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Review

Ghrelin acylation and metabolic control☆

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ABSTRACT

Since its discovery, many physiologic functions have been ascribed to ghrelin, a gut derived hormone. The presence of a median fatty acid side chain on the ghrelin peptide is required for the binding and activation of the classical ghrelin receptor, the growth hormone secretagogue receptor (GHSR)-1a. Ghrelin O-acyl transferase (GOAT) was recently discovered as the enzyme responsible for this acylation process. GOAT is expressed in all tissues that have been found to express ghrelin and has demonstrated actions on several complex endocrine organ systems such as the hypothalamus–pituitary–gonadal, insular and adrenal axis as well as the gastrointestinal (GI) tract, bone and gustatory system. Ghrelin acylation is dependent on the function of GOAT and the availability of substrates such as proghrelin and short- to medium-chain fatty acids (MCFAs). This process is governed by GOAT activity and has been shown to be modified by dietary lipids. In this review, we provided evidence that support an important role of GOAT in the regulation of energy homeostasis and glucose metabolism by modulating acyl ghrelin (AG) production. The relevance of GOAT and AG during periods of starvation remains to be defined. In addition, we summarized the recent literature on the metabolic effects of GOAT specific inhibitors and shared our view on the potential of targeting GOAT for the treatment of metabolic disorders such as obesity and type 2 diabetes.

Contents

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1. Ghrelin

Ghrelin was discovered in 1999 by Kojima and colleagues from extracts of rat gastric tissue as an endogenous ligand for the growth hormone secretagogue receptor (GHSR)-1a. *Ghre* is the Proto-Indo-European root of the word 'grow' and *relin* means release, referring to the ability of ghrelin to enhance GH release [50]. Besides its potent stimulatory effect on GH secretion, ghrelin has also been shown to play a pivotal role in metabolism and energy balance [8,79,90,94]. Ghrelin regulates short-term energy homeostasis by increasing hunger and food intake. This action is thought to be mediated by the activation of hypothalamic neuropeptides NPY/Agrp neurons [22,78]. In addition, ghrelin has been implicated in long-term energy balance by promoting weight gain and adiposity [19,90,94]. Importantly, the ghrelin system is also actively involved in the regulation of glucose metabolism and insulin secretion as demonstrated in rodents and humans [13,14,58,86,87]. Moreover, a wide variety of effects such as modulating immune function [24], water balance [41], gastric emptying and gastric acid secretion [56], cell proliferation [5], memory [16], anxiety [10], sleep [97], energy expenditure [88], bone metabolism [55], reproductive function [15,89] and cardiovascular function [30] have been ascribed to ghrelin. Most of these physiologic functions associated with ghrelin are thought to be mediated by AG binding to the (GHSR)-1a. Desacylated ghrelin (DAG) does not bind to the classical ghrelin receptor. Its precise physiologic function has been questioned. However, (GHSR)-1a independent effects of AG and DAG have been described suggesting the existence of new undiscovered orphan receptors [40,91,93].

The main source of ghrelin in the organism is the stomach, where 65–90% of the circulating ghrelin is synthesized [7,27]. The second major producing organ for ghrelin is the small bowel. Small amounts of this peptide are also secreted in the lung, pancreas, hypothalamus, pituitary, breast, kidney, ovary, prostate, liver, testis, fat, placenta, adrenal gland, muscle, and heart, allowing for its diverse and complex hormonal function [35]. The ghrelin receptor is also widely expressed in rodents and humans. However, the highest expression of GHSR-1a is in the central nervous system (CNS), particularly the hypothalamus and pituitary gland, areas essential for ghrelin action on energy regulation and GH secretion [46].

The human ghrelin gene is located on chromosome 3 (3p25-26) with 4 exons and 3 introns. Several products of the ghrelin gene exist. Among them, the most relevant ones are obestatin, DAG and desglut-14 ghrelin. The ghrelin gene generates a pre-propeptide consists of 117 amino acids (AA) (pre-proghrelin), which after several modifications produces 'proghrelin' (1–94 AA). The prohormone convertase (PC) 1/3 is responsible for the formation of mature ghrelin through limited proteolytic cleavage at a single arginine [51,104]. As a co-product of ghrelin, obestatin was proposed by Zhang et al. to reduce food intake and body weight [102]. However, subsequent studies failed to reproduce these results and the physiological role of this hormone remains to be defined [17,63,77].

The post-translational acylation has been shown to occur independently of the proteolytic processing of the ghrelin peptide [104]. The hydroxyl group of serine-3 is acylated with an n-octanoic acid or another medium-chain fatty acid (MCFA). This ghrelin modification is entirely unique to ghrelin and is required to activate the ghrelin receptor, (GHSR)-1a [50]. The enzyme that is responsible of ghrelin acylation was identified by two independent groups and was named the ghrelin O-acyltransferase (GOAT) [39,100].

2. Ghrelin O-acyltransferase

GOAT is a porcupine-like enzyme that belongs to membrane-bound O-acyltransferases (MBOAT) superfamily [43], previously known as MBOAT4. The human GOAT gene is located on chromosome 8p12 with 3 exons of 13.02 kb extension. This gene produces a protein with 435 AA of which the Asp307 and His338 residues are essential for its function and have been conserved in other MBOAT family members [39,100]. GOAT, like ghrelin, is highly conserved across vertebrates from zebrafish to humans. Amino acid sequence assays that compare GOAT structure in different species revealed a 90% homology for human and rodent and 59% for mammalian and zebrafish. Additionally rodent and zebra fish GOAT can successfully acylate human ghrelin. These findings highlight the relevance of the highly conserved structure of GOAT and its functionality across species [39,100]. With regard to the enzymatic property, the optimal temperature for biochemical reactions is 37–50 °C whereas the enzymatic activity of GOAT is abolished at 60 °C. The optimal pH for maximal specific activity is at 7.0–7.5, in concordance with the physiological range in the human body [66,100]. The deacylation process in the organism is carried out by a recently identified and characterized acyl-protein thioesterase 1/lisophospholipase I released mainly from the liver and stimulated by bacterial lipopolysaccharide LPS [74]. Earlier work has also demonstrated that ghrelin is desacylated by carboxylesterase in rat serum and by butyrylcholinesterase in human serum, suggesting that this process is mediated by more than one esterase [23].

Both missense variants of unknown functional significance in the ghrelin gene [42,48,53,95] and loss of function mutations of the ghrelin receptor gene [44,47,67,68,96] have been described. However, the impact of these genetic variances on energy balance remains controversial given the limited phenotypic data available. In a recent report, a loss of function mutation of the GOAT gene has been linked to increased risk of anorexia nervosa in humans [60], indicating its potential relevance in clinical diseases.

2.1. Substrates of GOAT

2.1.1. Desacylated ghrelin

DAG has similar structure as AG but does not have the acyl group in the Ser-3 position [50]. Since the presence of the acyl group is necessary for the binding of ghrelin to GHSR-1a, DAG does not stimulate GH secretion [50] and has therefore been considered as an inactive peptide. Conversely, a variety of (GHSR)-1a independent effects of DAG have been reported: enhancing insulin secretion from insulin cell lines [38], stimulation of osteoblast growth [25] and spinal cord neuronal precursor cell proliferation [73], promotion of intracytoplasmatic lipid accumulation in human visceral adipocytes [70], suppression of isoproterenol-induced lipolysis in rat adipocytes [59], and inhibition of cell death in cardiomyocytes and endothelial cells [11]. Even though DAG does not stimulate GH secretion, the GH response to ghrelin administration is diminished in transgenic (Tg) mice that overexpress DAG. These mice are also smaller than the wild-type (WT) animals [6]. DAG has also shown both orexigenic and anorexigenic effects depending on the experimental conditions used [9,18,57,93]. It is generally accepted

that DAG carries mainly anorexigenic properties mediated by an increase in cocaine–amphetamine-related transcript and urocortin expression in the paraventricular and arcuate nucleus in the hypothalamus [9]. Moreover, Tg mice that overexpress DAG have lower body weight and a slower lineal growth comparing to their WT littermates [6]. The effect of DAG on the GI tract has not been well studied but it seems to have opposite effects as AG. DAG does not affect gastric acid secretion [28,81] but has been shown to inhibit gastric emptying [9,18]. It has been speculated that a specific orphan receptor may be responsible for the (GHSR)-1a independent effects ascribed to DAG on the GI tract [91,93].

AG and DAG show a similar tissue distribution pattern. Both protein levels are most abundant in the gut, especially in the stomach tissue [45]. In gastric tissue, the ratio DAG:AG is 2:1 while the ratio has been reported higher in the systemic circulation [45,49]. Furthermore, the plasma AG:DAG can vary during different nutritional states such as fasting and feeding [45,49,84] depending on the method of sample processing used. Taken together, these observations suggest that the physiological function of ghrelin may be mediated by the control of ghrelin acylation.

2.1.2. Medium chain fatty acid (MCFA), medium chain triglyceride (MCT) and ghrelin acylation

Recently the emerging role of lipid sensing mechanism in the regulation of energy balance has come to focus [3,34,49,75]. An example of this regulation is that N-acylphosphatidylethanolamines (NAPE), a gut-derived circulating factor induced by fat ingestion, have been shown to inhibit food intake [34]. Similarly, oleylethanol amide (OEA), an endogenous lipid and a peroxisome proliferator-activated receptor-alpha (PPAR-α) agonist that is released from the small intestines after fat infusion, was found to decrease meal frequency and inhibit food intake [75]. Recent *in vitro* data suggest that fats can directly modify gastric ghrelin secretion from isolated rat stomach [3]. Furthermore, evidence also suggests that not only ghrelin but also GOAT expression and activity are modified by dietary lipids, in particular by the availability of MCFA [49].

In natural foods, MCTs are found in milk, vegetable oils (coconut and palm), and butter, and have a unique chemistry structure to allow for easy digestion. Before GOAT was discovered, it had been reported that the ingestion of either MCFAs or MCTs increased the stomach concentrations of AG [62]. This finding was confirmed by subsequent studies indicating the importance of ingested MCFAs in ghrelin acylation. Mice that were fed a diet rich in glycerol triheptanoate, a MCT constituted heptanoic acid (C7:0) that is not synthesized *de novo*, were able to produce C7-AG. Interestingly, concentrations of C7-AG in stomach and blood in these mice were higher than C8-AG (the preferred MCFA by GOAT) than those fed a control diet [49]. Mice on the MCT diet also produced higher amounts of AG in blood than control mice on standard chow. Furthermore, the presence of MCT is necessary for a massive production of AG in Tg mice that overexpress ghrelin and GOAT in the liver, highlighting again the importance of MCT availability. Moreover, it has been shown that the acyl donor for the GOAT mediated ghrelin acylation is n-octanoyl Co-A, not n-octanoic acid or Co-A alone, and that n-octanoyl Co-A produces a negative feedback on AG production [101]. Notably GOAT can catalyze the acyl modification not only with an n-octanoyl group but also with different length acyl side chains to produces n-hexanoyl, n-heptanoyl and n-decanoyl ghrelin depending on the MCT substrate availability in the diet [62]. It has been reported that GOAT has a preference for n-hexanoyl Co-A over n-octanoyl-Co-A even when the amount of endogenous n-hexanoyl ghrelin is very low as compared to n-octanoyl ghrelin in mouse [66].

Ghrelin acylation can be modified by nutritional status such as fasting and feeding and by dietary lipids. The availability of ghrelin and MCFAs are rate-limiting steps for the acylation process. A better understanding of the ghrelin acylation process can provide important knowledge on the role of ghrelin on short- and long-term energy regulation.

2.2. Tissue expression and specific regulation of GOAT

In addition to gastric tissue, the expression of GOAT has been described in many organ tissues at different levels depending on the species studied, namely duodenum, colon, pancreas, heart, kidney, muscle, tongue, testis, thymus, adipose tissue, adrenal gland, chondrocytes, pituitary, hypothalamus, placenta and ovary [4,31,36,37,39,65,71,72,80,83,100].

2.2.1. Gastrointestinal tract

Sakata et al. demonstrated that GOAT was localized within ghrelin producing cells in the stomach and duodenum of mice, proving information about the site of action for GOAT [72]. The pattern of ghrelin and GOAT expression have been found to be very similar in all tissues studied, however, the GOAT mRNA levels in the stomach are more than two- fold lower than ghrelin mRNA. Interestingly, it was estimated that about 15% of the GOAT expressing cells in the gastric mucosa did not coexpress ghrelin, suggesting the existence of other endogenous substrate for GOAT [72]. The ability of GOAT to acylate other substrates apart from ghrelin was suggested by others who found a subset of GOAT expressing cells in gastric mucosa (ECL cells) that were different from the ghrelin expressing cells (X/A cells) [83]. However, it is known that only a short amino acid sequence (e.g. 5 amino acids) with an unblocked amino terminal is sufficient to be recognized and acylated by GOAT [66,101]. Genome scans provide evidence that this 5 amino acid motif is only found in ghrelin. Therefore, it is likely that ghrelin is the only peptide that is acylated by GOAT.

2.2.2. Hypothalamic-pituitary axis

There is evidence that GOAT expression is regulated by GHRH, somatostatin (SST), and leptin in mouse primary pituitary cultured cells but not by IGF-1, NPY, insulin or DAG [31]. Interestingly, AG is able to increase GOAT expression via a positive autocrine feedback loop in the same culture system. In addition, a significant decrease in GOAT mRNA level in the pituitary, not in the hypothalamus, was observed in diet-induce-obesity (DIO) and *ob*/*ob* mice; an upregulation of GOAT mRNA was found after 24 h of fasting in the pituitary and after 48 h in the hypothalamus [31]. These findings raise the possibility of a tissue-specific regulation of GOAT in different models of obesity and states of energy balance. The biological relevance of this enzyme in the CNS may be related to its role in the coordination of neuroendocrine response to metabolic stress.

2.2.3. Pancreas

GOAT is expressed in pancreatic islets but without co-localization with insulin-producing cells [4]. Exposing INS-1 cells to insulin inhibits GOAT mRNA expression and protein levels. Importantly, insulin was found to inhibit the pancreatic transcription and translation of GOAT via the mTOR signaling pathway. Inhibition of mTOR signaling by rapamicyn dose- and time-dependently increases GOAT expression in INS-1 cells, while activation of mTOR by leucine decreases GOAT mRNA levels. Furthermore, the effect of insulin on GOAT expression in the pancreas can be seen also *in vivo*, consistent with the findings *in vitro*. The authors speculated that insulin might act as a repressor for AG production via a direct action on GOAT, providing a mechanism for the regulation of intra-islet ghrelin secretion [4].

2.2.4. Reproductive system

GOAT is expressed in the testes and ovaries. Protein and mRNA of GOAT have been detected in human myometrium of the uterus and is up-regulated during pregnancy [65]. Ghrelin may act as an orexigenic cue to maintain a positive energy balance during pregnancy and to ensure the metabolic needs of fetuses are met. Interestingly GOAT mRNA levels have been shown to be regulated by testosterone in isolated rat stomach [1].

2.2.5. Chondrocytes

Gomez et al. studied the influence of several hormonal and chemical stimuli on GOAT expression in cultured murine and human chondrogenic ATDC-5 and T/C-28a2 cell lines and showed that bacterial lipopolysaccharide (LPS) treatment decreased GOAT expression. However, neither GH nor ghrelin regulates GOAT mRNA expression in these cell lines [36]. Future studies are needed to better understand the role of ghrelin/GOAT signaling on the skeletal system.

2.2.6. Gustatory system

Recently, GOAT was found to be present in the gustatory system, in taste buds of the mice tongue [80]. Using genetic mutant mouse models (ghrelin, GOAT and GHSR null mice), the researchers showed that taste responsiveness to salty and sour flavors was modulated by the ghrelin-GOAT system. These intriguing findings suggest that taste perception is linked to periphery energy balance and that the ghrelin/GOAT system may play a role in regulating chemosensing [3,80].

2.2.7. Systemic circulation

Activity and protein levels of GOAT in the circulation are difficult to measure because this enzyme is membrane bound. Recently, an antibody against an extracellular loop of the GOAT was developed [82]. Studies using this antibody suggest that GOAT is released into the plasma. However, the specificity of this antibody has not been tested in GOAT-deficient mice and requires further characterization. Until reliable and highly specific anti-GOAT antibodies are made, GOAT production can be estimated by measuring the GOAT gene *MBOAT4* mRNA level. Further, GOAT activity can be measured indirectly by quantifying the amounts of DAG and AG in tissues and in circulation.

2.2.8. Factors that regulate GOAT expression

An interrelationship between ghrelin/GOAT and the mammalian target of rapamicyn (mTOR), an intracellular fuel sensor that is critical for cellular energy homeostasis, has recently been described [21,26,99]. Inhibition of mTOR signaling leads to an increase in plasma ghrelin, gastric GOAT mRNA levels and food intake [99].

Based on the previously reported leptin regulation on the ghrelin system [64] and the well known antagonistic action of these hormones on food intake, some investigators studied the role of exogenously administered leptin on gastric GOAT mRNA levels in rodents under different experimental conditions [31,37]. Gonzalez et al. found a potent stimulation of GOAT mRNA expression with leptin treatment in 48 h food deprived rats, an effect that was not reproducible in fed conditions [37]. The authors speculated that the low levels of leptin in the fasting state might act as a repressor for GOAT. Gahete et al. also reported similar results in a mouse model of leptin deficiency (*ob*/*ob*) where leptin administration significantly increased gastric GOAT mRNA levels as compared to the pair-fed controls [31].

In addition to fasting and feeding, gastric GOAT expression is regulated in a rodent model that mimics most of the clinical symptoms of systemic infections induced by LPS [52,82]. LPS injection suppresses plasma GOAT levels and upregulates gastric GOAT protein levels but not its expression in rats. These effects are accompanied by an inhibition of AG and DAG in plasma by 53 and 28% respectively, resulting in a decreased AG/DAG level. This work raises the possibility of an extracellular acylation process of ghrelin and suggests that GOAT regulation by LPS could be responsible for some of the anorexic effect of systemic infection [82].

2.3. GOAT and the metabolic control

2.3.1. Nutritional status

Studies investigate the nutritional regulation of gastric GOAT expression have yielded conflicting results. Some studies in mice showed that the expression pattern of GOAT is similar to that of the ghrelin gene, with an increased expression after 24 h or 48 h of fasting and in states of negative energy balance, and a low expression in states of positive energy balance [31,99]. However, in another study in rats, GOAT expression was found to be unchanged after a 48 h of food deprivation [37]. Kirchner et al. also found that gastric GOAT expression is highest when mice were fed *ad libitum* and low after 12, 24, 36, and 48 h of fasting. The decreased GOAT expression is in parallel with the low AG concentration in blood during prolonged fasting [49]. In the state of chronic malnutrition, Gonzalez et al. found that GOAT expression in the gastric mucosa and plasma AG levels were significantly increased after 21 days of 70% food restriction in rats [37]. However, this finding is contradictory at least in part to the previous observation in Wistar rats where after 5 months of 35% dietary restriction, the levels of GOAT mRNA from the whole stomach were significantly decreased [69]. The differences in experimental conditions, the duration of fast, the amount of caloric restriction, and the site of tissue sample collection may be responsible for the discrepancy in the literature.

In humans, total ghrelin levels are high in patients with severe malnutrition or anorexia nervosa [61]. Similar to observations made in rodents, after a long term fasting of 62–64 h, AG remained low while DAG was at the peak preprandial levels in healthy subjects suggesting a separated regulation process for ghrelin secretion and acylation [54]. Taken together, observations made in rodents suggest that ghrelin acylation is influenced by food intake and the availability of fatty acids in the diet. Therefore, GOAT may function as a modifier for the adaptive response to feeding and fasting.

2.3.2. Post-natal development

Developmental changes of GOAT in different tissues have not been well studied. Gonzalez et al. studied the expression of gastric GOAT in 10, 25 and 60 day old male rats and failed to find any difference between these experimental groups [37]. Conversely, other groups have shown an age dependent increase in gastric GOAT mRNA levels parallel to body weight gain in both male and female rats that were 2, 4, 6 and 8 weeks of age. These changes in GOAT mRNA level correlate with AG secretion levels from gastric explants [1] and are in concordance with previous work revealing a 21-fold higher GOAT expression in 7-month old as compared to 5-week old rats [69]. In addition, GOAT expression has been reported to be higher in male than female rats that were both 8 weeks old. The earlier developmental maturation in females may be the explanation for the gender difference in GOAT mRNA expression. Conversely, breastfeeding suppresses gastric GOAT mRNA levels in male, but not in female, rat pups [1]. It has been proposed that GOAT may function as a defense of body weight during early development by modifying the ratio of AG/DAG ratio [1]. Further studies are needed to elucidate the role of GOAT in the regulation of energy homeostasis and other physiological functions during development in both genders.

2.3.3. Body weight and lipid metabolism

Several bodies of work provided evidence that the endogenous GOAT-ghrelin system plays a role in the control of energy balance and metabolism [1,4,12,37,49,99,103]. AG administration leads to increased food intake, body weight and fat accumulation. Similar to the ghrelin knockout [ghrelin(−/−)] mice, GOAT(−/−) mice on a chow diet have normal body weight, fat mass, food intake, fat free mass and body length as WT that are on a standard chow diet [49,85,98,103]. The lack of body weight and growth phenotype is probably due to the developmental compensation in the mutant mouse model. However, an improved glucose tolerance and diet induced obesity resistance phenotype was observed in the ghrelin(−/−) mice [98,105].

To dissect the role of GOAT in metabolic control and energy balance, Kirchner et al. studied the phenotype of GOAT(−/−) mice fed a high fat diet (58% of calories from fat). In the absence of AG, these mice had lower body weight but similar body composition compared to the WT [49]. To test the hypothesis whether GOAT activity depends on the diet composition and specifically on the amount of MCFAs, they fed the GOAT(−/−) mice a diet that contained 10% of calories from glyceryl trioctanoate and glyceryl tridecanoate, MCT constituted for octanoic (C:8) and decanoic (C:10) acids respectively [49,62]. On this diet, the GOAT(−/−) mice had lower body weight and lower fat mass than the WT controls. Interestingly, this difference was due to higher energy expenditure instead of a result of decreased food intake. To uncover the physiological function of this enzyme, Tg mice that overexpress ghrelin and GOAT in the liver were generated and they have high levels of DAG in the circulation but lack the octanoyl-modified form of ghrelin. This is probably due to the lack of MCFAs in the liver under normal dietary conditions. When a triglyceryl octanoate diet was fed to these animals, their circulating concentration of AG significantly increased accompanied by a higher body weight and fat mass in contrast to the WT controls, reinforcing the importance of diet composition on ghrelin acylation. Furthermore, food intake did not differ between WT and Tg mice, but energy expenditure was significantly lower during both the dark and light phase in the Tg mice. These data indicates that Tg mice oxidize less fat than the WT. Interestingly the overexpression of human ghrelin in this mouse model is inducible and reversible by specific diets [49].

In summary, the available evidence suggests that GOAT is involved in the regulation of energy balance and its activity is influenced by the availability and composition of dietary fatty acids. The effect of GOAT is accomplished by modulating the process of ghrelin acylation, i.e. AG production. The ghrelin system is involved in meal preparation to optimize energy storage and may serve as a lipid sensor to signal the brain for the abundance of calories rather than functioning as a meal initiation signal as previous thought [29,49,76].

2.3.4. Glucose metabolism

Like ghrelin, GOAT is expressed in rat and human islets and has been implicated in the regulation of glucose metabolism and insulin secretion [12]. In addition to GHSR-1a, the intra-islet GOAT system may play a role in mediating the effect of ghrelin on insulin secretion. Insulin has been shown to inhibit GOAT mRNA and protein expression in INS-1 cells via the mediation of mTOR signaling [4]. Intriguingly, islets isolated from mice and humans treated with a GOAT inhibitor showed an enhanced glucose-stimulated insulin secretion and a reduction in blood glucose. This was accompanied by a 20-fold decrease in uncoupling protein (UCP)-2 mRNA level [12]. AG has been shown to suppress insulin secretion and impair glucose tolerance in both rodents and humans [86,87,92] whereas DAG has been shown to have opposing effects of AG on glucose tolerance, insulin secretion and insulin sensitivity in rodents and even in humans [32,33]. The metabolic phenotype of GOAT(−/−) mice that lack AG appears to be dependent on nutritional states. GOAT(−/−) mice that were subjected to a 6-h fast showed normal glucose tolerance [49]. However, after a 16-h fast, these mice demonstrated improved glucose tolerance and increased insulin secretion [103]. Furthermore, when the GOAT(−/−) mice was subjected to a 60% caloric restriction, they developed severe hypoglycemia and were morbid bound after 7 days when their body fat was down to 2% while the WT animals were able to maintain their blood glucose. The inadequate GH secretion in the GOAT(−/−) mice was thought to be the explanation for their inability in preserving blood glucose. Infusion of either ghrelin or GH normalized blood glucose in GOAT(−/−) mice and prevented death [103]. This finding indicates a potentially important protective role of endogenous ghrelin against hypoglycemia in the state of severe energy deficiency. More studies are needed to confirm these findings.

In summary, the role of endogenous ghrelin in the control of glucose homeostasis, while potentially one of the hormone's most exciting ones, remains controversial and mechanistically poorly understood. A proposed role in protection from hypoglycaemia during severe caloric deprivation remains to be confirmed.

2.4. GOAT as a therapeutic target

Metabolic disorders such as obesity and type 2 diabetes are major public health threats. Given the known effect of ghrelin on appetite stimulation, fat accumulation and glucose homeostasis, it is logical to speculate that ghrelin receptor antagonism would be beneficial for metabolism. However, there are some disadvantages associated with this approach. First, antagonizing its action might increase AG levels and thus change the AG/DAG ratios with the subsequent repercussions; second, the main ghrelin site action is the brain so the putative drug based in ghrelin antagonism should had a lipid moiety to cross the BBB decreasing the drug bioavailability and third, ghrelin acts on several complex endocrine axis and ghrelin mimetics and (GHSR)-1a antagonists could introduce multiple unwanted side effects. Furthermore, several of the physiologic functions of ghrelin occur in the CNS thus targeting GOAT instead of the ghrelin receptor could avoid possible CNS side effects as previously seen with other anti-obesity drugs [2,20]. Therefore, GOAT inhibition is considered to be the most promising therapeutic target in the ghrelin system for modulating body weight and glucose control to date.

2.4.1. GOAT analog and the metabolic control

A GOAT specific inhibitor, bisubstrate analog GO-CoA-Tat, has recently been developed. This analog is composed by three elements: ghrelin and octanoyl Co-A (substrates for GOAT) binding with a non cleavable bridge and a third piece namely Tat sequences that allow penetrate the analog within the cell where ghrelin octanoylation take place [12]. This analog bisubstrate inhibits AG but not DAG *in vitro* and *in vivo*. In GOAT/preproghrelin transfected HeLa cells the maximum inhibition is at 24 h of incubation, suggesting that ghrelin exists in pre-existing reservoirs within the cell. *In vivo* the maximum efficiency of AG inhibition is after 6 h of intraperitoneally injection in wild types animals on a MCT diet [12].

Chronic GO-Co-A-Tat treatment in mice prevented weight gain as observed in vehicle treated mice on a MCT-rich high fat diet. Moreover, the GO-CoA-Tat treated animals displayed significant lower fat mass but not lean mass. They also displayed lower blood glucose as well as lower levels of insulin-like growth factor 1, which is consistent with the effect of endogenous AG in modulating the somatotropic axis. GO-CoA-Tat did not significantly alter body weight, fat or lean mass in GOAT(−/−) mice in comparison to WT mice [12].

This analog has an impact on glucose tolerance and insulin secretion *in vivo* and *in vitro*. Mice pretreated with the GOAT analog displayed a significant increase in insulin secretion and a blood glucose reduction that were not seen in the GOAT(−/−) mice. These results support the hypothesis that GO-CoA-Tat regulates glucose metabolism through AG inhibition. An enhanced insulin secretion was also observed in human pancreatic islets cells pretreated with the analog [12].

3. Conclusions

The recent discovery of GOAT along with the multiple and pleiotropic roles played by several AG/DAG isoforms of ghrelin not only meant a significant step forward in endocrinology, obesity and potentially diabetes research, but also opened up new and exciting ways to dissect the molecular underpinnings of novel ghrelin actions. Recent experimental evidence suggests a relevant role of DAG, although currently unknown, as indicated by the high levels of this hormone versus AG in normal conditions and especially in GOAT knockout mice. Other point to take into account is the possible existence of other possible unknown receptors for ghrelin as suggested by some studies with DAG and some with AG. As mentioned above, the advances in basic ghrelin research have improved our understanding of body weight regulation and are generating new approaches for controlling specific diseases. GOAT could be a suitable target within the ghrelin system with some advantages over targeting the ghrelin receptor. Ghrelin has two characteristics which have attracted a considerable research community: (A) it is a unique peptide that undergoes a posttranslational modification with an octanoic acid being added and (B) it is the only potently orexigenic peripheral hormone known to date. Thus targeting GOAT or enzymes relevant for the deacylation process could be a promising strategy for the development of future therapeutics. Improved insight into ghrelin and GOAT physiology is furthermore an important requirement, which could lead to additional pharmacological opportunities. It for example remains an open question if ghrelin can be acylated in cells outside the stomach or the duodenum or to which extent GOAT may be able to utilize endogenous fatty acid as substrates during food deprivation. Additional genetically modified animal models and new technology to manipulate gene expression *in vivo* in adult animals will help to reach a more complete understanding of the GOAT/ghrelin system in metabolic control.

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