uroguanylin, and lymphoguanylin may also serve within intrarenal signaling pathways controlling cGMP production in renal target cells. Thus we propose that guanylin regulatory peptides participate in a complex multifactorial biological process that evolved to regulate the urinary excretion of NaCl when dietary salt levels exceed the body's physiological requirements. Guanylin, uroguanylin, and/or lymphoguanylin may also serve within intrarenal signaling pathways controlling cGMP production in renal target cells. Thus we propose that guanylin regulatory peptides participate in a complex multifactorial biological process that evolved to regulate the urinary excretion of NaCl when dietary salt levels exceed the body's physiological requirements. This highly integrated and redundant mechanism allows the organism to maintain sodium balance by eliminating excess NaCl in the urine. Uroguanylin, in particular, may be a prototypical “intestinal natriuretic hormone.”

THE BODY MAINTAINS SODIUM BALANCE in the face of remarkably diverse levels of NaCl in our diets. During periods of reduced dietary NaCl intake, renal sodium conservation is subserved via activation of the renin-angiotensin-aldosterone endocrine axis to prevent dissipation of body stores. However, when dietary NaCl intake is excessive, renal sodium excretion is enhanced to eliminate the excess NaCl absorbed by the digestive tract. The increased urinary excretion of sodium is most pronounced postprandially when the kidney slowly excretes excess NaCl after a salty meal. Physiological mechanisms contributing to this remarkable postprandial natriuresis are likely to be numerous and also redundant considering the importance of maintaining sodium balance. New findings are shedding light on a novel endocrine axis that links the intestine and kidney, thus contributing to sodium homeostasis when excess salt is consumed. A “natriuretic factor” has been postulated to exist within the digestive system as one explanation for the increased urinary sodium excretion elicited by oral salt administration (3, 41). Such a natriuretic hormone was unexpectedly discovered from
investigations into the cellular mechanisms underlying a cholera-like diarrhea caused by enterotoxigenic bacteria. The intestinal natriuretic factor that was identified is a small peptide named uroguanylin (27). Uroguanylin is similar in primary structure to the heat-stable enterotoxigenic bacteria. E. coli ST peptides activate membrane guanylate cyclase (GC) signaling molecules located on apical membranes of enterocytes lining the small and large intestine (16, 32, 38, 39, 46, 54, 60). Hyperstimulation of cGMP production by E. coli ST elicits large increases in intestinal fluid and electrolyte secretion, causing a characteristic watery form of diarrhea. For about a decade, these cell-surface receptors for ST peptides were thought to be a peculiarity of the intestinal mucosa serving as inopportune targets for bacterial toxins. Then, E. coli ST-activated receptor GC signaling molecules were discovered in two kidney cell lines (i.e., OK, PTK-2) and in the kidney, liver, and testes of North American opossums, Didelphis virginiana (20, 21, 38, 69). These findings predicted that endogenous, ST-like peptides exist to physiologically regulate the enzymatic activity of membrane receptor GCs in both the intestine and kidney, thus influencing epithelial cell function via the intracellular second messenger, cGMP. Discovery of a novel cGMP signaling pathway in the kidney activated by bacterial STs received little attention for a while, perhaps because this cGMP mechanism was identified in representative species from Metatheria, the marsupials. However, the recent discovery of three different (ST-like) peptides that activate receptor GC signaling molecules in the kidney, intestine, and other epithelia reveals that a uroguanylin peptide-regulated cGMP signaling pathway is fundamental to the kidney, intestine, and other organs of all mammals. Guanylin, uroguanylin, and lymphoguanylin are endogenous peptides that serve as physiological regulators of their cognate (i.e., ST) receptors on target cells within the intestine, kidney, and other epithelia (9, 19, 27). Guanylin regulatory peptides are the topic of this brief review, which concentrates on the regulation of renal function by uroguanylin acting in an endocrine axis linking the intestine and kidney for regulation of sodium balance. Evidence is also provided for the existence of another signaling mechanism for guanylin peptides made in the kidney to act locally and stimulate the enzymatic activity of receptor GCs on nearby target cells in the nephron. Thus both endocrine and/or intrarenal paracrine mechanisms may function to control receptor GC activity and influence ion transport processes via cGMP in the kidney.

GC Signaling Molecules

Two different kinds of GC enzymes were originally identified that produce cGMP for various intracellular signal transduction pathways (29, 68). Soluble, cytosolic GCs are heterodimers containing heme that serve as intracellular receptors for nitric oxide (NO). In contrast, plasma membrane GC proteins are cell-surface receptors for endogenous peptides. The membrane GCs have four main regions, consisting of a highly variable NH2-terminal domain that binds peptide ligands, a single membrane-spanning region, a kinase-like domain, and a conserved catalytic region located at the COOH terminus of the protein (39). Two different classes of endogenous peptides have been identified that control the enzymatic activities of membrane receptor-GCs. Discovery of these peptides stems from the seminal studies of E. coli ST as diarrhea-inducing peptide toxins and the subsequent demonstration of a class of natriuretic peptides in the myocardium (11, 16, 32). Guanylin and atriopeptin families of peptides consist in each case of three active peptides derived from six different genes. However, atriopeptin and guanylin peptides are distinctly different classes of GC-regulating agonists. One GC has been identified as a receptor for atriopeptins A and B (GC-A), whereas GC-B serves as a receptor for atriopeptin C (39). Two additional GC receptors have been identified by isolation of cDNAs from intestine encoding GC-C (61) and from kidney encoding OK-GC (45). Both GC-C and OK-GC are receptors for the guanylin peptides. Four other membrane GCs have been identified by cloning of cDNAs from either sensory tissues or intestine. These membrane GC signaling molecules are presently classified as orphan receptors. The invertebrate, Caenorhabditis elegans, has 26 different genes encoding membrane GC enzymes; thus it may be predicted that mammalian genomes will contain ~100 genes comprising the total family of cell-surface receptor GC proteins (2). Clearly, there are far more receptor GCs to be identified than are presently known. It is also likely that completely new classes of regulatory peptides will be discovered that act physiologically as endogenous agonists to control cGMP production via the cell-surface GC signaling molecules that are either presently classified or will soon be classified as orphan receptor GCs. Thus cGMP can be derived from a large number of different cell-surface GCs that are expressed in different cells, tissues, and organs of the body. These discoveries provide a type of combinatorial complexity to the cGMP signaling molecules that are available in different cells and tissues for the purpose of regulating a variety of discrete cellular functions via cGMP. Moreover, these findings also elevate the overall physiological importance of the membrane GC-cGMP signaling mechanism that will ultimately rival the contributions to biological regulation made by the more widely recognized intracellular cGMP pathway derived from NO-regulated forms of GC-receptors.

Discovery of Guanylin Peptides

The first endogenous, ST-like peptide identified was isolated from the intestinal mucosa of rats and named guanylin (9). Guanylin is a 15-amino-acid peptide that was purified from intestine by using a cGMP bioassay with T84 intestinal cells to detect the active peptides in extracts of jejunum. The cGMP signaling pathway activated by E. coli ST that was previously identified in OK and PTK-2 cell lines and in renal proximal tubules provided a rationale for guanylin to exist in both
tubular filtrate and the urine of opossums (20, 21, 69). The urinary forms of "guanylin" were subsequently isolated, and sequence analyses revealed that the bio-
logically active peptides in urine were merely related to guanylin. Distinct differences in primary structure of the biologically active peptides suggested that a novel ST/guanylin-like peptide was present in opossum urine (27). This was confirmed when a guanylin-like peptide was subsequently isolated from both intestinal mucosa and urine of opossums. The dominant urinary peptide was named uroguanylin, indicating its biological source and peptide family relationship. Uroguanylin was then isolated from human and rat urine (14, 37), intestinal mucosa of rats and opossums (28, 43), and plasma obtained from both opossums and humans (13, 30).

Uroguanylin is the major guanylin family peptide found in urine because guanylin is either undetectable or occurs at much lower concentrations than urogu-
nylin in the urine of mammalian species examined to date (14, 27, 34–37). A third member of the guanylin family of peptides was identified recently by molecular cloning of cDNAs, encoding an uroguanylin-like pep-
tide that we named lymphoguanylin (19). This unique peptide was synthesized and shown to be biologically active. Like the atropxin class of cGMP-regulating peptides, at least three different guanylin peptides exist to regulate the production of cGMP by activation of membrane receptor GCs in target tissues.

Precursor Structures of Guanylin Family Peptides

cDNAs encoding polypeptide precursors of guanylin, uroguanylin, and lymphoguanylin have been isolated, demonstrating that the active peptides are located at COOH-terminal ends of these proteins (Fig. 1 and Refs. 13, 15, 31, 43, 47, 49, 70, 71). Proguanylin and prouro-

Fig. 1. Precursor polypeptide structures containing biologically ac-
tive guanylin peptides. Models for preprohormone forms of rat guanylin, opossum uroguanylin, and opossum lymphoguanylin are shown. These prepropeptides are 115, 109, and 109 residues long, respectively. At COOH terminus of each polypeptide is biologically active peptide provided, as are the amino acid sequences using single-letter abbreviations for amino acids.

hydrolysis of peptide bonds between serine and valine residues of opossum preproguanylin and preprogu-
ylin. Cleavage sites in rat and human preproguanylin are located between glycine and valine residues and between serine and valine residues in preproguanylin (31, 43, 47, 49, 70, 71). Primary structures for the biologi-

cally active forms of rat guanylin and opossum forms of uroguanylin and lymphoguanylin are compared with the amino acid sequence of E. coli ST in Fig. 2. Uroguanylin and guanylin have four conserved cyste-
ines in each active peptide domain. Disulfide bonds are formed between the first to third and second to fourth cysteines in each peptide, and these disulfides were once thought to be required for optimal peptide potencies in the stimulation of cGMP production in vitro (9, 27). E. coli ST has the same disulfide pairings as guanylins plus a third disulfide bond linking an additional pair of cysteine residues. This structural feature may contribute to the apparently higher potencies of ST peptides for activation of intestinal receptor GCs compared with potencies of guanylin and urogu-
ylin (9, 14, 27, 37). Lymphoguanylin has a striking difference in primary structure because it has three cysteines, which can form only a single intramolecular disulfide bond (19). A lymphoguanylin peptide containing a single disulfide between the first and third cysteine residues was synthesized and tested for biologi-
cal activity. Lymphoguanylin activates GC-C receptors of T84 intestinal cells and OK-GC receptors in OK kidney cells (19). It may be concluded that the guanylin peptide family can be divided into three distinct subclasses on the basis of the number of intramolecular disulfides in the biologically active molecules. In the order of their discovery, class 1 peptides contain the bacterial STs with three disulfide bonds, class 2 pep-
tides consist of guanylin and uroguanylin with two disulfides, and the class 3 peptides are exemplified by lymphoguanylin containing a single disulfide bond (Fig. 2).

Guanylin Peptides: Actions in the Kidney

Activation of renal receptor GCs by administration of E. coli ST increases urinary cGMP excretion, reflecting the stimulation of cGMP production in uroguanylin target cells (20, 21). In the isolated perfused kidney, treatment with uroguanylin, guanylin, or ST elicits
Guanylin is less potent than either uroguanylin or ST as a natriuretic and diuretic agonist; however, guanylin does cause a substantial increase in urinary K⁺ excretion. Lymphoguanylin has been tested in perfused kidneys from rats, and this peptide also stimulates urinary Na⁺, K⁺, and H₂O excretion (17). Treatment of mice with intravenous uroguanylin or ST stimulates urinary Na⁺, K⁺, and H₂O excretion, but guanylin is ineffective at doses that were tested (24). Thus guanylin family peptides elicit the renal responses by direct actions on the kidney, as elucidated by using the isolated perfused kidney model and also act in vivo to regulate kidney function. Moreover, E. coli ST is a potent mimic of uroguanylin in the kidney as it is in the intestine (18, 20, 21, 24). These effects on renal function suggest that uroguanylin, in particular, can act to regulate urinary NaCl excretion in vivo; thus uroguanylin has appropriate biological activities to participate in the physiological regulation of sodium homeostasis. A natriuretic factor such as uroguanylin was postulated to exist in the 1970s (21). These studies demonstrate that oral loads of NaCl cause substantially greater increases in the urinary excretion of sodium compared with the natriuresis produced by intravenous salt. A natriuretic factor released from the digestive system into the bloodstream after salt ingestion was postulated as an explanation for these findings. A primary physiological role of the kidney is to excrete excess salt in the urine to maintain sodium balance when dietary NaCl intake exceeds the needs of an animal. The renin-angiotensin-aldosterone system plays a key role in conserving sodium when salt intakes are low, but the physiological factors responsible for the remarkable natriuresis secondary to high dietary NaCl are uncertain because of the complexity of salt homeostasis mechanisms in the body. Iso- lation of uroguanylin from urine and subsequent demonstrations that this peptide has natriuretic activity by direct actions on the kidney suggest that uroguanylin may participate in the physiological maintenance of sodium balance in vivo (14, 18, 24, 27, 37).

For uroguanylin and/or guanylin to serve as an “intestinal natriuretic hormone” in an endocrine axis linking the intestine and kidney, the peptides should be found in the bloodstream (3, 41). These peptides circulate because biologically active uroguanylin and inactive prouroguanylin peptides have been isolated from plasma and identified by the unique properties of their NH₂-terminal sequence analyses (13, 30, 34–36). Proguanylin levels in plasma are markedly elevated in patients with chronic and severe cases of renal failure, and proguanylin has been isolated from hemodialysate solutions (40, 53). Biologically active guanylin has not yet been isolated from plasma (13). Values for plasma proguanylin are reported to be in the 30- to 40-pM range, whereas plasma uroguanylin is ~5–7 pM in the plasma of normal humans, suggesting that guanylin is more abundant than uroguanylin in the circulation (34–36). Both proguanylin and uroguanylin/prouroguanylin levels are markedly increased in chronic renal failure (30, 36, 51, 53). The severity of chronic renal disease correlates with the magnitude of increases in both plasma proguanylin and uroguanylin concentrations. Uroguanylin levels are also increased in the nephrotic syndrome (34). It may be concluded that proguanylin and uroguanylin/prouroguanylin are cleared from the circulation by the kidney and that reduced functioning renal mass and decreased glomerular filtration rates (GFR) lead to substantial increases in the concentrations of these peptides in plasma. Circulating forms of uroguanylin and prouroguanylin are thought to be a major source of the urinary forms of biologically active uroguanylin (14, 27, 35, 37, 52). Both of these peptides can enter renal tubules by glomerular filtration. Prouroguanylin in the tubular lumen is then converted to active uroguanylin by tubular endoproteases because prouroguanylin is not detected in the urine by using sensitive RIA (35, 52). Guanylin is undetectable in human and rat urine and is present in opossum urine at much lower concentrations than is uroguanylin (14, 27, 37). If circulating levels of proguanylin are substantially greater than plasma uroguanylin as measured by RIA, it may be concluded that the absence of guanylin in urine is due to hydrolysis of filtered proguanylin by chymotrypsin-like endoproteases within the renal proximal tubule (1). Guanylin is sensitive to cleavage and inactivation by chymotrypsin, which hydrolyzes the peptide bond’s COOH terminal to the aromatic residues that are unique to guanylin peptides (4, 24, 26, 27; Fig. 2). In contrast, uroguanylin and ST have asparagine residues at this position in the active peptides. Thus inactivation of guanylin in tubular filtrate may be a physiologically protective mechanism that prevents filtered guanylin from acting on receptor GCs located on apical membranes of tubular target cells (15, 20, 21, 38). In this way, circulating uroguanylin could serve as the main intestinal natriuretic hormone in a novel endocrine axis that influences sodium homeostasis in the postprandial state. Such a mechanism involving hydrolysis of filtered guanylin by tubular proteases may also contribute to the lower potency of guanylin as a natriuretic and diuretic peptide when tested in the perfused kidney ex vivo or administered to animals in vivo (14, 24). Uroguanylin and guanylin have similar potencies in the activation of OK-GC receptors expressed naturally in the OK cell line or when expressed experimentally in HEK-293 cells transfected with OK-GC cDNAs (15, 45). Thus inactivation of guanylin by the kidney may explain the relatively low potency of this peptide as a diuretic and natriuretic peptide in vivo.

Uroguanylin serves as a circulating hormone for the purpose of regulating postprandial NaCl excretion, oral salt intake should increase the plasma levels of uroguanylin. Recent experimental evidence reveals that high-salt diets significantly stimulate uroguanylin, cGMP, and sodium excretion in human urine (35). Quantitative increases in uroguanylin excretion were
significantly correlated with the magnitude of increases in urinary sodium and cGMP. It is likely that increased urinary excretion of uroguanylin elicited by high-salt diets is due to increased secretion of uroguanylin from the digestive system, causing increases in plasma levels of both uroguanylin and prouroguanylin. Specific cells in gastrointestinal mucosa that produce uroguanylin and/or guanylin are specialized enteroendocrine cells (EC) located in the upper small intestine (6, 51, 55, 67). These unique enterocytes can secrete peptides and other cellular products into both plasma and the intestinal lumen. Guanylin is also highly expressed in goblet cells (8, 42). Cells with high concentrations of prouroguanylin were observed in characteristically shaped EC-like cells of opossum duodenum, as detected by using both immunocytochemistry with antibodies that react with the NH$_2$ terminus of prouroguanylin and by in situ hybridization histochemistry (Fig. 3). Other mammalian species also produce high concentrations of uroguanylin located within intestinal EC cells, although it should be emphasized that other intestinal cell types also contain uroguanylin. The stomach is another potential site for uroguanylin secretion from the gastrointestinal (GI) tract into plasma because uroguanylin has been recently localized to EC-like cells within the gastric mucosa (51). The stomach and upper small intestine are intuitively appropriate sites for NaCl-modulated secretion of uroguanylin into plasma. A key experimental approach to test the postulate that uroguanylin is an intestinal natriuretic hormone linking the GI tract and kidney could use the “Carey protocol” and compare the effects of oral vs. intravenous salt loads on plasma and urine concentrations of uroguanylin (3, 41). Oral NaCl loads should stimulate increases in plasma and urinary levels of uroguanylin to a greater extent than intravenous salt if uroguanylin is indeed an endocrine factor involved in maintaining sodium balance. Major questions that can be addressed in the future should focus on cellular and molecular mechanisms involved in the regulation of uroguanylin secretion from the GI tract. For example, how do the GI cells that produce uroguanylin detect NaCl? Is there a GI salt-sensor mechanism specific for Na$^+$ or for Cl$^-$, or does a cellular osmoreceptor account for the perception (or enteric taste) of dietary salt (57)? Are neural, endocrine, and/or paracrine pathways involved in salt-detecting mechanisms that influence uroguanylin secretion? How might a negative-feedback mechanism be constructed to function in this postulated endocrine pathway so that uroguanylin secretion can be subsequently reduced when excess salt is excreted in urine and sodium balance is restored in the postprandial state? A desire to better understand the fundamental aspects of uroguanylin endocrinology should guide future investigations into physiological processes governing both the secretion and cleavage of prouroguanylin to form the biologically active uroguanylin peptides in the body. Answers to some of these questions will provide key insights into important physiological mechanisms that help balance renal sodium excretion to match the dietary intake of salt.

Cellular and Molecular Mechanism of Uroguanylin Action

Molecular identity of the receptors for uroguanylin. The first cell-surface receptor for guanylin peptides to be identified at the molecular level is GC-C (61). Expression of GC-C cDNAs in vitro produces a membrane GC that is activated by ST peptides. GC-C is expressed throughout the GI mucosa, and this receptor GC is clearly an important target for guanylin and...
uroguanylin secreted into the intestinal lumen and for ST peptides secreted by enteric bacteria (7, 42, 46, 54, 60, 63). However, GC-C mRNA is not highly expressed in kidney, and GC-C may not actually participate in cGMP signaling pathways of renal target cells that contribute to the observed diuretic and natriuretic responses to either uroguanylin or ST in vivo (18, 24, 44). Deletion of GC-C genes in GC-C-KO mice reveals that intestinal fluid secretion responses to ST in vivo are greatly diminished, perhaps even completely lost (48, 62). In marked contrast, the natriuretic responses to uroguanylin and ST assessed in vivo are essentially retained in mice with no GC-C protein (5). Accordingly, GC-C does not appear to be a major receptor-GC for uroguanylin in the kidney. The renal cGMP signal transduction pathway for uroguanylin was first identified in cultured opossum kidney (OK) and potoroo kidney (PTK-2) cell lines; thus receptor GCs expressed in OK cells were postulated to be a major form of renal receptor GCs for guanylin peptides (20, 21, 69). Recently, cDNAs encoding membrane receptor GCs expressed in OK cells were isolated by molecular cloning by using RNA preparations from OK cells and kidney cortex to produce cDNAs for use as a template in a PCR-based cloning strategy. OK-GC cDNAs encoding a 1,049-amino-acid protein that belongs to the membrane GC family of proteins were isolated and sequenced. OK-GC cDNAs encoding a 1,049-amino-acid protein that belongs to the membrane GC family of proteins were isolated and sequenced (45). When the OK-GC cDNAs are expressed in cultured cells, a membrane protein of ~160-kDa size was produced and detected by Western assays. Expression of OK-GC cDNAs, either transiently in COS cells or stably in HEK-293 cells, produced a cell-surface GC that is activated by uroguanylin, guanylin, and E. coli ST. Northern hybridization assays reveal that OK-GC mRNA transcripts of ~3.8 kb are most abundant in kidney cortex and intestinal mucosa, but mRNAs are also detected at lower levels in renal medulla, adrenal gland, the heart, and urinary bladder. OK-GC is quite different from GC-C in the ligand-binding domains, with the OK-GC protein exhibiting only ~50% identity with rat, human, and porcine forms of intestinal GC-C (61, 66). OK-GC and GC-C proteins share >90% identity when their cyclase catalytic domains are compared, indicating that these two GC signaling molecules may constitute a subclass of receptors for the guanylin peptides within the larger family of membrane GC proteins. OK-GC may be a novel receptor GC for guanylin and ST peptides that plays an important physiological role in renal cGMP signaling. A eutherian homologue of OK-GC expressed in the renal tubules of GC-C-KO mice could be responsible for the apparently normal natriuretic and diuretic responses to uroguanylin and ST observed in these animals (5). A counterpart to OK-GC in renal tubules of GC-C-KO mice could be identified by cDNA cloning experiments to address an important question concerning the molecular identity of a major type of uroguanylin receptor-GC in the kidney of eutherian mammals. Identification of an opossum homologue of eutherian GC-C will also test the hypothesis that at least two different receptor-GCs for guanylin and uroguanylin are present in all mammals, indicating that both guanylin receptors evolved in common ancestors to both Eutheria and Metatheria.

Nephron location of cells containing receptor GCs. Locating specific cells in the kidney that have receptors for guanylin and ST peptides will advance our understanding of the actions of uroguanylin and ST on kidney function. In particular, the nephron localization of uroguanylin target cells will help define potential cellular mechanisms of uroguanylin action that result in the stimulation of Na\(^+\), Cl\(^-\), K\(^+\), and water excretion in vivo. Tubular epithelial cells expressing high levels of uroguanylin-binding sites have been labeled with \(^{125}\)I-ST, which is a very specific and high-affinity radioligand for membrane receptors in both the opossum kidney (15, 20, 21, 38) and mammalian intestine (33, 38, 46). In situ receptor autoradiography reveals that \(^{125}\)I-ST binds to receptors located on proximal tubular cells, with the highest apparent receptor density observed on cells of straight (i.e., pars recta) compared with convoluted portions of proximal tubules in the opossum kidney (Fig. 4). Proximal tubular localization of receptors labeled with \(^{125}\)I-ST also occurs in the mesonephros of developing animals as well as in the adult metanephros (38). Uroguanylin, guanylin, and unlabeled ST all compete effectively for binding sites labeled with \(^{125}\)I-ST, indicating that \(^{125}\)I-ST binds to all of the detectable receptors for guanylin peptides identified by using this method. No receptors for \(^{125}\)I-ST were detected in glomeruli in these experiments. It is likely that the OK-GC protein accounts for some of the specific binding sites for \(^{125}\)I-ST observed in proximal tubules, but it is also possible that other receptor-GCs labeled by \(^{125}\)I-ST in situ are expressed in opossum kidney (45).

In situ receptor autoradiography with \(^{125}\)I-ST is a useful method for detection of uroguanylin/ST receptors that are expressed at high density, such as those found in the intestine of mammals or in the opossum kidney.
kidney. It is also likely that this radioligand binding assay does not detect all of the functional receptors for uroguanylin in the kidney or in other putative target tissues. For example, the kidneys of American alligators have robust cGMP responses to ST and guanylin in vitro, but this tissue does not exhibit detectable binding sites for \(^{125}\)I-ST by using either an in situ or the in vitro binding assays (unpublished observations). Moreover, rat and mouse kidneys respond to uroguanylin and ST with large increases in urinary Na\(^+\), K\(^+\), and H\(_2\)O excretion (18, 24, 44), but we have not readily detected renal receptor GCs in these species by using in situ or in vitro binding assays with \(^{125}\)I-ST as the uroguanylin-like radioligand (unpublished observations). However, these \(^{125}\)I-ST binding assays work quite well when applied to intestinal tissues from these species, indicating that this method labels receptors that are expressed at very high densities on target cells within the intestinal mucosa (33, 46). Thus future experiments need to address a basic question concerning the nephron locations for target cells containing functional receptor-GCs for uroguanylin and guanylin in the kidney. The opossum is a useful animal model for this purpose because it exhibits a relatively high density of uroguanylin/guanylin receptors in the kidney. This is well demonstrated by the considerable cGMP responses of either renal tissue or OK cells to guanylin and ST peptides in vitro, and by interaction of \(^{125}\)I-ST with renal receptor sites in situ, and because of a relatively high level of OK-GC mRNA expression in the kidney cortex (15, 20, 21, 38, 45). Elucidation of the cellular loci for OK-GC along the nephron can now be accomplished by using both in situ hybridization histochemistry as well as immunocytochemistry assays to compare the specific distribution of cells containing OK-GC receptors with the patterns of binding sites for \(^{125}\)I-ST detected by in situ receptor autoradiography (15, 21, 38). Although uroguanylin may elicit natriuretic, diuretic, and kaliuretic actions by activation of OK-GC in proximal tubular target cells, it is also possible that other parts of the nephron have functional receptor GCs for the guanylin peptides. Localizing receptors for these peptides on specific target cells along the nephron in kidneys from GC-C-KO mice that retain diuretic and natriuretic responses to uroguanylin and ST in vivo should also provide new insights into the renal mechanisms of action of uroguanylin. A complete description of the molecular repertoire of receptor-GCs for the guanylin family of peptides, together with elucidation of the cellular localization of these receptors along the longitudinal axis of nephrons, is needed to explore several fundamental questions pertaining to the renal mechanisms of action for guanylin regulatory peptides.

**cGMP Signal Cascade**

Experiments designed to elucidate details pertaining to the cellular and molecular mechanisms of guanylin action have been conducted most extensively in the intestine. Results of these investigations may guide future studies of mechanisms for guanylin-GC-cGMP signaling in the kidney. A key receptor GC for these peptides in the GI tract is GC-C because GC-C-KO mice have no detectable fluid secretion responses to ST in vivo (48, 62). However, one or more additional receptors for guanylin/ST peptides are expressed in the intestine. Specific binding sites for \(^{125}\)I-ST are found in intestinal brush-border membranes (BBM) isolated from GC-C-KO mice (48). These BBM proteins are likely to be novel receptors for guanylin and uroguanylin that are produced by cells in the intestinal mucosa. Intracellular cGMP influences transepithelial Cl\(^-\) and HCO\(_3\)\(^-\) secretion in the intestine by interacting with either cGMP-dependent protein kinase II (PKG II) or cAMP-dependent protein kinase II (PKA II) that serve as receptors for cGMP in uroguanylin/guanylin target cells (23, 65). Disabling the gene encoding PKG II results in a loss of fluid secretion responses to E. coli ST in vivo and a marked decrease in anion secretion (Isc) responses of intestinal mucosa to ST measured in vitro (56). However, Isc responses to ST are partly retained in the intestine of PKG II-KO mice, indicating that alternate downstream signaling pathways exist for cGMP-dependent regulation of Cl\(^-\) and/or HCO\(_3\) secretion by guanylin peptides. It was previously shown in cultured cell models of intestinal epithelia that cGMP derived from GC-C activates Cl\(^-\) secretion via binding to and activation of PKA II rather than by activation of PKG II in the intestinal cell lines (22). Another potential cGMP signaling mechanism for regulation of intestinal PKA by cGMP is through cGMP binding to sites on cGMP-regulated phosphodiesterase enzymes in guanylin target cells, leading to decreased cAMP hydrolysis with increased levels of intracellular cAMP that activate PKA. A fourth possibility for regulation of cellular function by cGMP is through the direct interaction of this cyclic nucleotide with putative cation channels, although this cellular mechanism has not been demonstrated yet for the guanylin peptides (10).

A key substrate for phosphorylation by either PKG II or PKA II is the apical membrane-localized cystic fibrosis transmembrane conductance regulator (CFTR) protein expressed in the intestine (23, 58). Mutation of CFTR genes, resulting in either loss of the protein or modification of its activity, underlies the genetic disease of cystic fibrosis (CF). CFTR is one member of a large family of ABC proteins that transport small molecules across cell membranes in an ATP-dependent fashion. This protein also has anion-conducting properties; thus CFTR has been postulated to serve as channels for Cl\(^-\) and HCO\(_3\)\(^-\) secretion across apical plasma membranes of epithelial cells. Transgenic mice with disabled CFTR genes have marked reductions of intestinal Cl\(^-\) and HCO\(_3\)\(^-\) secretion responses to uroguanylin (33). Thus CFTR plays an important role in cellular anion secretion responses to uroguanylin and guanylin, serving as a key substrate for phosphorylation by PKG II and/or PKA II enzymes in the intestine. A role for cGMP-dependent regulation of renal CFTR in the signal cascade pathways leading to the diuretic, natriuretic, and kaliuretic responses to uroguanylin and ST.
is unclear, although CFTR is quite clearly expressed in the kidney (50, 64). Although major alterations in renal function of CF patients do not occur, CFTR could play a role in the cellular mechanism of uroguanylin-regulated transport in the kidney as it clearly does in the intestine. The apical membrane-associate PKG II molecule, which plays an important role in the signaling cascade of enterocytes, is also expressed in the kidney, where it could have a similar function. One possibility is that OK-GC receptors for uroguanylin, PKG II receptors for cGMP, and CFTR substrate proteins for PKG II-mediated phosphorylation are localized together in the apical plasma membranes of uroguanylin/ guanylin target cells in the kidney. This signaling machinery could provide the intracellular cGMP to activate PKG II and/or PKA II to phosphorylate CFTR, which in turn influences Cl− and/or Na+ transport in the renal tubular cells that are targets for the guanylin family peptides. Whether this cellular pathway involves the activation of Cl−- and/or HCO3− secretion in renal tubules as previously elucidated for the corresponding pathway in the intestinal epithelium is presently uncertain (25, 33). Defining the renal transport mechanisms that are influenced by such a postulated cGMP-mediated signal cascade in nephron target cells is an important topic that should guide future research into the renal mechanism of action of uroguanylin.

Intrarenal Signaling Mechanism for Lymphoguanylin

Recent studies reveal that mRNAs for guanylin and/or uroguanylin and their receptor GCs are expressed in many different tissues of the opossum (15). During the course of these studies, it was noticed that a novel guanylin/uroguanylin-like mRNA is expressed in spleen and testes. This mRNA is longer than either the 0.8-kb guanylin mRNA or the 1.2-kb uroguanylin mRNA. Several cDNAs were subsequently isolated, by using a homology-PCR-cloning strategy, that contain open-reading frames encoding a polypeptide that is 84% identical to preprouroguanylin, but shares only 40% identity with preproguanylin (19). This peptide was named lymphoguanylin because the cDNAs were first isolated from spleen, and the polypeptide is clearly a new member of the guanylin peptide family. A major structural difference found in lymphoguanylin is a tyrosine (109) located at the COOH terminus of this peptide. All guanylin and uroguanylin peptides identified have cysteine at this position. Disulfide bonds connecting the first and third cysteines and linking the second to fourth cysteines in the peptide molecules were thought to be required for biological activity of guanylin and uroguanylin (9, 27). Replacement of cysteine (109) with tyrosine (109) in lymphoguanylin is a substantial difference in molecular structure that places the peptide in a new subclass within the guanylin family of bioactive peptides (Fig. 2). Lymphoguanylin is closely related to uroguanylin because both peptides have a pair of acidic residues in their NH2-terminal domains. Also shared among lymphoguanylin, uroguanylin, and ST peptides are conserved asparagine residues. Guanylin differences have aromatic amino acids at this position. Another difference is the methionine (104) substitution in lymphoguanylin for the valine (104) of uroguanylin. The signal peptide domain in preprouroguanylin is 87% identical to the NH2-terminal region of preprolymphoguanylin (19). Lymphoguanylin was synthesized and oxidized to form a single disulfide bond between cysteine (68) and cysteine (106). Synthetic lymphoguanylin with this disulfide stimulates cGMP production in T84 human intestinal cells, but its potency is less than that of either uroguanylin or guanylin. All three peptides are full agonists in the stimulation of the intestinal form of GC-C expressed in T84 cells. However, OK-GC receptors in OK cells are relatively poorly activated by synthetic lymphoguanylin compared with stimulation by guanylin and uroguanylin. Unlike guanylin and uroguanylin, which were isolated as active peptides, lymphoguanylin has been identified by molecular cloning (19). The primary structure of lymphoguanylin in vivo could be different from the synthetic peptide that was initially prepared and tested in these cGMP bioassays.

Northern assays detected lymphoguanylin mRNAs of ~1.6 kb, with the highest level of transcripts found, surprisingly, in the kidney and in both atria and ventricles of the heart. Kidney cortex has higher levels of lymphoguanylin mRNAs than renal medulla. This finding is also consistent with higher expression of OK-GC mRNAs in renal cortex compared with medulla (45). In situ receptor autoradiography also reveals a markedly higher density of receptor sites labeled with 125I-ST in cortex compared with medulla of opossum kidney (15, 21, 38). Taken together, these findings suggest that an intrarenal paracrine mechanism for lymphoguanylin-mediated activation of intracellular cGMP signaling is present in the opossum kidney that may function similar to the receptor-GC mechanism influenced by local production of guanylin and uroguanylin in the intestine. Eutherian homologues of opossum lymphoguanylin have not been described thus far, but uroguanylin and guanylin mRNAs are expressed in kidney tissue from both eutherian and metatherian types of mammals (15, 67, 71). Uroguanylin mRNAs are relatively abundant in murine kidney, and treatment of mice intravenously with uroguanylin substantially increases the urinary excretion of water, Na+ and K+ in this species (24, 67). It may be postulated that uroguanylin is made within the murine kidney and secreted locally to regulate tubular function by activation of receptor GCs located nearby in tubular cells of mice, and by analogy, in other eutherian species as well. Uroguanylin produced in the kidney may be secreted into tubular filtrate, and this peptide together with uroguanylin derived from the plasma by glomerular filtration may account for the high levels of biologically active uroguanylin excreted in urine (14, 27, 35, 37, 52). At least one class of renal receptors for the guanylin peptides, OK-GC, is localized to apical plasma membranes of proximal tubular cells; thus uroguanylin can be delivered to these receptors in vivo via tubular...
filtrate (15, 20, 21, 45). By this route, uroguanylin from the plasma and uroguanylin or lymphoguanylin made within the kidney could activate target cells and influence renal function via intracellular cGMP. Lymphoguanylin may not appear in the urine at levels similar to either uroguanylin or guanylin in the opossum because this peptide was not isolated in previous experiments that purified both uroguanylin and guanylin from urine (27). Thus lymphoguanylin could be degraded to an inactive peptide, or it may be present at levels below those required for detection, purification, and NH₂-terminal sequence analysis. A number of minor peaks of bioactive peptides were obtained when uroguanylin was first isolated from opossum urine, but these peptides were not sequenced because inadequate amounts were obtained from large batches of urine (27). Thus lymphoguanylin may be present in urine, and future experiments may identify this peptide and provide insights into the primary structure of the peptide produced in vivo. These experiments together with investigation into the possibility that a lymphoguanylin homologue occurs within eutherian mammals should provide insights into a possible intrarenal signal transduction pathway that utilizes lymphoguanylin to regulate renal cell function and contribute to Na⁺ homeostasis.

Conclusions

Discovery of cGMP signaling pathways in the kidney and identification of three novel regulatory peptides have provided new insights into cellular mechanisms for the cGMP-mediated control of kidney function by guanylin family peptides. An endocrine axis involving uroguanylin released from the GI tract into the circulation may link the digestive system with the kidney as one means of influencing body sodium balance (Fig. 5). Uroguanylin is a candidate for the intestinal natriuretic factor that was postulated to be released from the GI tract on oral ingestion of NaCl to explain the simple physiological observation that oral salt elicits much larger natriuretic responses than does intravenous salt administration. Enhanced urinary salt and water excretion after a meal also is influenced by increases in renal blood flow, GFR, and filtered load stemming from complex regulatory mechanisms involving multiple factors that include the guanylin and atriopeptin families of cGMP-regulating peptides as well as NO produced within the kidney (11, 12, 59). Because the guanylin and atriopeptins regulate cellular function via activation of membrane GCs and NO activates cytosolic GCs, it may be proposed that intracellular cGMP plays a key role in regulating salt and water excretion by the kidney in the postprandial state. An intrarenal axis for local production of guanylin peptides mirrors a similar mechanism for atriopeptins and NO as autocrine and/or paracrine regulators of kidney function. It is likely that additional cGMP-regulating peptides will be identified in the future, in view of the fact that <10% of membrane GCs that exist in the mammalian genome have been identified at the present time. Only one-half of the membrane GCs identified by molecular cloning are now classified as orphan receptors. Thus we conclude with the notion that our knowledge of the full complement of cGMP-regulating hormones and their cognate receptor GCs that exist in the body is grossly inadequate. Additional
peptide hormones, like the guanylinls and atriopeptins, will surely be identified in the future, and these peptides may have physiological actions on the kidney mediated by membrane GC signaling molecules that stimulate intracellular cGMP signaling cascades to influence renal function.

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