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Review article

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GUANYLIN AND RELATED PEPTIDES

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Guanylin and uroguanylin are short peptides homologous to heat-stable enterotoxins of *Escherichia coli* and other enteric bacteria. Guanylin and uroguanylin are synthetized from the respective prepropeptides mainly in gastrointestinal mucosa and are secreted both into intestinal lumen and into the blood. Luminally secreted peptides stimulate chloride and bicarbonate secretion in the intestine through the mechanism involving guanylate cyclase C receptor, cyclic GMP, protein kinase G and cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. Bacterial enterotoxins, which have greater potency than endogenous peptides, induce excessive fluid secretion into intestinal lumen leading to secretory diarhea. Uroguanylin is expressed mainly in enterochromaffin cells of duodenum and proximal small intestine whereas guanylin is abundant in goblet cells of colonic epithelium. Uroguanylin and guanylin increase urinary sodium and potassium excretion both as circulating hormones and as paracrine mediators produced within the kidney. Uroguanylin functions as "intestinal natriuretic hormone" which is secreted in response to oral sodium loading and maintains sodium balance during postprandial period. Plasma and urinary concentrations of guanylin and uroguanylin increase in renal failure and heart failure. Guanylin peptides possess antiproliferative activity in intestinal cells culture and their expression decreases in colonic carcinoma indicating that their deficiency may contribute to the pathogenesis of this disease.

Key words: *guanylin, uroguanylin, lymphoguanylin, heat-stable enterotoxin, guanylate cyclase C*

INTRODUCTION

Guanylin, uroguanylin and lymphoguanylin are three closely related peptides discovered initially in gastrointestinal tract which regulate electrolyte and water transport in intestinal and renal epithelia through cyclic GMP-dependent mechanism. Their discovery was associated with studies concerning the pathogenesis of bacterial diarhea. Enterotoxigenic strains of bacteria such as *Escherichia coli* and *Yersinia enterocolitica* produce heat-stable enterotoxins (STs) which stimulate chloride secretion leading to fluid

accumulation in gastrointestinal lumen and secretory diarhea. This type of diarhea is a major cause of morbidity and mortality among children, especially in developing countries and a major health problem in domestic animals. In adult humans this mechanism is responsible for "traveller's diarhea". Bacteria producing enterotoxins derive a substantial evolutionary advantage because toxin-induced diarhea promotes fecal contamination of drinking water and provides an efficient mechanism for the infection of new hosts.

It was found in 1978 that heat-stable toxin of *E. coli* stimulated cyclic GMP synthesis in intestinal epithelial cells (1, 2). Although guanylate cyclase activity was described in mammalian tissues about a decade earlier, ST was the first known regulator of this enzyme. The fact that bacterial peptide stimulated synthesis of intracellular second messenger suggested the existence of specific ST receptors in the gut, however, the investigations in this field did not develop for the next 10 years. At the end of 1980s Forte *et al.* (3, 4) found specific ST binding sites in intestine and other tissues of North American opossum (*Didelphis virginiana*). The identification of ST receptors in extragastrointestinal tissues, such as kidney, testis and airway epithelium, which are not exposed to enterotoxins has suggested that their endogenous agonist(s) must exist. The first of them, guanylin, was isolated from rat intestine in 1992 (5). Subsequently, uroguanylin was isolated from opossum urine (6). Recently the third member of this peptide family, lymphoguanylin has been identified by molecular cloning (7). Unlike two other peptides, lymphoguanylin has not been isolated from mammalian tissues so far.

The existence of guanylin and related peptides in both metatherian (marsupial) mammals such as opossum and in eutherian (placental) mammals which diverged about 130 mln years ago suggests that this peptide system appeared early in vertebrate evolution. This is also supported by identification of guanylin-like peptides and specific receptors binding ST, guanylin and uroguanylin in all mammalian species, birds, reptiles and fish investigated so far (8—12).

The aim of this review is to summarize the current knowledge about guanylin and related peptides with special emphasis on their role in water-electrolyte balance, their involvement in pathology and recent developments in this field since several excellent reviews covering the earlier literature are available (11, 13, 14—20).

STRUCTURE OF GUANYLIN PEPTIDES

Mature guanylin consists of 15 aminoacids. Rat and mouse peptides have identical aminoacid sequences and human guanylin differs from them only in one residue (21). Human uroguanylin contains 16 residues whereas rat, mouse and opossum uroguanylins consist of 15 aminoacids. Guanylin possesses

aromatic aminoacid at 9th position which makes this peptide sensitive to chymotrypsin digestion between this and next residue, whereas ST and uroguanylin are resistant to this protease because they posses asparagine at this position (22). Guanylin and uroguanylin contain two intramolecular disulfide bonds necessary for biological activity (*Fig. 1*).

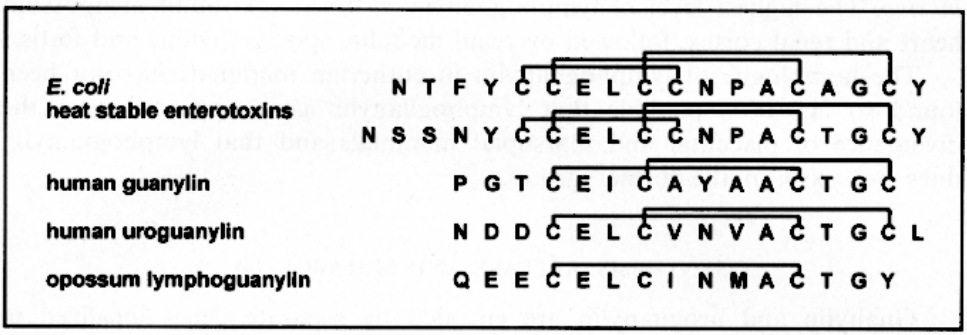

Fig. 1. Structure of guanylin and related peptides. Position of disulfide bonds is indicated. Aminoacid sequence presented using single-letter abbreviations.

E. coli ST_a, was initially purified as 18-aminoacid peptide. Later, homologous 19-aminoacid form of ST_a was characterized. Both share the same fourteen C-terminal residues and differ only in the remaining 4—5 N-terminal aminoacids which are not necessary for biological activity. ST_a contains three disulfide bridges making it more active than endogenous peptides.

Recently, the third member of guanylin peptide family has been identified by cDNA cloning from opossum spleen and has been named lymphoguanylin (7). Complementary DNA encodes 109 aminoacid preprohormone (preprolymphoguanylin) which demonstrates 84% homology with opossum preprouroguanylin and 40% homology with opossum preproguanylin. This prohormone is predicted to be cleaved to 15-residue active peptide containing only three cysteines (unlike in other peptides lymphoguanylin contains tyrosine at its C-terminus). Therefore it is possible to form only one disulfide bridge within this molecule. Synthetic lymphoguanylin containing SS bond between 4th and 12th residue is less potent agonist than either guanylin or uroguanylin (7). Lymphoguanylin analogue with cysteine to serine substitution at 7th position has been synthetized. This substitution eliminates the possibility of alternative SS bond alignments and facilitates the formation of single SS bridge and proper folding of the peptide. The potency and efficacy of this analogue is comparable to uroguanylin. Thus, the family of guanylin-related peptides can be divided into three subfamilies containing three (bacterial enterotoxins), two (guanylin and uroguanylin) or one (lymphoguanylin) disulfide bonds. Lymphoguanylin is, however, more similar to uroguanylin than to guanylin because: 1) aminoacid sequence of its prohormone demonstrates higher degree

of homology to the former peptide, 2) possesses two acidic glutamate residues near its N-terminus, 3) contains internal asparagine at the position occupied by aromatic aminoacid in guanylin molecule, which makes lymphoguanylin resistant to chymotrypsin digestion. Until now lymphoguanylin has not been isolated from tissues, therefore it is not clear whether this peptide is synthetized *in vivo*. The highest level of lymphoguanylin mRNA was found in opossum heart and renal cortex, followed by renal medulla, spleen, thymus and testis.

The homologue of lymphoguanylin in eutherian mammals has not been found so far. It is possible that lymphoguanylin gene appeared after the divergence of placental and marsupial mammals and that lymphoguanylin does not exist in the former group.

SYNTHESIS, RELEASE AND METABOLISM

Guanylin and uroguanylin are encoded by separate genes localized in humans on chromosome 1 p34-p35 (23, 24). Both genes have similar structure, they consist of three exons separated by two introns. Guanylin and uroguanylin are initially synthetized in the form of prepropeptides (*Fig. 2*). Human preproguanylin consists of 115 aminoacids. Cleavage of 21 N-terminal residues leads to formation of 94-aminoacid proguanylin (25, 26). Human preprouroguanylin contains 112 aminoacids including 26-aminoacid signalling peptide and 86-residue prouroguanylin sequence (24, 27). *In vitro* prouroguanylin is converted to uroguanylin by chymotrypsin, this enzyme may be involved in uroguanylin processing also *in vivo* because uroguanylin is produced in large amounts in duodenum where chymotrypsin is delivered with pancreatic juice.

Undoubtedly, the main source of guanylin and uroguanylin is gastrointestinal tract which contains the highest levels of respective mRNAs and mature proteins among all tissues (26, 28, 29). Intestinal mucosa is probably the main or the only source of circulating peptides. Rat guanylin mRNA was also found in adrenal gland, kidney and uterus/oviduct (28), whereas uroguanylin transcript is present in lung, pancreas and kidney (29, 30). It seems that extragastrointestinal guanylin/uroguanylin play only local, paracrine role and have little or no contribution to circulating pool of peptides.

Peptides produced in intestinal mucosa are released both apically, into the intestinal lumen and basolaterally toward blood vessels (13). Studies *in vitro* demonstrate that isolated rat colonic mucosa secretes guanylin and proguanylin into the apical compartment and the amounts of prohormone are about 15-fold greater than of bioactive peptide. Guanylin but not proguanylin is also released toward basolateral side but its amount is about 6-fold lower than secreted apically (31). Isolated vascularly perfused rat colon secretes guanylin both into intestinal lumen and much lower amounts into portal effluent (32).

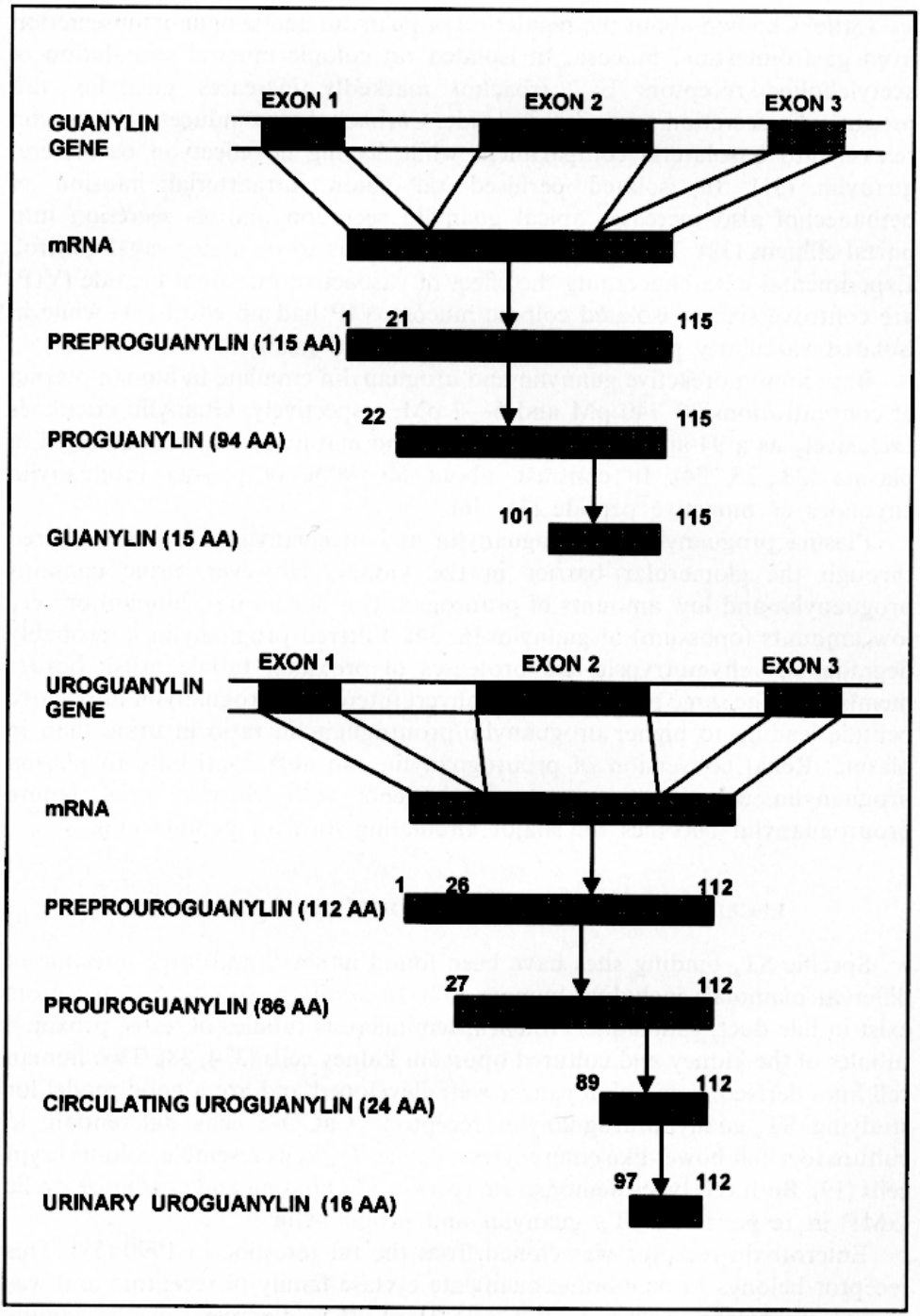

Fig. 2. Synthesis of human guanylin (top) and uroguanylin (bottom).

Little is known about the regulation of guanylin and uroguanylin secretion from gastrointestinal mucosa. In isolated rat colonic mucosa stimulation of acetylcholine receptors by carbachol markedly increases guanylin and proguanylin secretion toward apical side. Carbachol also induces proguanylin release into basolateral compartment while having no effect on basolateral guanylin (31). In isolated perfused rat colon intraarterial infusion of bethanechol also increases apical guanylin secretion and its secretion into portal effluent (32). Thus guanylin secretion seems to be under vagal control. Experimental data concerning the effect of vasoactive intestinal peptide (VIP) are controversial, in isolated colonic mucosa VIP had no effect (31) while in isolated vascularly perfused rat colon stimulated guanylin secretion (32).

Both immunoreactive guanylin and uroguanylin circulate in human plasma at concentrations 30—40 pM and 5—7 pM, respectively. Guanylin circulates exclusively as a 94-aminoacid prohormone, no mature guanylin is detected in plasma (23, 33, 34). In contrast, about 60—90% of plasma uroguanylin circulates as bioactive peptide (35, 36).

Plasma proguanylin, prouroguanylin and uroguanylin are freely filtered through the glomerular barrier in the kidney. However, urine contains uroguanylin and low amounts of prouroguanylin but no (rat, human) or very low amounts (opossum) of guanylin (6, 34). Filtered proguanylin is probably degraded by chymotrypsin-like proteases of proximal tubule brush border membrane. The same proteases may convert filtered prouroguanylin into active peptide leading to higher uroguanylin/prouroguanylin ratio in urine than in plasma. Renal conversion of prouroguanylin can also contribute to plasma uroguanylin concentration since in patients with chronic renal failure prouroguanylin becomes the major circulating form of peptide (36).

RECEPTORS AND SIGNAL TRANSDUCTION MECHANISMS

Specific ST_a binding sites have been found in small and large intestine of different mammals including humans (37). In addition, specific ST_a receptors exist in bile duct, gallbladder, trachea, seminiferous tubules of testis, proximal tubules of the kidney and cultured opossum kidney cells (3, 4, 38). Two human cell lines derived from colon cancer were developed and are a good model for studying ST_a/guanylin/uroguanylin receptors. CaCO-2 cells differentiate in culture to small bowel-like enterocytes whereas T_{84} cells resemble colonic crypt cells (19). Both cell types demonstrate specific ST_a binding and synthetize cyclic GMP in response to ST_a, guanylin and uroguanylin.

Enterotoxin receptor was cloned from the rat intestine in 1990 (39). This receptor belongs to membrane guanylate cyclase family of receptors and was named guanylate cyclase C (GC-C). Two other members of the family, guanylate cyclase A (GC-A) and B (GC-B) had been identified previously and

bind specifically atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP), respectively. Membrane guanylate cyclases are single-chain peptides with four domains: N-terminal extracellular domain responsible for ligand binding, short hydrophobic transmembrane domain, and two intracellular domains: kinase-like domain and C-terminal catalytic domain which transforms guanosine triphosphate to cyclic GMP. Aminoacid sequence of extracellular domain differs significantly within this receptor family and these differences are responsible for ligand specificity. Other parts of receptor demonstrate higher degree of homology. However, the degree of homology between GC-A and GC-B is higher than between each of these receptors and GC-C. GC-A and GC-B share about 40% homology within extracellular domain and about 90% homology within intracellular domains whereas the degree of homology between GC-A/GC-B and GC-C within these domains are 10% and 55%, respectively. Consequently, while GC-A can bind CNP but with lower affinity than ANP and GC-B can bind ANP with lower affinity than CNP, no cross-activation between cardiac natriuretic peptide receptors and guanylin receptors was observed. GC-C differs from GC-A and GC-B also in additional C-terminal tail containing about 70 aminoacids which is not found in cardiac peptide receptors. Human GC-C was subsequently cloned from intestine (40), T_{84} cells (41) and from CaCO-2 cells (42). Comparison of primary structure of human and rat GC-C reveals 71% homology within extracellular domain and 91% homology within intracellular domains (*Fig. 3*). Thus, between-species degree of homology of GC-C is higher than within-species homology between GC-C and natriuretic peptide receptors. Therefore, human guanylins act on rat receptors and *vice versa*. The rank order of GC-C ligands potency in most experiments is ST_a > uroguanylin > guanylin.

GC-C mRNA is expressed not only in gastrointestinal mucosa but also in adrenals, brain, neonatal and regenerating liver, placenta, testes, airway epithelium, spleen, thymus and lymph nodes (43—45). However, mice lacking GC-C despite having largely impaired intestinal secretory response to ST_a are otherwise healthy (46). It indicates that either guanylin peptides act also on other receptors or the lack of guanylin signalling is compensated effectively by other regulatory mechanisms. On the other hand, the fact that GC-C receptor which mediates severe and potentially life-threatening diarhea was not eliminated during long vertebrate evolution suggests its important physiological functions.

It is possible that GC-C is not the only guanylin receptor. Several ST_a binding proteins with different molecular weights and different ligand affinities have been described in intestinal epithelial cells (47). It is not clear whether these proteins represent different splice variants of GC-C transcript, proteolytic processing of receptor protein, different degree of glycosylation (48) or even the products of separate gene(s). The attractive but unresolved question is also

whether the receptors specific for each of guanylin peptide exist, analogously to natriuretic peptide receptors.

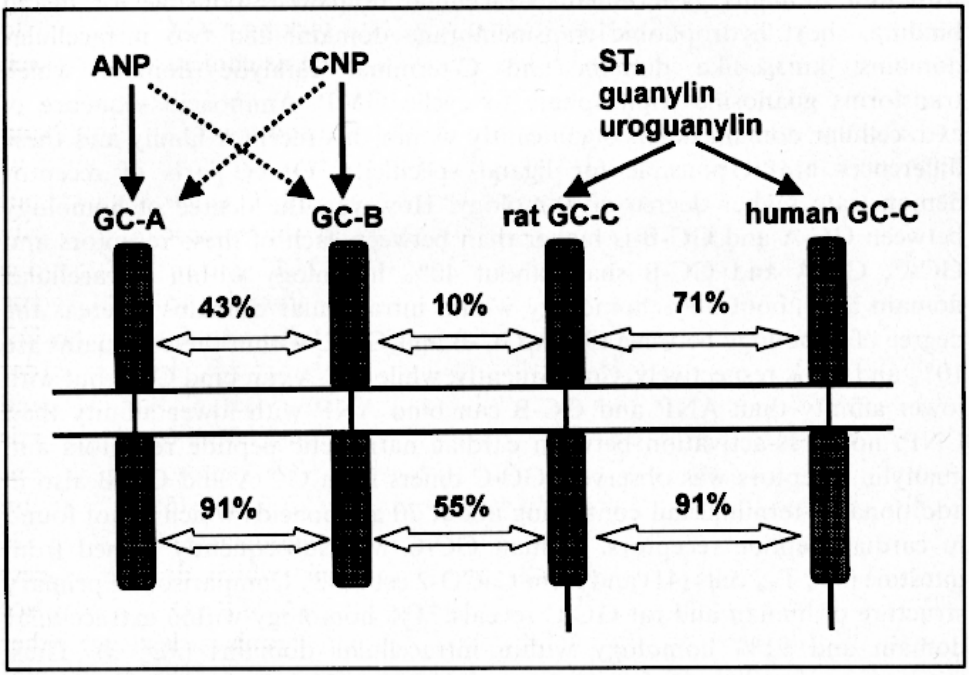

Fig. 3. Structure and sequence homology within membrane-bound guanylate cyclase receptors family. Guanylate cyclase A (GC-A) and guanylate cyclase B (GC-B) of the rat as well as rat and human GC-C are demonstrated.

GUANYLIN PEPTIDES IN THE GASTROINTESTINAL TRACT

Gastrointestinal tract is a primary site of guanylin peptides synthesis and their main target tissue. The well evidenced hypothesis states that guanylin/uroguanylin are released apically by intestinal epithelial cells, act on receptors in apical membrane and stimulate chloride and, in some segments, bicarbonate secretion. Ion movement causes osmotic shift of water leading to increased fluid accumulation within the gut lumen. Thus guanylin peptides act locally in autocrine ("luminocrine") and paracrine manner. Controversies exist about the precise cellular sources of these peptides, their mechanisms of action and their role in physiology of different intestine segments.

Secretory effect of guanylin and related peptides was observed in studies performed on cultured intestinal cells (5, 6, 49, on isolated tissues (25, 50 and *in vivo* (2, 51, 52). In addition to stimulating fluid secretion, guanylin peptides increase luminal bicarbonate secretion and elevate luminal pH, especially in duodenum.

The effect of guanylin peptides in the intestine is mediated by GC-C and cGMP. ST_a, guanylin and uroguanylin elevate cGMP concentration in intestinal epithelial cells (5, 6, 50, 53). The secretory effect is mimicked by membrane-permeable cGMP analogues (2). Mice lacking GC-C receptor do not respond to ST_a whereas other secretagogues such as cholera toxin as well as cGMP act normally (46, 54).

Intestinal chloride secretion is a vectoral transport of Cl^- ions from basolateral to luminal compartment and consists of two steps: chloride entry to the enterocyte through the basolateral membrane and its subsequent output across luminal surface (35) (*Fig. 4*). Chloride enters the enterocyte primarily through basolateral bumetanide-sensitive Na^+, K^+, $2Cl^-$ cotransporter and is secreted into intestinal lumen through cystic fibrosis transmembrane conductance regulator (CFTR). CFTR may also transport HCO_3^- Alternatively, bicarbonate secretion can occur through brush border membrane Cl^-/HCO_3^- exchanger. CFTR is the primary target of cGMP and also of cAMP mediated secretory response. Secretory effect of guanylin is abolished by CFTR blockers (56, 57). Anti-CFTR antibodies prevent cGMP-induced increase in chloride secretion in CaCO-2 cells (58). Reduction of CFTR expression in T_{84} cells with antisense oligonucleotides to CFTR mRNA diminishes ST_a-induced Cl^- secretion. Cells which do not express CFTR do not respond to cGMP until transfected with CFTR cDNA (59). Mice lacking CFTR gene have virtually no response to agonists elevating either cGMP or cAMP concentration (57. 60). Heterozygous $CFTR^{+/-}$ mice demonstrate reduction of ST_a secretory effect by about 50% (61). Because both cGMP and cAMP secretory responses are impaired, CFTR null mice (unlike GC-C null mice) suffer from severe obstruction which results in high mortality (62). Finally, patients with cystic fibrosis are resistant to ST_a-induced diarhea (63). It is suggested that the involvement of CFTR in enterotoxin-induced diarhea may explain high incidence of cystic fibrosis gene in population. According to this hypothesis, CF heterozygotes are less sensitive to bacterial diarhea and therefore CF gene was not eliminated during the evolution.

It seems that the main signalling cascade, at least in the small intestine is stimulation of protein kinase G II (PKGII) by cGMP and subsequent phosphorylation of CFTR by this kinase (64, 65). In $PKG^{-/-}$ mice the secretory response of the intestine to ST_a is markedly reduced in both *in vivo* and *in vitro* studies (66). Like GC-C null mice, mice lacking PKGII are otherwise healthy indicating that other transduction pathways, such as cAMP-protein kinase A, may compensate cGMP-signalling deficit. Mechanisms other than PKG may also mediate the effect of guanylin peptides, especially in colon where PKGII expression is low (67). First, high concentrations of cGMP may cross-activate protein kinase A. This mechanism is suggested by studies on cultured T_{84} and CaCO-2 cells in which the

secretory effect of guanylin and cGMP analogues is prevented by inhibitors of protein kinase A (58, 59, 68). Another possible mechanism is inhibition of cAMP-degrading phosphodiesterase by cGMP leading to elevation of this nucleotide and stimulation of PKA (67, 69) (*Fig. 4*).

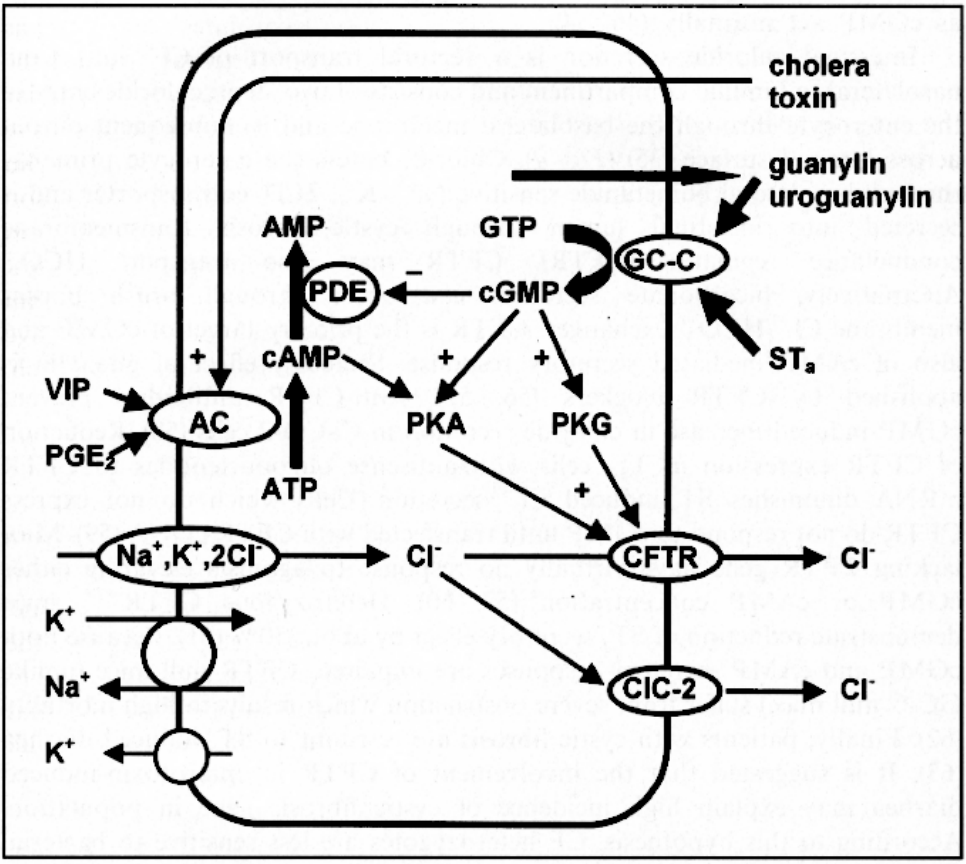

Fig. 4. The mechanism of chloride secretion by enterocyte and its regulation by guanylin peptides, heat-stable enterotoxins and heat-labile enterotoxins (see text for details). ST_a — heat-stable enterotoxin, GC-C — guanylate cyclase, C, PKG — protein kinase G, PKA — protein kinase A, CFTR — cystic fibrosis transmembrane conductance regulator, CIC-2 — chloride channel, PDE — phosphodiesterase, AC — adenylate cyclase, VIP — vasoactive intestinal peptide, PGE_2 prostaglandin E_2.

Heat-stable toxins of *E. coli* and other bacteria bind to GC-C receptors with higher affinity than endogenous peptides and act as their molecular mimics stimulating chloride secretion through the same mechanism and leading to excessive fluid accumulation within intestinal lumen and secretory diarhea. In contrast, cholera toxin activates adenylate cyclase and elevates

intracellular cyclic AMP concentration stimulating protein kinase A, which phosphorylates CFTR — the common final pathway for both cGMP and cAMP-mediated secretory responses (*Fig. 4*). Thus, cholera toxin evokes intestinal secretion in the mechanism similar to vasoactive intestinal peptide (VIP) and prostaglandin E_2 which also stimulate adenylate cyclase (55).

The distribution of guanylin and uroguanylin in gastrointestinal tract was studied extensively. Three points need to be addressed: 1) distribution along the longitudinal axis, 2) distribution along the villus/crypt axis, and 3) cellular sources of peptides. With regard to first point, guanylin and uroguanylin have complementary, although to some extent overlapping distribution. Guanylin mRNA is expressed throughout the intestine from duodenum to distal colon but maximal expression is observed in colon and lowest in duodenum, both in rats (21, 30, 53) and in humans (25). The level of uroguanylin mRNA is highest in duodenum and jejunum and decreases progressively in the ileum and cecum (43, 53, 70, 71). The similar results were obtained when the level of respective prohormone proteins was measured (72). Guanylin is not found in the stomach, whereas uroguanylin mRNA is very low here (53).

Cellular distribution of guanylin peptides has been studied by immunohistochemistry and in situ hybridization. Uroguanylin is mainly produced by enterochromaffin cells of the small intestine — a kind of enteroendocrine cells which also produce serotonin (71). These cells secrete their mediators both in apical and basolateral directions which is consistent with dual function of uroguanylin as luminocrine and circulating hormone. Uroguanylin is also produced by enterochromaffin-like (ECL) cells in the stomach, which regulate HCl secretion by releasing histamine in response to gastrin and acetylcholine (73). Immunohistochemical studies suggest that guanylin is produced mainly in mucus-producing goblet cells; most but not all goblet cells also express guanylin (74). Intravenously administered guanylin stimulates mucus secretion by crypt goblet cells (75).

The distribution of guanylin/uroguanylin receptors in the gastrointestinal tract was also investigated. Specific ST_a binding sites are present in apical membranes of intestinal epithelium from duodenum to distal colon. In humans the amount of receptors is highest in proximal small intestine and decreases progressively toward distal direction (9). The expression of guanylin receptors in intestine is highest in newborns and neonates which explains their special sensitivity to bacterial diarhea (76).

Intestinal expression of uroguanylin and to a lesser extent, guanylin demonstrates circadian rhythm with peak levels during the dark (active) photoperiod in the rat. Maximal changes are observed in ileum, less pronounced in jejunum and colon. The level of GC-C mRNA is also maximal during the dark phase (77). It is not clear whether these changes result in changes of peptides secretion and what is their mechanism, in particular

whether they are caused by photoperiod or rhythmicity of food intake. In humans urinary uroguanylin excretion is highest during early morning (78).

The activity of guanylin and uroguanylin is pH-dependent. In cultured T_{84} cells guanylin is 10-fold more potent in elevating cGMP at pH 8 than at pH 5, whereas uroguanylin is more potent in acidic than in alkaline conditions. In alkaline environment guanylin is more potent than uroguanylin and the opposite is observed in acidic conditions (79). ST_a is slightly more potent in acidic than in alkaline pH but the difference is not so striking as in the case of endogenous peptides. ST_a is, however, more potent than either guanylin or uroguanylin in both acidic and alkaline conditions. These relationships correspond with peptides distribution along gastrointestinal tract. Uroguanylin, which is highly expressed in duodenum, is probably the main regulatory peptide in this segment which receives acidic chyme from the stomach, and by stimulating HCO_3^- secretion may contribute to neutralization of luminal fluid. In addition, uroguanylin is resistant to chymotrypsin present in large amounts in duodenum. When one moves distally toward alkaline environment of distal small intestine and colon, guanylin becomes more active.

In addition to regulating intestinal secretion, guanylin peptides may be also involved in the regulation of gut motility. Guanylin inhibits cholecystokinin--induced contractility of guinea pig cecal muscular layer in a dose-dependent manner and does so at concentrations about 10-fold lower than required for the regulation of chloride secretion (80).

GUANYLIN PEPTIDES, THE KIDNEY AND WATER-ELECTROLYTE BALANCE

The role of guanylin peptides is not restricted to gastrointestinal tract but they may also function as circulating hormones involved in the regulation of water-electrolyte metabolism. Intravenous administration of ST_a, guanylin or uroguanylin into mice dose-dependently increases urinary sodium, potassium and water excretion without changes in glomerular filtration rate and renal blood flow (51, 81). Natriuretic effect of guanylin is less pronounced than of two remaining peptides but guanylin induces comparable increase in kaliuresis. In isolated perfused rat kidney, uroguanylin increases urine output, natriuresis and kaliuresis, glomerular filtration increases only after the high doses (82). The effect of guanylin is dose-dependent: lowest doses increase potassium excretion selectively, moderate doses increase also sodium excretion and high doses increase glomerular filtration rate (82). ST_a is about 10-fold more potent than uroguanylin in isolated kidney preparation (82, 83). Lymphoguanylin was also tested in this model and found to increase slightly urine output and natriuresis as a result of decrease in proximal tubular sodium and chloride reabsorption. Lymphoguanylin had only slight effect on potassium excretion (84). Synthetic form of lymphoguanylin containing only two cysteine residues is much more potent than 3-cysteine lymphoguanylin, although both possess disulfide bridge

between the same residues. Its potency in isolated rat kidney is comparable to uroguanylin (85).

The mechanism of renal action of guanylin peptides has not been studied so extensively as in the intestine and most hypotheses arose from the extrapolation of gastrointestinal data. The results presented above suggest that guanylin peptides inhibit tubular sodium and/or potassium reabsorption or stimulate potassium secretion. In general, the effect of these peptides on specific nephron segments has not been investigated so far and therefore the precise sites of action along the nephron and involved receptors are not elucidated. Autoradiographic studies localize ST_a binding sites to apical membranes of proximal tubule of opossum (3, 4). In the rat, specific guanylin binding sites have been observed in the apical membranes of proximal tubules and, unlike in opossum, also in basolateral side of thin limb of Henle's loop and collecting ducts (86). ST_a increases urinary cGMP excretion, cGMP synthesis in kidney slices of opossum and in cultured opossum kidney cells (3, 4). However, initial Northern blotting studies demonstrated no or very low level of GC-C mRNA in the kidney of eutherian mammals (39). Moreover, in GC-$C^{-/-}$ mice intestinal secretion in response to ST_a is markedly impaired whereas natriuretic response is normal (81, 87). These results suggested an alternate, GC-C independent signalling pathway in the kidney. In 1999 the second guanylate cyclase C receptor was cloned from opossum kidney cells and was named OK-GC (opossum kidney guanylate cyclase) (88). This receptor is distinct from GC-C but its aminoacid sequence shares 59%, 71%, 89% and 94% homology with the latter within extracellular, transmembrane, kinase-like and catalytic domains, respectively. Its mRNA is present in large amounts in the renal cortex and intestinal mucosa, lower expression is observed in renal medulla, heart and adrenal medulla. It was suggested that OK-GC was a specific renal receptor for guanylins. However, its homologue in eutherian mammals has not been found so far and the homologue of GC-C has not been identified in opossum, therefore it is possible that OK-GC is simply the species-specific opossum form of GC-C and not the organ-specific receptor. Studies performed on isolated rat tubular segments using RT-PCR method demonstrate GC-C mRNA with highest expression in cortical collecting duct, slightly lower expression in proximal convoluted tubules, medullary thick ascending limb, medullary collecting duct and thin limb, lower level in glomeruli and very low in cortical thick ascending limb and papillary collecting duct (89). In contrast to these data, guanylin and uroguanylin have no effect on electrolyte transport and cGMP level in principal cells of rat cortical collecting duct (90). GC-C mRNA was also identified in the medulla of human kidney (36). Thus, the question what receptor mediates renal effect of guanylin remains open. At present it may be concluded that guanylins act through GC-C and possibly via some additional cGMP-linked or even cGMP-independent receptors.

The possibility of specific renal receptors for each peptide should also be considered and is suggested by several facts. First, guanylin, uroguanylin and lymphoguanylin differ in natriuretic/kaliuretic effect ratio as described above. Second, atrial natriuretic peptide administered before guanylin enhances its natriuretic effect whereas ameliorates natriuretic activity of uroguanylin (91). Third, guanylin and uroguanylin have the opposite effects on freshly isolated mouse cortical collecting duct cells: guanylin depolarizes whereas uroguanylin hyperpolarizes these cells. (92). Interestingly, GC-C mRNA was not found in these cells. However, one should also take into account the possibility that both peptides act *in vivo* through the same receptor but with different potencies in different nephron segments due to differing pH values, as described in the intestine.

Intracellular mechanism of action of guanylins on renal tubular transport is not clear. The mechanism analogous to operating in the intestine, involving cGMP, protein kinase G and CFTR may operate since both PKG and CFTR are expressed in the nephron (89, 93). However, the distribution and functional role of CFTR in the kidney is not clear since cystic fibrosis is not associated with renal dysfunction. In addition to CFTR, other chloride channels such as CIC-5 are expressed in brush border membranes of proximal convoluted tubule and in apical membranes of collecting duct intercalated cells and may be involved in chloride secretion (94), chloride channel stimulated by cGMP has been described in proximal tubules (95). It is possible that guanylin peptides regulate tubular sodium reabsorption rather than chloride secretion. Guanylin and uroguanylin decrease the expression of Na^+, K^+-ATPase γ subunit in the mouse kidney (81). Atrial natriuretic peptide and nitric oxide which also elevate intracellular cGMP decrease Na^+, K^+-ATPase activity through PKG--dependent mechanism (96).

Guanylin peptides affect renal function as circulating hormones, but are also produced in the kidney and act locally in auto- and paracrine fashion. As described earlier, circulating proguanylin, prouroguanylin and uroguanylin are freely filtered through glomerular barrier. Guanylin is not found in urine due to degradation of peptide by proximal tubule brush border proteases, this mechanism may protect renal receptor from this peptide circulating at high concentration leaving them ready to bind uroguanylin, which is suggested to be the main renal hormone in this family. Thus, filtered uroguanylin, supplemented by uroguanylin liberated from filtered prohormone by proximal tubule brush border proteases, is likely to bind receptors found in apical membranes of proximal tubule (and possibly other nephron segments) thereby affecting tubular transport. However, the evidence of local intrarenal production of peptides also exist. Guanylin mRNA is found in all tubule segments of the rat kidney with highest expression in medullary collecting duct followed by thin limb and proximal convoluted tubule, intermediate levels are

expressed in medullary and cortical thick ascending limb and cortical collecting duct and lowest in papillary collecting duct. Uroguanylin mRNA is highest in medullary collecting duct, intermediate in thin limb, medullary and cortical thick ascending limb and cortical collecting duct, low in proximal convoluted tubule and virtually undetectable in proximal straight tubule, distal convoluted tubule and papillary collecting duct (89). Immunoreactive uroguanylin has been localized in distal tubules of the rat kidney but it is not clear whether this reflects local synthesis of the peptide or only its internalization and catabolism since no prouroguanylin was found in these cells (97). Immunohistochemical studies demonstrate uroguanylin also in proximal and distal tubules as well as in collecting ducts of the human kidney (98). Lymphoguanylin mRNA was also found in the kidney (7) but lymphoguanylin is not found in urine. Thus, it is likely that locally produced guanylin peptides affect ion transport in tubule segments in which the role of cGMP (triggered by ANP and NO) in transport regulation is well documented, such as proximal tubule, thick ascending limb and medullary collecting duct, but also in segments such as thin limb in which no regulatory mechanism has been previously described. The example of such dual endocrine/paracrine regulation exists in natriuretic peptide system; apart from ANP produced in the heart, its N-terminally extended form, urodilatin is synthetized within the kidney. Some of similarities and differences between natriuretic peptides and guanylins are depicted in *Table 1*.

Table 1. The major similarities and differences between natriuretic peptide and guanylin peptide systems.

	Natriuretic peptides	Guanylin peptides
Members of the family	ANP BNP CNP	guanylin uroguanylin lymphoguanylin
Major site of synthesis	Heart (ANP, BNP)	Intestinal mucosa
Intrarenal production	+ (urodilatin)	+
Regulation by sodium balance	+	+
Receptors	NPR-A (GC-A) NPR-B (GC-B) NPR-C	GC-C ?specific renal receptor
Major second messenger	cGMP	cGMP
Natriuretic activity	+	+
Inhibition of aldosterone secretion	+	+
Regulation of intestinal secretion	−	+
Expression within the lymphatic system	+	+
Antiproliferative activity	+ (vascular smooth muscle cells)	+ (intestinal epithelial cells)
Vasodilating activity	+	?

The abbreviations used are: ANP — atrial natriretic peptide, BNP — brain natriuretic peptide, CNP — C-type natiuretic peptide, NPR-A — natriuretic peptide receptor A (guanylate cyclase A, GC-A), NPR-B — natriuretic peptide receptor B (guanylate cyclase B, GC-B), GC-C — guanylate cylase C.

To serve as the effective regulators of sodium metabolism, guanylin peptides should be regulated by sodium balance. Some facts supporting such regulation have been presented. High-sodium diet increases urinary uroguanylin and cGMP excretion and natriuresis correlates with urinary uroguanylin and cGMP in humans (36). Low sodium diet administered to the rat for 1 week decreases the expression of guanylin and its receptors in distal colon whereas high sodium diet has no effect (99). Urinary uroguanylin excretion is highest in early daytime hours, the period commonly including meals containing sodium, and lowest at night when no sodium is delivered (78). It is not clear how the intestine senses sodium balance but it is likely that local sodium concentration is more important than volume status, at least in the short run. Oral administration of 2% saline to normal humans increases urinary uroguanylin excretion after one hour whereas intravenous administration of the same NaCl volume has no effect (100). Perfusion of isolated rat intestine with saline stimulates guanylin and uroguanylin secretion into luminal perfusate and mesenteric vein (101). However, states characterized by chronic hypervolemia such as congestive heart failure (78) and chronic renal failure (36) are also associated with elevated plasma and urinary guanylin peptides. Whether sodium balance affects only intestinal or also renal guanylin system is not clear. Because both uroguanylin (102) and lymphoguanylin (7) mRNAs are expressed in the heart at least in opossum, the interesting possibility should be considered that one or both are secreted together with ANP in response to sodium overload and act synergistically on renal sodium excretion (14). If so, cardiac guanylins could partially contribute to natriuretic effect of atrial extracts described originally by deBold *et al.* in 1981 (103). In fact, it was noticed that factors stimulating endogenous ANP release induced greater natriuresis than exogenous ANP suggesting the existence of an additional factor released by cardiac tissue (104). To test this hypothesis, the effect of volume status on cardiac uroguanylin expression and the contribution of cardiac uroguanylin to circulating pool of the peptide should be investigated. Preliminary studies show the presence of uroguanylin mRNA in atria and ventricles of humans with congestive heart failure (78).

It was found in 1970s that oral salt loading increased greater natriuresis than intravenous administration of comparable sodium amounts (105—107). Natriuresis after salty meal is essential for maintaining sodium balance, in particular to prevent marked postprandial hypervolemia. The authors suggested that some humoral factor is secreted by the intestine in response to oral sodium and regulates renal sodium excretion. Uroguanylin is a good candidate for "intestinal natriuretic hormone" because: 1) is synthetized in intestinal mucosa, 2) is secreted into the bloodstream, 3) has natriuretic effect in the kidney, 4) its production is regulated by sodium balance.

In the rat GC-C mRNA is absent or very low in the liver during adult life (28, 44) but is evidently present in fetal and neonatal liver (76). The level of hepatic GC-C mRNA and radiolabelled ST_a binding decreases progressively after birth and reaches virtually undetectable levels by 3—4 postnatal week. Since guanylin is not synthetized in the liver, hepatic GC-C receptors must be activated by circulating hormones or another, yet unidentified ligand. Hepatic GC-C mRNA is upregulated after partial hepatectomy and during liver regeneration after partial hepatic necrosis induced in the rat by toxic agents such as carbon tetrachloride and tarpentine (44, 108). These data suggest that GC-C-is involved in liver growth and regeneration but the precise role for guanylin peptides in this process is not clear. Because cGMP has potent antiproliferative effect in different cells including hepatocytes, it is possible that GC-C signalling contributes to self-limitation of hepatocytes proliferation after liver injury.

Guanylin is produced by nonciliated secretory cells of airway epithelium of bovine, guinea pig, and rat (109) and GC-C receptor is also present in airway epithelial cells (28, 38, 45). Guanylin stimulates chloride secretion by cultured human bronchial epithelial cells. Interestingly, the effect is not mediated by CFTR because is synergistic with CFTR-regulating cAMP agonists and is sensitive to outwardly rectifying chloride channel (but not to CFTR) blockers (110). This observation opens the possibility of guanylin peptides application in therapy of mucoviscidosis. Uroguanylin and guanylin cause relaxation of guinea-pig tracheal smooth muscle cells, prevent antigen-induced bronchoconstriction and reduce microvascular leakage in trachea, bronchi and intrapulmonary airways in sensitized animals (111). Intravenous or inhaled uroguanylin is also effective against bronchoconstriction induced by leukotriene C4 (112).

Guanylin mRNA has been detected in chromaffin cells of rat adrenal medulla, especially in norepinephrine secreting cells. Whether guanylin is secreted together with catecholamines and whether adrenal medulla contributes to circulating guanylin remains to be established (113). In the adrenal cortex, guanylin binding sites have been demonstrated in zona glomerulosa but not in other layers. Guanylin has no effect on ACTH-regulated steroid secretion but dose-dependently inhibits aldosterone secretion by zona glomerulosa cells in response to angiotensin II and potassium.

Chronic zinc deficiency in rats is associated with increased intestinal uroguanylin mRNA expression and increased prouroguanylin concentration. The number of prouroguanylin expressing cells is increased and the cells are scattered throughout the villus in zinc-deficient rats whereas in zinc-adequate rats these cells are restricted to tips of villi (114, 115). It is suggested that overproduction of uroguanylin may contribute to diarhea often accompanying zinc deficiency.

In addition to crucial role of GC-C receptors in infectious diarhea, guanylin peptide system may be also involved in the pathogenesis of non-bacterial diarhea. Plasma concentration of guanylin is increased in patients with symptomatic carcinoid tumor and immunoreactive guanylin is present in carcinoid tissue suggesting that oversecretion of this hormone is partially responsible for carcinoid-associated diarhea. In contrast, plasma guanylin in patients with Crohn's disease and ulcerative colitis is normal (116).

Guanylin peptide system is markedly affected in renal diseases. In chronic renal failure, plasma and urinary uroguanylin increase proportionally to disease severity, correlate with plasma creatinine and are the highest in patients on hemodialysis (36). In addition, whereas in healthy persons mature uroguanylin is the only peptide excreted in urine and is more available than its precursor in plasma, in renal failure patients prouroguanylin becomes the dominating peptide both in plasma and in urine (35). Plasma concentration of proguanylin is also markedly elevated in uremic patients (34, 117). Plasma guanylin peptides are filtered through highly-permeable hemodialysis membranes; their plasma concentration decreases during dialysis and they appear in hemofiltrate (117, 118). Increase in guanylin peptides in renal failure results probably from the combination of increased secretion due to chronic volume overload and decreased renal clearance. Nephrotic syndrome is accompanied by high plasma and low urinary uroguanylin (119).

The most intriguing findings, however, concern the involvement of guanylin peptides and their receptors in the pathogenesis of intestinal neoplasia. Guanylin and uroguanylin mRNAs are almost absent in human colorectal carcinoma in contrast to adjacent normal colonic epithelial cells (120–122). In colonic polyps, the expression of guanylin peptides is markedly reduced but not completely absent, i.e. intermediate between normal tissue and adenocarcinoma. In contrast, GC-C receptors are normally expressed in adenocarcinoma cells (123). Uroguanylin dose-dependently inhibits proliferation of cultured T_{84} and CaCO-2 cells and induces their apoptosis (121). Orally administered uroguanylin decreases the number and diameter of colonic polyps in a mouse model. Thus, guanylin peptides are probably involved in the regulation of intestinal epithelial cells turnover and their

deficiency may contribute significantly to the development of intestinal neoplasia. In addition, administration of GC-C agonists is a potentially promising therapy in colonic carcinoma. It is also suggested that chronic or repeated infections with enterotoxin-producing bacteria may protect the host against colorectal carcinoma and contributes to low incidence of colorectal cancer in developing countries (121). Expression of GC-C mRNA in lymph nodes which do not contain histologically confirmed metastases may predict disease recurrence in patients with colorectal carcinoma (124). GC-C mRNA has been also detected in plasma in 80% of patients with colonic carcinoma suggesting possible diagnostic application (125).

CONCLUSIONS

The discovery of guanylin peptides markedly expanded our knowledge about cGMP-mediated regulatory mechanisms. Their involvement in gastrointestinal and water-electrolyte regulation is well documented, although many questions are still open. The role of these peptides in lymphatic, respiratory, reproductive and central nervous system is much less clear. Guanylin peptide family is affected in several disease states and first possibilities of diagnostic and therapeutic applications have appeared recently. Much work is still to be done. Are there specific receptors for each guanylin peptide and if not why two (or three) peptides with similar activity exist? Is lymphoguanylin synthetized *in vivo*, especially in eutherian mammals? Is there any effect of guanylins on blood vessels in which cGMP has well known vasodilating activity? These are only the examples of questions which need to be addressed in the future.

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