REVIEW

Uroguanylin: a new actor in the energy balance movie

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Abstract

Uroguanylin (UGN) is a potential target in the fight against obesity. The mature protein is released after enzymatic cleavage from its natural precursor, proUGN. UGN is mostly produced in the gut, and its production is regulated by nutritional status. However, UGN is also produced in other tissues such as the kidneys. In the past, UGN has been widely studied as a natriuretic peptide owing to its involvement in several different pathologies such as heart failure, cancer and gastrointestinal diseases. However, recent studies have suggested that UGN also acts as a regulator of body weight homeostasis because it modulates both food intake and energy expenditure. This ultimately results in a decrease in body weight. This action is mediated by the sympathetic nervous system. Future studies should be directed at the potential effects of UGN agonists in regulating body weight in human obesity.

Key Words

- ▶ uroguanylin
- ▶ body weight
- ▶ obesity
- ▶ energy balance

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Introduction

Energy homeostasis to maintain optimum body weight is implemented through the interaction between various neuroendocrine systems, and the gastrointestinal–brain axis has been revealed as a key mediator of this regulation (Cummings & Overduin 2007). The gastrointestinal tract (GI tract) releases several peptides that act as hormonal and humoral signals—mainly in response to food intake—which reach different regions of the brain and carry information about the nutritional status of the whole organism. Such signals can be translated into feelings of satiation or hunger to regulate appetite. Satiety signals are potential targets for the design of anti-obesity drugs, which would greatly advance the fight against obesity (Strader & Woods 2005).

A recently discovered satiety peptide called uroguanylin (UGN) or GUCA2B (guanylate cyclase activator 2B) comprises 16 amino acids and is secreted as a prohormone (proUGN) from duodenal epithelial cells into the lumen. It undergoes postprandial enzymatic conversion into its active form. Once activated, UGN acts as an agonist of the transmembrane receptor guanylyl cyclase 2C (GUCY2C) on intestinal epithelial cells, increasing intracellular levels of cyclic guanosine monophosphate (cGMP) (Fruhbeck 2011, Valentino *et al.* 2011) (Fig. 1).

Guanylin or (GUCA2A, guanylate cyclase activator 2A) is an analog of UGN that also binds to the GUCY2C transmembrane receptor. It is a 15-amino acid peptide that is secreted as a prohormone, proguanylin, from

Figure 1

Mechanism of action of the GUCY2C-mediated signaling pathway. (1) Agonists of GUCY2C (guanylin (GN), uroguanylin (UGN), *Staphylococcal* enterotoxins (STs), linaclotide, plecanatide, and dolcanatide) activate the GUCY2C expressed on the apical surface of the cells, hydrolyze guanosine triphosphate (GTP), and increase the production of intracellular cyclic guanosine monophosphate (cGMP). (2) Elevated cGMP concentrations activate cGMP-dependent protein kinase, type 2 (PKG-II) and inhibit the activity of the cAMP-phosphodiesterase PDE3, thereby cross-activating cAMP-dependent protein kinase (PKA), leading to the phosphorylation and activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, which stimulates the secretion of chloride (Cl⁻) and bicarbonate (HCO₃⁻) ions in the lumen. (3) The CFTR-mediated secretion of Cl⁻ and HCO₃⁻ ions is accompanied by the efflux of positive ions (Na⁺ and H⁺), which follow the electronegative osmotic gradient.

colonic epithelial cells and is cleaved into its active form (Schulz *et al.* 1990, Currie *et al.* 1992).

All GUCY2C analogs are responsible for decreasing sodium and water permeability, which increases chloride secretion owing to the phosphorylation of the cystic fibrosis transmembrane receptor (CFTR). This process is regulated by elevated levels of cGMP within epithelial intestinal cells. Overproduction of uroguanylin or guanylin leads to acute diarrhea, which is typically found in bacterial infections when *Staphylococcal* enterotoxins (STs) bind to the GUCY2C receptor (Currie *et al.* 1992, Forte & Currie 1995).

Moreover, the pharmacological industry has created synthetic agonists of UGN that also bind to the GUCY2C receptor. Examples include linaclotide, which is used for the treatment of irritable bowel syndrome, retains many structural features of human bacterial enterotoxins (Brancale *et al.* 2017), and plecanatide and dolcanatide, which have many of the structural and functional characteristics of the endogenous ligand uroguanylin (Shailubhai *et al.* 2015).

Regulation of intestinal UGN production

The enterochromaffin cells (EC cells) of the intestine are the main source of UGN in humans and rats (Brenna *et al.* 2016). To a lesser extent, enteroendocrine cells (EE cells) are also a source of UGN. UGN is released from the gastrointestinal tract immediately after food intake (Valentino *et al.* 2011). Very prolific UGN mRNA expression in the small intestines of rats has been reported (Date *et al.* 1999), although lower levels have

also been found in their stomachs (Folgueira *et al.* 2016b). Extraintestinal tissues such as the pancreas, adrenal glands, lungs and testis are also sources of UGN (Laney *et al.* 1992, Miyazato *et al.* 1996, Blanchard & Cousins 1997, Li *et al.* 1997, Nakazato *et al.* 1998).

The intestinal levels of UGN are directly regulated by nutritional status and correlate with UGN levels in the plasma (Folgueira *et al.* 2016b). In mice, circulating levels of proUGN increase in response to the ingestion of nutrients (Valentino *et al.* 2011). According to the postprandial release of UGN, animals in a fasting state have reduced levels of UGN in the duodenum, which correlates with decreased plasma UGN levels, compared with animals fed *ad libitum* (Folgueira *et al.* 2016b). Furthermore, when fasted animals are re-fed, the intestinal and plasma levels of UGN are restored. These regulation of UGN by energy availability is dependent on leptin, an hormone synthesized and secreted in proportion to the amount of adipose tissue, whose levels are decreased in fasting states and restored after refeeding (Trayhurn *et al.* 1995). The following evidence supports this: first, UGN expression in the intestine was increased after intraperitoneal leptin injection at doses of 0.5 µg per g body weight every 12 h for 3 days (Folgueira *et al.* 2016b). Second, in leptin-deficient ob/ob mice, UGN levels remained unaltered after fasting or refeeding, indicating that nutritional status alone cannot regulate UGN production when leptin is not present (Folgueira *et al.* 2016b). Third, leptin-deficient ob/ob mice have low levels of intestinal and plasma UGN compared with their wild-type counterparts, and leptin treatment restores UGN levels in the ob/ob mutants to those found in the wild-type mice (Folgueira *et al.* 2016b). Finally, a

classical model of hyperleptinemia (obese animals fed a high-fat diet) exhibits high levels of circulating leptin and increased levels of UGN in the intestine and plasma.

In addition, it has been suggested that a reduction in intestinal levels of UGN following intake of a high-calorie diet silences hypothalamic GUCY2C and regulates satiety. A novel mechanism has been proposed that involves a role for UGN in the disturbance of the gut–brain axis that occurs in obesity (Kim *et al.* 2016). However, GUCY2C receptors in the hypothalamus are not affected by obesity, which suggests that centrally administered UGN is effective in treating obesity. It is important to note that divergent circulating prouroguanylin levels have been found in human obesity compared to rodents with diet-induced obesity. In this sense, and accordingly to the findings in ob/ob mice, recent clinical studies have found decreased proUGN levels in both adolescent (Di Guglielmo *et al.* 2017a,b) and adult (Rodriguez *et al.* 2016) obese subjects. UGN downregulation in obesity is associated with endoplasmic reticulum (ER) stress (Kim *et al.* 2016). In line with this observation, a recent study

has found that inflammation correlates with decreased circulating proUGN levels female adolescents with obesity (Di Guglielmo *et al.* 2017b). In addition, circulating proUGN levels are also decreased in human obesity in relation to adiposity, with weight loss achieved by bariatric surgery inducing a significant increase of circulating proUGN (Rodriguez *et al.* 2016).

In human obesity, the intestinal expression of guanylin peptides is not only regulated by leptin, but also by insulin and sexual hormones. In this regard, obese patient with type 2 diabetes exhibit increased protein expression of proguanylin in the small intestine compared with obese normoglycemic patients (Rodriguez *et al.* 2016). Moreover, female obese adolescents exhibit an increased intestinal immunostaining of uroguanylin and guanylin compared with age-matched male pairs (Di Guglielmo *et al.* 2017a).

In summary, nutritional status is a key factor in regulating UGN production, and leptin is a direct regulator of UGN production. Taken together, the evidence indicates that UGN is a part of the intestine–brain–adipose tissue network that regulates energy homeostasis and body weight (Fig. 2).

Figure 2

Regulation of intestinal uroguanylin (UGN) production by nutritional status is dependent of leptin levels. Food deprivation reduces UGN production in the duodenum, an effect that is reversed by the resumption of feeding or leptin treatment. Consistently, in an ob/ob mouse model of leptin deficiency, UGN expression is reduced in the duodenum and the levels revert after leptin treatment. Diet-induced obese mice with elevated levels of leptin exhibit increased UGN levels in the intestine compared with lean mice.

Centrally administered UGN and energy homeostasis regulation

It has been suggested that UGN plays a role as a satiety factor in the brain–gastrointestinal axis (Fruhbeck 2011) regulating food intake and energy homeostasis, and ultimately body weight. It is widely known that the integration of all neurohormonal signals produced in the periphery and the regulation of the appetite occur mainly in the hypothalamus. Accordingly, it has recently been discovered that once uroguanylin is secreted by the gut into the circulation, it targets the GUCY2C receptor of the hypothalamus, activating anorexigenic pathways and producing satiety in mice (Valentino *et al.* 2011).

Pharmacological effects of acute UGN administration

It has been shown that systemic administration of a GUCY2C agonist reduces food intake in wild-type mice, but this effect has not been observed in GUCY2C-deficient mice. It has also been shown that GUCY2C activation in the central nervous system—especially in the hypothalamus—through the specific administration of a GUCY2C agonist reduces food intake (Valentino *et al.* 2011). In spite of these findings, reports assessing

the metabolic role of endogenous UGN and its receptor have shown controversial findings. A study indicated that GUCY2C receptor knockout mice have elevated levels of body fat as a consequence of increased food intake (Valentino *et al.* 2011). In contrast, a following study showed that GUCY2C-knockout mice had unaltered body weight, adiposity and glucose tolerance (Begg *et al.* 2014). Therefore, the physiological significance of the UGN-GUCY2C system for the control of energy homeostasis is not fully understood.

Pharmacological effects of UGN chronically administered

The putative anorectic action of UGN when administered at short term did not occur when UGN was administered for longer periods. The treatment of obese mice with centrally administered UGN during one week induces a reduction in body weight, independently of food intake (Folgueira *et al.* 2016a). The decrease in body weight found after UGN administration was accompanied by a reduction in body fat and was due to an increase in energy expenditure. Accordingly, the thermogenic program in brown adipose tissue (BAT) was activated as demonstrated by an increase in the main thermogenic markers (uncoupling protein 1 (UCP1), uncoupling protein 3 (UCP3), PR domain-containing 16 (PRMD16) and peroxisome proliferator-activated receptor gamma coactivator 1- α (PGC1- α) in the BAT of UGN-treated obese mice (Folgueira *et al.* 2016a). Furthermore, the central administration of UGN also induced the conversion of white adipocytes into brown adipocytes, a phenomenon named browning (Folgueira *et al.* 2016a). This was demonstrated by the increase in UCP-1 levels in the subcutaneous adipose tissue. In addition to this thermogenic effect, centrally administered UGN also stimulated lipid mobilization in subcutaneous adipose tissue indicated by the higher expression of pHSL (Folgueira *et al.* 2016a). Together, the different effects in white adipose tissue and BAT, which are mediated through the sympathetic nervous system, contribute to the reduction in body fat and body weight described in obese mice after treatment with centrally administered UGN. Besides the lipolytic action of uroguanylin through central mechanisms, the white adipose tissue expresses GUCY2C receptors and both guanylin and uroguanylin exert a direct action on human visceral adipocytes stimulating lipolysis through the activation and phosphorylation of HSL and the upregulation of several genes involved in lipid mobilization (Rodriguez *et al.* 2016).

Several studies have shown that β -adrenergic receptors constitute a key factor in the regulation of adipose tissue metabolism by the autonomous nervous system. Because the parasympathetic nervous system is not involved in the effect of centrally administered UGN at a peripheral level in adipose tissue, it is thought that the sympathetic nervous system plays a role. Accordingly, there is no effect following a central injection of UGN in the knockout mice for the three β -adrenergic receptors (β 1, β 2 and β 3) with regard to body weight, fat mass or brown and white adipose tissue metabolism.

However, the effects of central UGN were not just limited to adipose tissue, because increased gastric motility was observed, as demonstrated by an increase in the amount of fecal matter. This gastric effect occurs via the vagus nerve, which is thought to be the main connection between the gastrointestinal tract and the brain (Seoane *et al.* 2007). Accordingly, the gastric effect of centrally administered UGN disappears in mice that have undergone surgical vagotomy. However, in such resected mice the effects of UGN on reducing body weight and fat mass, and increasing the thermogenic program and browning are still present, which suggests that these effects are the result of a mechanism that is independent of the parasympathetic nervous system (Folgueira *et al.* 2016a).

Physiological role of UGN

The physiological role of UGN in energy balance regulation is actually reinforced by the most recent published works. It was described that, centrally administered UGN induces weight loss and a reduction in fat mass in obese mice, independently of food intake. The effects of central UGN in peripheral organs are mediated by both branches of the autonomous nervous system, while the sympathetic nervous system controls fat metabolism in the adipose tissue and the parasympathetic nervous system regulates motility in the gastrointestinal tract (Fig. 3).

The effects of UGN on glucose homeostasis have not been fully elucidated. The only investigation of this aspect did not discover any effect of centrally administered UGN analogues on glucose homeostasis. In a study by Begg and coworkers (2014) UGN-deficient mice fed a high-fat diet did not show any alteration in glucose tolerance. However, when the mice were fed a mixed meal, they exhibited impaired glucose tolerance and elevated levels of glucose in the blood. Furthermore, the deficient mice also exhibited increased postprandial plasma insulin levels (Begg *et al.* 2014). In addition, it was recently described

Figure 3

Mechanism of central uroguanylin (UGN) action to reduce body weight and adiposity. The chronic central infusion of UGN reduces weight gain and adiposity in diet-induced obese mice, independently of food intake, and acts at different levels: centrally administered UGN induces brown adipose tissue thermogenesis, as well as browning and lipid mobilization in white adipose tissue through stimulation of the sympathetic nervous system. In contrast, brain UGN augments fecal output through the vagus nerve.

that circulating concentrations of proguanylin, but not prouroguanylin, are associated with markers of insulin resistance, such as glycemia or the HOMA index, and its intestinal protein expression is significantly increased in obese patients with type 2 diabetes (Rodriguez *et al.* 2016).

Future research should attempt to determine whether the loss of GUCY2C signaling is involved in the development of glucose intolerance and should elucidate the main mechanisms involved in glucose homeostasis due to UGN.

Other functions of UGN

Besides the emerging role of UGN in the gut–brain axis as a regulator of feeding, energy homeostasis, body mass and metabolism in rodents with normal physiology (Valentino *et al.* 2011, Folgueira *et al.* 2016a), other functions have been attributed to UGN-GUCY2C. These functions affect other physiological systems and are potential therapeutic targets in various pathologies including heart failure (HF), gastrointestinal diseases and cancer (Li *et al.* 2007, Lin *et al.* 2016).

It has been suggested that pro-uroguanylin (proUGN)—secreted from the intestine in response to oral salt loads—acts as an endocrine hormone on renal tubule epithelial cells as part of an entero-renal natriuretic axis (Forte *et al.* 1996). The infusion of both UGN and proUGN into rodents induces a natriuretic response activating the renal mechanisms involved in Na⁺ excretion (Fellner *et al.* 2016). It was initially proposed that the dietary ingestion of salt induces an increase in the secretion of proUGN by EC (Moss *et al.* 2008). proUGN is converted to UGN

in the intestine and the renal tubules (Hamra *et al.* 1996, Qian *et al.* 2008), leading to an increase of Na⁺ in the urine (Greenberg *et al.* 1997, Nakazato *et al.* 1998, Lessa *et al.* 2012) and a decrease in intestinal Na⁺ absorption (Joo *et al.* 1998, Toriano *et al.* 2011). As part of this entero-renal axis, UGN signaling acts by regulating Na⁺ homeostasis. Salt ingestion is not reflected in circulating proUGN levels and does not affect the gastrointestinal expression of uroguanylin (Fellner *et al.* 2016), but high levels of salt intake induce the synthesis of proUGN in the kidney; proUGN is subsequently delivered to the lumen of the nephron in its active form, UGN. The most recent findings on this topic suggest that the regulation of the UGN system by salt consumption takes place mainly in the kidneys and is not related to the intestinal mechanism controlling UGN production (Toriano *et al.* 2011). The natriuretic effect of UGN has been described in UGN-knockout mice, which have hypertension as a consequence of a delay in sodium excretion (Fellner *et al.* 2016).

Patients that have experienced HF have high plasma levels of proUGN, and this alteration is initially showed to be independent of hypertension or renal alterations. Furthermore, UGN levels tend to increase as the severity of the HF increases (Carrithers *et al.* 2000). It is thought that the increase in UGN levels associated with HF might be due to impaired renal clearance leading to accumulation (Carrithers *et al.* 2000). Moreover, acute renal impairment increases the level of circulating proUGN and reduces its conversion into the active form. This is due to a decrease in glomerular filtration (Qian *et al.* 2008).

HF patients have a combination of impaired renal response and reduced renal clearance of circulating

proUGN, resulting in diminished natriuretic and diuretic activity. The mentioned factors contribute to intravascular volume expansion, which explains the correlation between high prohormone levels and hypertension described, differently to that found by Carriethers and coworkers in a more recent study (Narayan *et al.* 2010).

The GUCY2C pathway is downregulated in patients suffering from chronic inflammatory bowel diseases (CIBD: Crohn's disease (CD) and ulcerative colitis (UC)); this downregulation is more significant when the clinical conditions worsen (Brenna *et al.* 2015). The GUCY2C signaling pathway plays a key role in the regulation of intestinal fluid and the balance of electrolytes. Guanylin and uroguanylin act by providing optimal intestinal mucosa hydration by secreting water, NaCl, and HCO₃⁻ (Lan *et al.* 2016). In this context, it has been proposed that GUCY2C signaling regulates the intestinal inflammatory response. Accordingly, the restoration of the silenced pathway is a promising approach to treating patients with the chronic diseases mentioned earlier (Lan *et al.* 2016). Currently, various promising drugs that are structurally similar to uroguanylin and guanylin and act as potential therapeutic analogs are being tested to treat chronic diseases. Linaclotide is one such drug; it is used to treat CIBD and chronic idiopathic constipation (CIC) (Ahsan *et al.* 2017). Two other analogs of uroguanylin—plecanatide and dolcanatide—can be used to ameliorate colonic inflammation in acute and chronic models of murine colitis, possibly through the suppression of proinflammatory cytokine production (Shailubhai *et al.* 2015). Furthermore, recent clinical studies have confirmed that preparations of both drugs for oral administration are minimally absorbed into the systemic circulation and act locally in the gut lumen, eliminating the toxicity concerns associated with the existing systemic IBD therapies (Shailubhai *et al.* 2013).

Considering that the renewal process of the intestinal epithelium goes through a cycle of proliferation, migration, differentiation, apoptosis and ultimately loss of epithelial cells into the lumen, GUCY2C signaling is believed to be important in maintaining the balance between apoptosis and regeneration. Therefore, treatment with dolcanatide results in minimal loss of crypts and low distortion of crypt morphology, suggesting less infiltration of inflammatory cells (Shailubhai *et al.* 2015).

Finally, IBD patients are susceptible to cancer, so the therapeutic candidates described earlier could be useful for delaying disease progression. GUCY2C has been proposed

as a tumor suppressor because it regulates the regenerative mechanism that maintains the integrity of the gut, which plays an essential role in the pathophysiology of colorectal cancer (Li *et al.* 2007). In obese mice, the loss of GUCY2C signaling is probably mediated by the activation of ER stress mechanisms induced by the consumption of excessive calories and the response of the unfolded protein (Lin *et al.* 2016).

In this context, one novel therapeutic approach to cancer prevention would be treatment with an oral preparation of linaclotide, a synthetic 14-amino acid peptide that has more than 60% amino acid identity with guanylin and uroguanylin (Weinberg *et al.* 2017). A secondary therapeutic approach has been proposed that comprises the oral administration of plecanatide, which acts via cGMP/GC-C signaling to mediate the downregulation of Wnt/b-catenin signaling within the colon, thereby delaying the progression of colorectal cancer. Furthermore, recent clinical studies suggest that plecanatide is a safe and orally active drug candidate, with promising potential for use in the treatment of various GI disorders and diseases (Shailubhai *et al.* 2013).

These findings represent a new role for GC-C agonists in the chemoprevention of inflammation associated with colorectal carcinogenesis in populations at risk, including obese individuals.

Concluding remarks

The UGN-GUCY2C system constitutes a promising target in the fight against obesity. The pharmacological utility of GUCY2C agonist was suggested by the initial findings in preclinical models. The central administration of UGN to obese mice induce a decrease in body weight independent of food intake, an effect mediated by the increase in energy expenditure as probed by the activation of the thermogenic program in BAT and the induction of lipid oxidation in WAT. Futures studies should be aimed to elucidate if in humans the beneficial effects found in preclinical models are reproduced.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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