The uroguanylin system and human disease

Hazim RAHBI*, Hafid NARAYAN*, Donald J. L. JONES† and Leong L. NG*‡
*Department of Cardiovascular Sciences, University of Leicester, Leicester Royal Infirmary, Leicester, U.K., †Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, U.K., and ‡Leicester National Institute for Health Research Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, U.K.

ABSTRACT

The uroguanylin system is a newly discovered endocrine/paracrine system that may have a role in the regulation of salt balance, appetite and gut health. The precursor pro-uroguanylin is predominantly synthesized in the gut, although there may be other sites of synthesis, including the kidney tubules. Products from pro-uroguanylin may mediate natriuresis following oral consumption of a salt load through both GC-C (guanylate cyclase C)-dependent and -independent mechanisms, and recent evidence suggests a role in appetite regulation. Local paracrine effects in the gut through GC-C stimulation may have tumour-suppressing actions through the regulation of cell proliferation and metabolism. Although most information on this system has been derived from knockout models, recent human studies have indicated possible roles in heart failure and renal failure. An improved understanding of the nature of its natriuretic, appetite and tumour-suppressing actions may facilitate the discovery of new therapies for heart failure, obesity and cancer prophylaxis.

INTRODUCTION

The control of sodium balance by the kidney is of vital importance to cardiovascular homeostasis. Although the RAAS (renin–angiotensin–aldosterone system) plays a major role in the regulation of renal sodium excretion, physiological experiments in the mid–1950s suggested the existence of other natriuretic factors [1]. Henry et al. [2] provided evidence for the presence of receptors in the left atrium that sensed the changes in circulating blood volume and affected the homeostatic responses of the kidney. Subsequent research led to the discovery of ANP (atrial natriuretic peptide) in the early 1980s [3]. Since then, numerous studies have provided insight into the important role of ANP in the regulation of blood volume and the protection against volume overload. However, it has been confirmed that there is a greater natriuretic response of the kidney to an oral salt load than to the same amount administered intravenously, which led researchers to believe, even before the discovery of the ANP system, that the intestine releases a natriuretic factor which stimulates natriuresis in postprandial periods of salt absorption [4,5]. This intestinal natriuretic system is likely to complement the action of ANP in the overall control of sodium homeostasis and hence blood pressure. The two candidates for this enterorenal system are the guanylin peptides guanylin and uroguanylin. The present review will predominantly discuss uroguanylin, but reference will be made to the structural homology of the two molecules.

GC (GUANYLATE CYCLASE) RECEPTORS

The natriuretic peptide family of receptors with GC (guanylate cyclase) activity has been well studied and includes GC-A and GC-B [6]. GC-A is sensitive to stimulation by ANP and BNP (brain natriuretic peptide), whereas GC-B is readily stimulated by CNP (C-type natriuretic peptide), but only slightly responsive to ANP or BNP [6].

Key words: appetite, cancer, guanylate cyclase, heart failure, natriuresis, uroguanylin.
Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CFTR, cystic fibrosis transmembrane regulator; CNP, C-type natriuretic peptide; GC, guanylate cyclase; HF, heart failure; IBS, irritable bowel syndrome; NHE, sodium/hydrogen exchanger; PKA II, cAMP-dependent protein kinase II; PKG II, cGMP-dependent protein kinase II; RT, reverse transcriptase.
Correspondence: Professor Leong L. Ng (email lln1@le.ac.uk).
An additional member of this family is GC-C, which was first identified as a receptor for the exogenous heat-stable enterotoxin *Escherichia coli* (STa) that causes secretory diarrhoea [7], commonly experienced as ‘Traveller’s diarrhoea’ in the West, but is responsible for a considerable burden of infant mortality in developing countries.

**DISCOVERY OF GUANYLIN PEPTIDES**

The identification of an exogenous ligand for the GC receptor in the intestines and other tissues prompted scientists to search for an endogenous ligand [8] for this receptor. Guanylin was first isolated from rat jejunum tissue tested for cGMP-stimulating activity and competitive displacement of radiolabelled STa on an *in vitro* T84 colonic cell line, and named in reference to its activity on a GC-linked receptor [9].

Guanylin mRNA is prevalent in the rat intestine, but detectable levels were also identified in the kidney, adrenal gland, uterus and oviduct [10]. Analysis using Northern and Western blots indicated that guanylin expression is low in the proximal small bowel, with a higher level of expression in the distal portion of the small bowel and throughout the colon [11].

The observation that kidney tissue extracts have cGMP-stimulating activity on T84 cells led to the search for a renal source of guanylin by testing fractions of opossum urine for T84 cell cGMP-stimulating activity. However, as well as identifying the 14-amino-acid opossum analogue of guanylin, a second distinct 15-amino-acid peptide was discovered, highly acidic and sharing only 53% homology with opossum guanylin. This peptide also potently stimulates cGMP production in T84 cells and was named uroguanylin due to its first source of identification in urine [12].

Uroguanylin is expressed abundantly in the intestinal epithelium as a precursor which undergoes enzymatic cleavage to yield the bioactive peptide [13].

The third member of the guanylin family of peptides, lymphoguanylin, was discovered by analysis of mRNA transcripts from opossum spleen, which revealed a novel peptide sharing 40% homology with guanylin and 80% with uroguanylin, but being relatively less potent than both at stimulating cGMP accumulation in the T84 cell bioassay [14]. Comparison of the amino acid sequences of guanylin, uroguanylin and lymphoguanylin as well as bacterial STa is shown in Figure 1.

Uroguanylin stimulates both chloride and bicarbonate anion transport in the duodenum [13]. Uroguanylin also affects the kidney [15]. 24-h Urinary excretion of uroguanylin was significantly higher in individuals on a high-salt diet (10 g/day) compared with those on a low-salt diet (7 g/day). There was also a significantly positive correlation between urinary excretion of uroguanylin and sodium, potassium, chloride or cGMP [16]. It has, therefore, been suggested that uroguanylin links the intestine and kidney in an endocrine fashion to regulate salt metabolism.

**ACTION OF UROGUANYLIN IN THE INTESTINE**

The role of the intestine in salt and water homeostasis did not receive much attention until the discovery of the guanylin/uroguanylin system. However, since then, it has been shown that uroguanylin stimulates transepithelial chloride secretion in T84 cells and intestinal tissue mounted in Ussing chambers through an increase in cGMP production [6,12]. It has also been shown that NaCl intake affects the expression of mRNA of uroguanylin in the intestine and this NaCl-induced increase in mRNA expression can occur 4 h following oral hypertonic gastric administration of NaCl [17].

In the intestine, the binding of uroguanylin to the GC-C receptor leads, via a complex cascade, to the secretion of salt and water to the intestinal lumen. The GC-C receptor has an extracellular ligand-binding domain and an intracellular domain with a region of homology to protein kinases and a guanylate cyclase catalytic domain [18,19].

Activation of the GC-C receptor by guanylin, uroguanylin or STa stimulates chloride secretion via activation of the CFTR (cystic fibrosis transmembrane regulator) anion channel, which is also responsible for bicarbonate excretion [20]. Anion channel activation occurs via two mechanisms, phosphorylation by PKG II (cGMP-dependent protein kinase II) or phosphorylation...
The uroguanylin system and human disease

Figure 2  Proposed intracellular mucosal signalling pathway in gastrointestinal epithelial mucosal cells

Activation of GC-C receptors by guanylin peptides induces cGMP production which stimulates chloride and bicarbonate secretion by CFTR and ClC channels, while inhibiting the NHE2 channel, reducing sodium uptake. The overall effect is to increase net water secretion into the intestinal lumen.

by PKA II (cAMP-dependent protein kinase II) [20–23]. cGMP may also inhibit PDE III (phosphodiesterase III), increasing intracellular cAMP and thus activating PKA II [24]. As a minor degree of chloride secretion still occurs from intestinal mucosa from CFTR−/− mice, further chloride secretion may occur via the ClC channel [25].

As well as promoting chloride and bicarbonate ion secretion, cGMP additionally reduces sodium uptake by inhibiting the apical NHE (sodium/hydrogen exchanger) NHE2. The overall effect is to increase net water efflux from epithelial cells into the intestinal lumen [26]. These pathways are illustrated in Figure 2.

The activity of uroguanylin in the intestinal lumen is influenced by luminal pH, where it was observed that uroguanylin is more potent at pH 5 than pH 8 [27]. Interestingly, uroguanylin is also expected to be involved in the regulation of the microclimate pH in the jejunum. This has been based on the observation that a microclimate pH gradient is present at the apical surface of enterocytes along the crypt–villus axis, which is induced by the generation of bicarbonate from crypt cells and the production of hydrogen ion by the villus enterocytes [28]. GC-C mRNA and GC-C activity have been reported to exist in the crypt and villus enterocytes of the jejunum, which suggests that uroguanylin acts in an autocrine or a paracrine fashion to control the microclimate pH [28]. Uroguanylin is not only present in the intestines, but it has been identified in other organs such as the stomach, kidney and pancreas. It has been shown that uroguanylin-containing cells in the intestine are enterochromaffin cells [29]. These are widely distributed in the intestine and, when stimulated, release serotonin and substance P both apically (into the lumen) and basolaterally (into the circulation). This characteristic of the enterochromaffin cells makes it possible for uroguanylin to be delivered luminally to GC-C receptors and to other tissues such as the kidney via the circulation [29]. Taken together, this led researchers to propose uroguanylin as the primary candidate for an enterorenal system that stimulates natriuresis in response to an oral salt load. However, recent evidence has suggested that the inactive pro-peptide pro-uroguanylin plays an important role in inducing natriuresis through generating peptide fragments, in the renal tubules, which are more effective at stimulating natriuresis than uroguanylin [30]. This will be explored further in a later section of the present review.

ACTION OF UROGUANYLIN IN THE KIDNEY

Uroguanylin has been proposed as an intestinal natriuretic factor as the administration of uroguanylin increased renal sodium excretion [15] and the ablation of the uroguanylin gene in mice led to impaired salt excretion and increased blood pressure [31]. Carrithers et al. [15] have shown that uroguanylin induced natriuretic, kaliuretic and diuretic effects in mice in a dose- and time-dependent fashion. Significant natriuresis occurred following the infusion of uroguanylin for 30 min with no change in glomerular filtration rate, plasma creatinine, urine osmolality, heart rate or blood pressure, which suggests that the action of uroguanylin is mediated via a tubular transport mechanism [15]. Further support for this hypothesis comes from Qian et al. [32], where the infusion of intact pro-uroguanylin resulted in significant natriuresis coupled with stable blood pressure.
and glomerular filtration rate. Uroguanylin circulates in the blood as the inactive precursor pro-uroguanylin, which is then processed intrarenally to the active form [32].

In contrast with the intestine, where the action of uroguanylin has been extensively investigated and the role of the GC-C signalling pathway has been confirmed, in the kidney, however, the mechanism of uroguanylin action is less well understood. Two fundamental issues will need to be addressed. The first question relates to the source of the uroguanylin that mediates the renal natriuretic and diuretic effects. Forte [33] proposed the intestine as the source of renally active uroguanylin. Support for this theory comes from the fact that the intestine secretes large amounts of pro-uroguanylin into the plasma [34] and infusion studies with radiolabelled recombinant pro-uroguanylin have shown that the circulating pro-peptide is processed intrarenally into uroguanylin, which is then excreted in the urine [32]. On the other hand animals fed on a high-salt diet had normal levels of plasma uroguanylin [35] and pro-uroguanylin [36] despite having increased urinary uroguanylin compared with control animals [35], which provides evidence in favour of a renal source of uroguanylin. This is consistent with the detection of uroguanylin-like polypeptides in the kidney [37,38]. However, as pre-pro-uroguanylin mRNA was only detected at very low levels in the kidneys [39,40], researchers thought that uroguanylin was not synthesized in the kidney, but rather absorbed from the plasma or filtered through the glomerulus [37].

Recently Qian et al. [38] showed that, despite the low levels of renal pre-pro-uroguanylin mRNA, the rat kidney contains significant amounts of authentic pro-uroguanylin (16% of the level in the intestine) and identified the distal tubule as its expression site. Qian et al. [38] proposed a number of explanations for this discrepancy. One possibility is an inherently high rate of pro-uroguanylin synthesis compared with the intestine. There could also be an increased rate of pro-uroguanylin processing, secretion and/or degradation in the intestine. Finally, a renal transport mechanism could import previously synthesized pro-uroguanylin from the plasma into the kidney, which is consistent with the assumption of an intestinal source of the renally active uroguanylin. However, the tracing of intravascularly infused radiolabelled recombinant pro-uroguanylin, despite showing the accumulation of high levels of radioactivity in the kidney, showed that most of the retained material was in its free amino acid form rather than intact pro-uroguanylin and was associated with the proximal tubule [32], which is in contrast with the previous report [38] where the distal tubule was identified as the site of expression for pro-uroguanylin. This supports the idea that plasma pro-uroguanylin does not contribute to the renal stores of the pro-peptide located within the distal tubule [38]. Collectively, the observations described above suggest that renally active uroguanylin is produced internally within the kidney, but at the same time does not dismiss the idea that the intestine could be contributing to the renal pool of uroguanylin. Interestingly, the identification of a distal site for uroguanylin expression indicates that uroguanylin has a distal physiological action [38], which could explain the results of a recent study by Moss et al. [30] that reported a natriuretic but an anti-kaliuretic effect following administration of pro-uroguanylin and uroguanylin. Similar results were obtained with other natriuretic hormones that act in the distal nephron, such as the atrial natriuretic hormone, which has been observed by Rabelink et al. [41] to induce natriuresis without accompanying kaliuresis. The anti-kaliuretic effect reported by Moss et al. [30] was inconsistent with earlier studies showing uroguanylin to induce kaliuresis [15,42]. Moss et al. [30] explained this disagreement by suggesting that reported uroguanylin-induced kaliuresis was seen in the context of diuresis, which provides an indirect flow-dependent stimulus for potassium excretion. In their study, the authors [30] also reported that this anti-kaliuretic effect was progressively reversed with the use of higher doses of uroguanylin or pro-uroguanylin. They concluded that, at the highest doses of uroguanylin and pro-uroguanylin, an anti-kaliuretic effect may be compensated for by increase flow in the distal tubules, which increases potassium excretion via a separate stimulus [30].

The second area of uncertainty concerns the uroguanylin receptor in the kidney. The action of uroguanylin through the GC-C receptor in the intestine, which subsequently stimulates cGMP, led researchers to assume a similar mechanism in the kidney. Support for this hypothesis comes from identifying abundant levels of mRNA encoding the GC receptor in the cortex of opossum kidney [43]. In addition, Kinoshita et al. [16] have shown a significantly positive correlation between the urinary excretion of uroguanylin and cGMP in response to a high-salt diet. In contrast, GC-knockout mice still exhibit significant uroguanylin-induced natriuresis which favours a GC-independent mechanism mediating the renal effects of uroguanylin [15,42]. Furthermore, Qian et al. [38] failed to detect significant expression of GC-C mRNA in the kidney. They also found that the infusion of the two isomers of uroguanylin Ugn A, a potent activator of the GC receptor, and Ugn B, a very weak agonist of this receptor, did not stimulate the excretion of urinary cGMP [38]. These results are consistent with reports from other laboratories where a GC-C-independent mechanism was evident in the rat kidney [45,46]. Therefore it is likely that the action of uroguanylin in the kidney may be mediated by a pertussis-sensitive G-protein-linked receptor in proximal tubule epithelial cells [46] and by
The uroguanylin system and human disease

Figure 3 Proposed proximal tubule intracellular signalling pathway
Uroguanylin stimulates sodium and potassium excretion into the tubule by both GC-C-receptor-dependent and -independent pathways, probably mediated by a G-protein-coupled receptor (GPCR). AQP, aquaporin; ENaC, epithelial sodium channel.

Figure 4 Proposed cortical collecting duct epithelial cell intracellular signalling pathway
Uroguanylin exhibits an anti-kaliuretic effect, as well as having natriuretic activity. AQP, aquaporin; GPCR, G-protein-coupled receptor; ENaC, epithelial sodium channel; ROMK, renal outer medullary potassium channel.

an arachidonic acid-linked G-protein-coupled receptor in collecting duct cells [47]. In addition, cortical collecting duct cells express the GC-G receptor, which may also be a receptor for uroguanylin [48]. Uroguanylin has also been shown to down-regulate apical expression of the pendrin chloride/bicarbonate exchanger in intercalated cortical collecting duct cells thus increasing chloride secretion, although the receptor and mechanism responsible for this remains to be elucidated [49,50].

Taken together, the studies described above suggest that the principal effects of uroguanylin in the kidney are independent of GC-C, but this receptor may still have an undefined role in the renal response to uroguanylin [38]. Alternatively, it can be involved in the signalling pathway of uroguanylin in the kidney under certain conditions, such as prolonged salt intake and this latter hypothesis is supported by Fonteles et al. [51], where prolonged salt ingestion in rats led to an expressive up-regulation of the GC-C receptor. The proposed intracellular signalling pathways in the proximal tubule epithelia and cortical collecting duct epithelial cells are shown in Figures 3 and 4.

ENDOCRINE FUNCTION OF UROGUANYLIN

Uroguanylin has been proposed as the primary candidate for an enterorenal system that has an important role in sodium homeostasis. However, a number of findings are not consistent with this hypothesis and tend to suggest that the propeptide pro-uroguanylin is the true mediator of this enterorenal axis.

Moss et al. [34] have shown that the levels of pro-uroguanylin both in gut extracts and in plasma are much higher than that of uroguanylin. The infusion of anaesthetized rats with recombinant human pro-uroguanylin increased sodium and fluid excretion significantly and, when measuring the pro-uroguanylin levels in the plasma, Moss et al. [34] found that the infusion protocol produced a 3-fold increase in plasma pro-uroguanylin, which is in contrast with the previous published studies of uroguanylin where it had to be infused at supraphysiological levels to induce a renal response. The levels of pro-uroguanylin in the circulation are much higher than that of uroguanylin, with the latter forming less than 3% of the total amount [34]. Pro-uroguanylin was five times more potent than uroguanylin in inducing natriuresis and this response was not correlated with the uroguanylin concentration in the urine, which increased by 5–10-fold following administration of a natriuretic dose of pro-uroguanylin and by 30–50-fold after a non-natriuretic dose of uroguanylin [30]. Taken together, this casts doubts on the fact that uroguanylin is the active metabolite of pro-uroguanylin, which is responsible for its renal excretory action. Pro-uroguanylin is processed in the kidney to a number of active metabolites. In rodents, uroguanylin-15 is considered to be the active form [37,52], but an 18-amino-acid variant has also been identified [40]. In humans, uroguanylin-16 is the main form [6], but again...
a 24-amino-acid form is also recognized in the plasma [53]. With regards to the opossum 13-, 14- and 15-amino-acid peptides have all been described [12]. It is important to note that the previously described active forms of uroguanylin have been identified through the activation of the GC-C receptor which, despite being essential for the secretory activity of uroguanylin in the intestine, is not required for the renal action of uroguanylin [42]. Therefore other peptide fragments of pro-uroguanylin that have been formerly classified as inactive on the basis of their inability to activate the GC-C receptor may possibly be mediators of an endocrine pathway that links the intestine and the kidney. This raises the possibility that uroguanylin−/− mice have impaired salt excretion as a result of loss of one of these fragments, rather than the absence of uroguanylin [30].

**SIGNALLING FOR UROGUANYLIN IN OTHER ORGANS**

In addition to its role in the intestine and the kidney, uroguanylin has been identified in other tissues. Fan et al. [39] managed to detect pre-pro-uroguanylin mRNA in the atria and the ventricles of the opossum heart. mRNA transcripts for uroguanylin and prouroguanylin were also detected in the reproductive system and brain [54]. Patients with cystic fibrosis have abnormalities in water and salt secretion in a variety of organs, including the intestine, the liver, the pancreas and the lung. Therefore uroguanylin may play a role in the abnormal handling of salt and water in these patients through the cystic fibrosis transmembrane conductance regulator [8].

**ROLE OF UROGUANYLIN IN HF (HEART FAILURE) AND HYPERTENSION**

Sodium balance is essential in the regulation of intravascular volume and thus guanylin may be involved in the pathogenesis of hypertension and HF. Uroguanylin-knockout mice have significantly higher blood pressure than wild-type animals, which is associated with reduced sodium excretion [31]. In a human study, urinary cGMP-stimulating bioactivity on T84 cells has been found to be greater in HF patients compared with controls, suggesting that there is increased production of guanylin peptides in HF, although the T84 cell bioassay is not specific for uroguanylin [54]. Plasma pro-uroguanylin levels are also elevated in HF patients compared with controls, most likely as a result of reduced renal clearance of the circulating peptide [55]. Drugs that improve the sensitivity of the kidney to uroguanylin could potentially form a new class of diuretics which could be used in the treatment of heart failure and hypertension.

Both ANP and especially BNP are used as biomarkers for HF, but a study that evaluated novel biomarkers for HF in patients presenting with acute shortness of breath showed a limited diagnostic value for pro-uroguanylin [56], which may indicate that elevated plasma levels in HF patients is a process that takes place over a long period of time, rather than in the short-term.

**ROLE OF GUANYLIN PEPTIDES IN COLON CANCER**

The intestinal epithelium undergoes continuous regeneration and differentiation along the crypt–villus axis, where stem cells are present at the bottom of crypts and the more differentiated enterocytes reside in the villus compartment [57]. The expression of guanylin and uroguanylin along the crypt–villus axis is also observed to be associated with the transition from proliferation to differentiated compartments [57]. Both guanylin and uroguanylin were found to be among the most commonly lost gene products early in colorectal tumorigenesis [58–61]. Furthermore, the loss of the GC-C receptor is associated with increased susceptibility to intestinal carcinogenesis in mice [62,63].

GC-C−/− and guanylin−/− mice show increased proliferation of colonic epithelial cells [64] and abnormal crypt architecture [65]. Loss of GC-C expression also leads to genomic instability and attenuated apoptosis [63]. Collectively, these observations suggest a role for the GC-C receptor as an intestinal tumour suppressor dysregulation of which, reflecting the loss of paracrine hormones, is important in the initial stages of the pathophysiology of colorectal tumorigenesis [66]. This raises the possibility of using oral GC-C receptor ligands as a treatment for colon carcinoma, one of the most common malignancies [67]. Indeed, the oral administration of uroguanylin to mice suppressed intestinal tumorigenesis through inducing apoptosis in human colon carcinoma cells in vitro and inhibiting the formation of polyps in the Min/+ mouse animal model of colorectal cancer in vivo [68]. In addition to its role as a suppressor of colorectal carcinogenesis, the GC-C receptor has the potential to be used as a marker for regional lymph node metastasis in colorectal carcinoma. It is widely accepted that spread to regional lymph nodes provides important information on disease prognosis [69,70]. Recurrence rates increase from 25% in patients who are free of regional lymph node spread (pN0) to 50% in those who have >four lymph node metastases [71,72]. However, a recurrence rate of 25% in pN0 patients suggests the presence of occult metastases to regional lymph nodes that have not been identified by histopathological examination [73]. Therefore a more precise way of detecting micrometastasis is warranted. The selective expression of GC-C receptors in normal
intestinal cells and the overexpression of this receptor in intestinal tumour cells can be used as a specific marker for colorectal cancer [73]. A case control study that examined the expression of GC-C mRNA, using RT (reverse transcriptase)–PCR, in lymph nodes of patients with node-negative colorectal cancer found that GC-C mRNA was associated with disease recurrence [74]. A larger prospective trial was set up to assess the role of GC-C using quantitative RT–PCR in patients with pN0 colorectal cancer in identifying occult metastases and defining the risk of recurrence following surgical treatment. The results suggested that GC-C expression in lymph nodes that have been histologically labelled as negative was independently associated with time to recurrence and disease-free survival [73]. Therefore the previously unrecognized role of the GC-C receptor and its endogenous ligands may form a new paradigm in the staging and treatment of colorectal cancer.

**ROLE OF UROGUANYLIN IN KIDNEY DISEASE**

In addition to its action in the kidney under normal circumstances, uroguanylin may also be involved in renal disease. Nakazato et al. [75] found that plasma levels of uroguanylin were higher in patients with chronic renal failure than in normal individuals. Similarly, Kinoshita et al. [16] reported that the 24-h urinary excretion of uroguanylin was significantly higher in patients with chronic renal failure or haemodialysis compared with the control group. Plasma uroguanylin was also elevated in these patients compared with their normal counterparts [16]. The elevation in plasma and urinary uroguanylin is probably a result of reduced renal clearance and impaired renal response.

**ROLE OF GUANYLINS IN APPETITE REGULATION**

The most interesting recent development in guanylin peptide research is in their role as potential mediators of appetite regulation. A detailed series of experiments by Valentino et al. [76] showed that not only did GC-C−/− mice have a greater weight and more adipose tissue than wild-type mice fed on the same diet, but they had increased fasting insulin associated with impaired glucose tolerance. Furthermore, when food was freely available, GC-C−/− mice exhibited hyperphagia with a diminished satiety response compared with wild-type animals. Intravenous STa, but not oral STa, induced satiety in wild-type but not in GC-C−/− mice, indicating that extra-intestinal GC-C receptors are responsible for this effect, with satiety induced by injections of pro-uroguanylin but not pro-guanylin in wild-type mice, indicating that uroguanylin appears to be the mediator. Added to this is the observation that plasma pro-uroguanylin levels increase after food intake and the satiety response can be blocked with pro-uroguanylin-neutralizing antibodies, strongly supporting the hypothesis that there is an endocrine axis linking the intestine with the brain, as GC-C receptors but not guanylin or uroguanylin are expressed in the hypothalamus.

These results are exciting in their implications and could lead to new treatments for obesity and the metabolic syndrome.

**ROLE OF UROGUANYLIN IN THE TREATMENT OF IBS (IRRITABLE BOWEL SYNDROME)**

IBS is a functional disease associated with abdominal pain and altered bowel activity. Infection with the heat-stable enterotoxin STa, a potent agonist of the GC-C receptor, is associated with increased intestinal fluid secretion and enhanced gut motility [77,78]. Pharmacological modulation of the GC-C receptor has been the target for drug therapy aimed to treat chronic constipation and constipation-predominant IBS [57]. Oral linaclotide, a novel agonist of GC-C, enhances gut secretion and transit [79]. In a Phase II randomized controlled trial, 35 women with constipation-predominant IBS were randomised to oral linaclotide (100–1000 μg once daily) or placebo. Linaclotide at a dose of 1000 μg was associated with a significant effect on ascending colon emptying half-time and overall colonic transient at 48 h. Both doses improved stool frequency, ease of passage, time taken to first bowel movement and decreased stool consistency. The study was not associated with any safety warnings [80]. In a larger multicentre trial that recruited 310 patients with chronic constipation, oral linaclotide was administered at doses of 75, 100, 150, 300 or 600 μg and compared with placebo. All of the doses improved the weekly rate of spontaneous bowel movements in comparison with placebo. Furthermore, the treatment improved the rate of complete spontaneous bowel movements, stool consistency, straining, abdominal discomfort and bloating. The only adverse effect in the study was diarrhoea, which was evident with the higher doses of linaclotide and led to the withdrawal of six patients [81]. Further studies assessing the role of uroguanylin and SP-333, a synthetic GC-C agonist, are now in the pre-clinical phase [57].

**SUMMARY**

The search for an endogenous ligand for the GC-C receptor led to the discovery of the uroguanylin system. Since then, numerous studies have shed the light on
the role of uroguanylin in salt handling in the intestine and kidney. The uroguanylin system has been proposed as a candidate for an enterorenal system that senses acute increases in dietary salt intake and signals the kidneys to increase urinary sodium excretion, while simultaneously reducing intestinal sodium absorption. In the intestine, the action of uroguanylin through the GC-C receptor leads via CFTR and NHE to salt and water excretion. However, in the kidney, uroguanylin acts primarily through a GC-C-independent mechanism.

A role for uroguanylin has also been implicated in the pathogenesis of hypertension, HF and renal disease. The use of uroguanylin as a biomarker for the diagnosis of HF in the acute setting seems less promising though.

The action of uroguanylin through the GC-C receptor in the intestine is not limited to controlling salt and water homeostasis. Uroguanylin has been suggested to be a potential appetite regulator, which can have implications in exploring new treatment options for obesity and the metabolic syndrome. The role of the GC-C receptor and its ligand uroguanylin in the prevention and treatment of colon cancer is of particular interest and could underscore novel treatment for one of the most common malignancies in the Western world. The modulation of the GC-C receptor has also been investigated as a possible treatment paradigm for chronic constipation and IBS, both of which are associated with increased morbidity and a major economic burden.

Finally, the expression of uroguanylin in various organs, such as atria, brain and reproductive system, suggests that uroguanylin has a physiological function in these organs, which has yet to be elucidated.

FUNDING

Our own work was supported by a British Heart Foundation Junior Research Fellowship [grant number FS/09/040 (to H.N.)], and the Leicester National Institute for Health Research Cardiovascular Biomedical Research Unit and the Van Geest Foundation (to L.L.N. and D.J.L.J.)

REFERENCES


