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Ghrelin: An endogenous ligand for growth hormone secretagogue receptor (GHS-R)

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ABSTRACT

Ghrelin is a peptide hormone secreted into circulation from the stomach. It has been postulated to act as a signal of hunger. Ghrelin injection acutely increases energy intake in lean and obese humans and chronically induces weight gain and adiposity in rodents. Circulating ghrelin levels are elevated by fasting and suppressed following a meal. Inhibiting ghrelin signaling therefore appears an attractive target for anti-obesity therapies. A number of different approaches to inhibiting the ghrelin system to treat obesity have been explored. Despite this, over a decade after its discovery, no ghrelin based anti obesity therapies are close to reaching the market. This article discusses the role of ghrelin in appetite control in humans, examines different approaches to inhibiting the ghrelin system and assesses their potential as anti-obesity therapies.

Keywords: Ghrelin, Growth Hormone Secretagogue Receptor, Obesity, Appetite.

INTRODUCTION

In 1999, ghrelin was discovered in gastric extracts as a natural ligand of the orphan growth hormone secretagogue receptor type 1a (GHS-R1a) [1]. Through this receptor, it acts as a growth hormone releasing peptide and food intake modulator [1]. GHS-R1a is expressed in the hypothalamus and pituitary, which is consistent with the GH-releasing and appetite stimulating effects of ghrelin [2]. However, it has been demonstrated that GHS-R1a is also expressed in other CNS areas and peripheral tissues, where ghrelin is also expressed; suggesting that ghrelin possesses many others affects besides the release of growth hormone (GH) and the stimulation of food intake. In fact, numerous studies have shown that ghrelin also affects energy and glucose homeostasis, gastrointestinal, cardiovascular, pulmonary and immune function, cell proliferation and differentiation and bone physiology [3,4]. Although the major active product of ghrelin gene is a 28-amino acid peptide acylated at the serine 3 position with an octanoyl group (C8:0), called simply ghrelin, recent developments have shown that ghrelin gene can generate various bioactive molecules besides ghrelin, mainly des-acyl ghrelin and obestatin, obtained from alternative splicing or from extensive post-translational modifications [5,6]. Although their receptors have not yet been identified, they have already proven to be active, having intriguingly subtle but opposite physiological actions to ghrelin [3,4].

GHS-R antagonists are expected to perform antiobesity functions by suppressing food intake and weight gain. In fact, small-molecule GHS-R antagonists and [D-Lys-3]-GHRP-6, which is one of the few known peptide antagonists of GHS-R, decrease food intake and weight gain via peripheral injection [7]. Because a peptide can be chemically

synthesized and is unlikely to act as an antigen, peptide drugs are attractive candidates to replace antibodies in drug therapies targeting specific molecules. The exploration of how novel peptides bind to a cell surface receptor from a randomized peptide library has been achieved using phage display [8]. Drugs developed from peptides that bind to thrombopoietin [9, 10] and erythropoietin receptors [11] were discovered by phage display and have been used in therapies [12].

DISCOVERY OF GHRELIN

GHSs are a family of small synthetic peptides and non-peptide molecules that stimulate the secretion of GH in several species, including in humans. In 1977, Bowers and colleagues developed the first GHS peptides derived from Met-enkephalin, which stimulated in vitro the release of GH from pituitary cells, although the potency of these peptides was rather weak [13, 14]. Further development led to the production of several new peptides with increased potency, including GH-releasing peptide-6 (GHRP-6, His–D-Trp–Ala–Trp–D-Phe–Lys-NH2) and hexarelin, both of which are active in vitro and in vivo [15, 16]. Based on the structure of GHRP-6, peptidomimetics of GHS such as MK-0677 (a spiroindoline with marked bioavailability and long-lasting effects after oral administration) were developed by Merck [15]. The primary action of the GHSs is the stimulation of GH release from the somatotroph acting through a mechanism distinct from that of GH-releasing hormone (GHRH). GHRP-6 activates the phospholipase C (PLC) pathway, resulting in an increase the intracellular Ca2+ through inositol 1,4,5-trisphosphate- (IP3)- mediated signal transduction, a pathway distinct from the adenosine-3',5'-monophosphate- (cAMP)-dependent protein kinase (protein kinase A, PKA) pathway utilized by the GHRH receptor [17–19]. In 1996, the Merck group identified and cloned the GHSR by utilizing expression cloning and exploiting their compound MK-0677. Changes in intracellular calcium concentrations were indicative of a positive response [20–22].

The second endogenous ligand for GHSR was also isolated from the rat stomach [23]. The ligand des-Gln14-ghrelin, a 27-amino acid peptide with an n-octanoyl modification at Ser3, is identical to ghrelin except for deletion of one glutamine, and is produced through alternative splicing of the rat ghrelin gene. Des- Gln14-ghrelin has the same potency as ghrelin for inducing increases in intracellular Ca2+ concentrations in GHSR-expressing cells, and for increasing plasma GH concentrations in rats. On the other hand, the unmodified, des-n-octanoyl form of ghrelin (desacyl ghrelin), has no effect on the elevation of intracellular Ca2+ and GH secretion. In structure–activity analysis, the octanoic acid is not the only modification group of the Ser3 side chain that can sustain the activity of ghrelin; some other fatty acid modifications can also maintain activity.

The Ghrelin Molecule

The fatty acid (n-octanoyl) side chain at Serin 3, a biochemical feature which is essential for ghrelin´s bioactivity, makes this gastrointestinal peptide hormone an endogenous factor unique in mammalian biology [24]. While cleaving more than 50% of its 28 amino residues starting from the C-terminal end of the ghrelin molecule (down to less than 14 amino residues) hardly influences binding or activation of its receptor in vitro, minor modifications of the postranslationally added n-octanoic acid already impair receptor binding or activation [25]. Aminoacid 28 (Arg) is naturally cleaved in an unknown percentage of stomach derived circulating ghrelin molecules, resulting in a 27 AA long peptide, which is still bioactive. As reported very recently, the octanoyl side chain occurs in at least two different sizes (C8 and C10), while the identity of a putative acyl-transferase that is presumably located in the stomach and should be responsible for the “activation” of ghrelin via octanoylation, is still at large [26]. Degradation processes are also believed to mainly involve enzymatic processes, since the half-life of ghrelin in circulation is estimated to be between 5 and 15 minutes. The only factor that has been shown to “deactivate” ghrelin is High Density Lipoprotein (HDL), which can bind and des-acylate ghrelin to a significant extent, thereby depriving it of its ability to bind and activate the ghrelin receptor GHS-R1a [27].

Control of Ghrelin Secretion

Regulation of ghrelin levels and action involves several mechanisms that are, at least in part, independent. As suggested by van der Lely and coauthors [28], these mechanisms include: 1) regulation of transcription and translation of the ghrelin gene; 2) regulation of post-translational processes of the ghrelin molecule (i.e. acylation and deacylation) and regulation of levels and/or activity of the putative enzymes involved in the post-translational processing; 3) secretion rates of the bioactive ghrelin molecules; 4) possible existence of ghrelin binding proteins and their effects on hormone’s bioactivity; 5) accessibility of target tissue (i.e. blood-brain barrier transport); 6) clearance or degradation of ghrelin by kidney or liver passage; 7) circulating concentration of additional endogenous ligands or other possibly cross reacting hormones; 8) ghrelin receptor(s) levels of expression and activity in target tissues.

Ghrelin and Energy Balance

Chronic Intracerebroventricular injection of ghrelin strongly stimulates feeding in rats and increases body weight gain [29]. Daily subcutaneous injection of ghrelin in mice induces a progressive increase in body weight, with a significant gain in fat mass but no change in lean body mass. This could result from a chronic decrease of fat oxidation as indicated by an increased respiratory quotient [30]. Because ghrelin can induce adiposity that is sustained during ghrelin treatment, ghrelin might participate in the long-term regulation of body mass. In humans, studies suggest that weight loss increases circulating ghrelin levels [31, 32]. GH is also known to be an important anabolic agent, and has been used to counteract muscle wasting associated with surgical stress, sepsis, glucocorticoid administration, and AIDS [33]. Ghrelin secretion counteracts further decreases in energy storage and prevents starvation or cachexia. Therefore, evaluation of the role of ghrelin in the pathogenesis and treatment of such cachectic conditions is warranted [34]. Ghrelin and GH might serve as anabolic signaling molecules during energy depletion.

GHRELIN AND THE CENTRAL REGULATION OF FEEDING

Ghrelin is the first identified hormone that acts as a starvation-signaling molecule from the stomach and stimulates feeding after peripheral administration. After ghrelin is administered to the CNS, neurons expressing the immediate-early transcription factor c-Fos are observed primarily in regions implicated in the regulation of feeding behavior, including the paraventricular nucleus, the arcuate nucleus (ARC), and the dorsomedial and ventromedial hypothalamic nuclei [29], suggesting that ghrelin might contribute to central control of energy homeostasis such as body temperature and energy expenditure. This distribution coincides with that of GHSR [35]. GHSR mRNA is expressed in 94% of the neurons in the ARC that express NPY, in 8% of cells that express pro-opiomelanocortin (POMC), in 30% of those that express somatostatin, and in 20–25% of those that express GHRH mRNA [36].

The ARC is an important site for translating input from diverse hormonal signals into behavioral and metabolic responses that powerfully influence energy balance [37]. NPY and AGRP—orexigenic molecules—are expressed in the same neurons in the medial ARC [38, 39], whereas POMC and the cocaine- and amphetamine-regulated transcript (CART) — anorexigenic molecules—are expressed in the lateral ARC [40], AGRP antagonizes the actions of α-melanocyte-stimulating hormone (α-MSH) at the melanocortin 4 receptor (MC4-R), and is therefore orexigenic [41, 42] (Figure 2). Intracerebroventricular injection of ghrelin leads to increases in the expression of both NPY and AGRP mRNAs, and pretreatment with NPY-specific or AGRP specific antibodies, or with a antagonists to NPY or AGRP receptors significantly inhibits ghrelin-induced feeding [29]. Because ghrelin injection did not alter the expression of POMC mRNA, these results indicate that ghrelin-dependent orexigenesis is mediated by the output of the ARC NPY–AGRP neurons. The ARC is a crucial target of leptin, an anorexia mediating molecule produced from adipose tissue [37]. Most NPY–AGRP–producing, or POMC–CART–producing neurons also express leptin receptors, and both types of neurons are regulated by leptin, albeit in an opposing manner [43]. Leptin inhibits ghrelin-induced feeding, and ghrelin substantially reverses the anorexic effect of leptin, indicating that ghrelin may antagonize leptin action in regulating the NPY–AGRP system (Figure 1).

Leptin stimulates the POMC anorexigenic pathway and inhibits the NPY–AGRP orexigenic pathway, resulting in reduced food intake. The effect of ghrelin in the hypothalamus is opposite to that of leptin. The orexigenic effect of ghrelin is mediated by activating on the output of the NPY–AGRP neurons. Fasting increases ghrelin and decreases leptin production, leading to the activation of the orexigenic pathway. This response might be important for the adaptation to fasting.

Effect on carbohydrate metabolism

The hypothesis that ghrelin could play a role in the regulation of glucose homeostasis and insulin secretion was based on the observation that, as shown in the previous sections, several biological activities of AG are mediated by the cholinergic system/vagus nerve, which also plays a pivotal role in the regulation of the endocrine pancreas. Moreover, ghrelin (including both AG and UAG) is expressed in pancreatic islets, where it is present already during fetal development, whereas it decreases during adulthood [44, 45]. The expression of GHS-R1a in the endocrine pancreas has been found by several groups [46, 47]. Furthermore, previous reports in the literature described an effect of synthetic GHSs on insulin and glucose levels, although these metabolic actions were supposed to be mediated by the neuro-endocrine activity that GHSs exert at pituitary level. In fact, the increase in plasma glucose levels induced by sustained treatment with GHSs in obese rats was thought to be due to GHSs-induced activation of hypothalamo-pituitary adrenal axis [48]. Similarly, chronic treatment with MK-0677, a non-peptidyl GHS, induced hyperglycemia and insulin resistance in lean, but not in obese, elderly subjects and this phenomenon was supposed

to reflect increased GH secretion [49]. However, a possible mediation by GH of the GHSs-induced modulation of glucose homeostasis was ruled out by the observation that GHRP-6, in fed conditions, induced a rise in glucose as well as in insulin and free fatty acid (FFA) levels in the presence of GH receptor antagonism by pegvisomant.

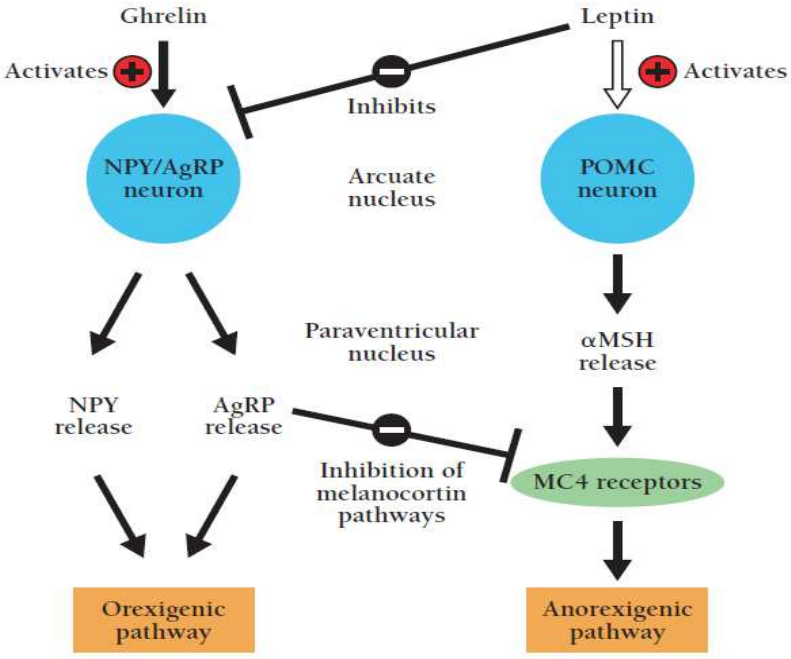

Fig 1: A simplified model of the feeding regulatory signaling of ghrelin and leptin

The first report showing an effect of ghrelin on glucose homeostasis was by Broglio et al., who observed that AG injection to healthy subjects induced an acute and significant increase in glycemia that was followed by a transient decrease in circulating insulin levels [50, 51]. These metabolic effects were not induced by UAG [52] or by a synthetic GHS, although the latter potently stimulated GH release to the same extent as AG [50]. This, along with the fact that glucose and insulin changes persisted over 2 hours after AG administration, in contrast with a more transient increase in GH levels, suggested that the metabolic actions of AG were GH independent.

Overall, these findings suggest that the gut hormone AG may exert a significant role in the regulation of insulin secretion and glucose metabolism. AG might integrate the General hormonal and metabolic response to fasting that, at least in humans, is accompanied by a clear-cut increase in GH secretion coupled with inhibition of insulin secretion and activation of mechanisms devoted to maintaining glucose levels [53].

Effect on lipid metabolism

Ghrelin has been reported to increase body fat, also independently of changes in food intake [54]. A specific effect of ghrelin on lipid metabolism was suggested by the observation that rodents treated with AG showed enhanced fat content independently of feeding behaviour, as assessed by magnetic resonance imaging (MRI), increased respiratory quotient (suggesting enhanced carbohydrates utilization and decreased fat utilization), dual energy x-ray absorptiometry (DEXA) and weight of omental and retroperitoneal fat pads [55, 56]. In fat tissue AG and UAG promote adipogenesis and inhibit lipolysis, whereas they also modulate lean tissue fat distribution and metabolism. In fact, AG as well as UAG was shown to favour adipogenesis when infused to rodent bone marrow [57]. The increase in respiratory quotient following both central and peripheral injection of AG is likely to reflect reduced whole body lipid oxidative utilization [54].

More recently, Barazzoni and colleagues [58] showed that sustained AG injection in rats (twice-daily for four days) modulates lipid metabolism also in non adipose tissues, including liver and skeletal muscle, increasing body weight, but not food intake. In the liver, AG induced lipogenic and glucogenic patterns of gene expression and triglyceride content, whereas the activity of the stimulator of FFA oxidation, AMP-activated kinase (AMPK), was reduced and

mitochondrial oxidative enzyme activities were unchanged [58]. In muscle, AG reduced triglyceride content, increased mitochondrial oxidative enzyme activities and increased mRNA encoding uncoupling protein-2, independent of changes in expression of fat metabolism genes and phosphorylation of AMPK. Thus, AG favors triglyceride deposition in liver over skeletal muscle, suggesting that AG could be involved in adaptive changes of lipid distribution and metabolism in the presence of caloric restriction and loss of body fat. AG treatment also significantly increased the mRNA levels of important regulators of tissue fat metabolism and content, such as peroxisome proliferator activated receptor (PPAR)-γ, in primary cultured rat differentiated adipocytes and in muscle [58, 59].

The actions of AG on lipid metabolism are unlikely to be mediated by GHS-R1a, since epididymal adipose tissue or isolated adipocytes did not express GHS-R1a mRNA, but showed a common high-affinity binding site recognized by AG and UAG and also synthetic, peptidyl and non-peptidyl GHSs . In keeping with this, bone marrow adipogenesis was stimulated also by UAG, but not by a potent GHS-R1a agonist [57].

In conclusion, both AG and UAG promote adipogenesis and inhibit lipolysis, probably acting via a yet unknown receptor, different from GHS-R1a.

Gastrointestinal function

Available data suggest that ghrelin affects many aspects of GI function, including exocrine secretion, epithelial protection and motility. Results regarding the effects of ghrelin on gastric acid secretion are equivocal, as stimulation (probably via vagal pathways stimulating parietal cells) [60], inhibition [61], and lack of effect [62] have been reported. It was suggested that conflicting data may reflect the presence of both stimulatory and inhibitory pathways, and that experimental conditions and models may determine how they balance out. The stimulatory action of ghrelin on gastric secretion may be important in preparing the stomach to process food. Ghrelin is also able to stimulate pancreatic protein secretion via central pathways [63].

Ghrelin may also stimulate cell proliferation and differentiation of the gastrointestinal epithelium. Indeed, a gastroprotective effect has been demonstrated in various models, which seems to depend mainly on vagal activity, sensory nerves and hyperaemia mediated by nitric oxide synthase–nitric oxide (NOS–NO) and cyclooxygenase–prostaglandin systems [64,65]. The protective effect has also been demonstrated in experimental colitis [66]. A recent study showed that, both intraperitoneally and centrally administered, ghrelin suppresses mucosal intestinal apoptosis in fasting rats [67].

Structural relationships between ghrelin and motilin, lead to evaluation of the motility effects of ghrelin. Indeed, it was demonstrated that ghrelin, like motilin, induces the migrating motor complex (MMC) and accelerates gastric emptying, both in humans and rodents [68,69]. Also, ghrelin seems to accelerate colonic motility [70]. Prokinetic actions of ghrelin are mediated by the cholinergic system, via central mechanisms and probably by the myenteric plexus [71].

Des-acyl ghrelin does not seem to influence gastric secretion [62]. Regarding to motility, des-acyl ghrelin inhibits gastric emptying without altering small intestinal transit [72]. Peripheral des-acyl ghrelin may induce this function by direct activation of brain receptor (CRF 2) by crossing the blood-brain barrier but not by the activation of vagal afferent pathways [72]. The effects on cell proliferation and differentiation of the gastrointestinal epithelium were not yet determined. Recently, obestatin was demonstrated to stimulate the secretion of pancreatic juice enzymes through a vagal pathway in anaesthetized rats [73]. As with the effects of obestatin on regulation of food intake, its effects on gastrointestinal motility are involved in great controversy with most studies reporting no effect [74] and others reporting a negative effect (in gastric and jejunal motility) under basal and ghrelin-stimulated conditions [75]. Further studies should clarify this issue and its effects on gastric exocrine secretion and epithelial protection.

Cardiovascular function

Numerous studies suggest that ghrelin has a wide array of cardiovascular activities. Regarding the macrocirculation, the vasoactive effects of ghrelin depend on the vascular territory. In the systemic circulation, ghrelin has a vasodilatory effect that is endothelium-independent [76,77] and involves peripheral and central mechanisms [78]. Indeed, intravenous injection of ghrelin in humans causes a significant decrease in mean arterial pressure, but does not change heart rate [79]. By contrast, ghrelin increases coronary perfusion pressure in rat hearts perfused using the Langendorf system and significantly increases pressure-induced myogenic tone in coronary arterioles [80]. In the

microcirculation, ghrelin increases vascular flow, and this action may affect other physiological functions of ghrelin [81].

Ghrelin is also able to improve endothelial function by inhibiting basal and TNF-a-induced chemotactic cytokine production, increasing nitric oxide bioactivity and inhibiting angiotensin II-induced migration of human aortic endothelial cells [82]. Salutary cardiotropic effects of ghrelin have been demonstrated in various experimental models. These may result not only from an increase in GH, appetite and vasodilation, and decrease in cytokine production, but also from direct effects of ghrelin on cardiomyocytes. In vitro, ghrelin decreases inotropism [83] and lusitropism [84], inhibits apoptosis of cardiomyocytes [85], improves myocardial function during ischemia/reperfusion and isoproterenol-induced injury [86] and reduces infarct size [87]. In healthy volunteers [88] and patients with chronic heart failure [89], ghrelin decreases systemic vascular resistance, which results in increased cardiac output as shown by increased cardiac index and stroke-volume index.

Immunomodulation

GHSR mRNA is expressed in several lymphoid organs [90] and in various leukocyte subsets including T and B cells, monocytes [91], suggesting that ghrelin might play some role in the generation and/or control of immune interactions. In fact, various studies demonstrated that ghrelin may modulate immune cell proliferation and activation and secretion of proinflamatory cytokines. Chronic injection of a ghrelin mimetic to old mice stimulated growth, differentiation and cellularity of the thymus, in addition to increasing T-cell production [92]. This resulted in an enhanced resistance to the initiation of neoplasms and subsequent metastasis in animals inoculated with lymphoma cells and an improved thymic engraftment in bone marrow transplant recipients [92]. In vitro studies showed that ghrelin inhibited ROS generation by human PMN in a dose-dependent manner [93]. Interestingly, ghrelin ameliorates pancreatic obiliary inflammation and associated remote organ injury induced by pancreatic obiliary obstruction, by inhibiting neutrophil action [94]. Ghrelin also modulates the production of proinflammatory cytokines. Recently, it has been shown that ghrelin and the GHS-R are found in human T cells and monocytes, where ghrelin specifically inhibited chronic, LPS- and leptin-induced synthesis of proinflammatory anorectic cytokines such as leptin, interleukin 1b (IL-1b), IL-6 and tumor necrosis factor a (TNF-a) [95,91]. These data have established a novel role for ghrelin in immune cell function as a negative regulator of inflammatory cytokine expression induced by cell activation by antigen, mitogens, or leptin, Onthe contrary, des-acylghrelin does not influence immune function [96], while effects of obestatin on immune system were not yet determined.

Bone physiology

Recent studies indicate that ghrelin is also involved in the regulation of bone growth and metabolism as one of its peripheral effects. It has been demonstrated that primary osteoblasts, as well as osteoblastic cell lines of various species, express GHS-R1a [97]. Evidence was also provided that ghrelin treatment directly stimulates fetal rat calvarial osteoblastic cells proliferation and differentiation, alkaline phosphatase activity and calcium accumulation in the matrix primary [97]. In addition, ghrelin increased bone mineral density of both normal and GH-deficient rats [98]. These observations show that ghrelin directly stimulates bone formation. Data regarding effects of des-acyl ghrelin and obestatin on bone physiology are very limited. A recent study demonstrated des-acyl ghrelin stimulates human osteoblasts proliferation in the absence of GHS-R1a [99]. Unlike ghrelin, obestatin does not exert any relevant activity in chondrocytes [100].

APPROACHES TARGETING THE INHIBITION OF THE GHRELIN SYSTEM

Several different approaches have been used to attempt to target the ghrelin system to ameliorate obesity:

Antagonizing the ghrelin receptor (GHS-R1a)

The ghrelin receptor, GHS-R1a, was first identified as the receptor through which growth hormone secretagogues (GHSs) act [101]. This discovery was made several years prior to the discovery of ghrelin. Development of GHS-R1a antagonists began with the identification of GHS-R1a and efforts and investment escalated following the discovery of ghrelin and elucidation of its role in energy homeostasis. It was hypothesized blocking ghrelin signaling through antagonizing the GHS-R1a would suppress pre-prandial feelings of hunger due to high ghrelin levels. A number of pharmaceutical companies have now developed GHS-R1a antagonists. In 2007 Bayer reported their GHS-R1a antagonists which were though to penetrate the central nervous system (CNS) reduced body weight in diet induced obese (DIO) mice when administered for 10 days [102,103]. Acute injection of their antagonists improved glucose tolerance [102,103]. By contrast another antagonist they had developed thought to have poor CNS penetration improved glucose tolerance but had weak effects on body weight. This suggests that for antagonists to

be effective in regulating body weight they need to cross the blood brain barrier. In addition it suggested effects on glucose homeostasis may be due to the GHS-R1a receptors in the pancreas. This is on accord with the suggestion that ghrelin's effects on energy homeostasis require activation of the GHS-R1a in the CNS possibly either the hypothalamus or the brain stem. Ipsen developed an antagonist, BIM28163, which antagonized the GHS-R1a in vitro and blocked ghrelin's stimulation of GH in vivo. However, BIM28163 caused weight gain rather than weight loss in rodents [104]. This highlights the complexities of the ghrelin system, but also suggests there may be potential to develop antagonist that independently block either ghrelin's effects on appetite or GH. GHRR1a antagonists that suppress appetite but not GH release may be more suitable as an obesity therapy. Further ghrelin antagonists have been developed by a number of other pharmaceutical companies including Abbott laboratories, Zentaris, Merck, Tranzyme, and Novo Nordisk. Few have reported successful in vivo results and we are not aware of any clinical trial where ghrelin antagonists have proved successful as an obesity treatment. This suggests even antagonist which are effective in rodent models, such as those developed by Bayer, have failed at later stages of testing/development. Despite this the search for more effective GHS-R1a antagonists is still ongoing. For example, very recently Amgen reported the development of a piperazinebisamide based GHS-R1a antagonists. They state the compound is now being used for in vivo proof of concept studies [105]. Thus there still belief GHS-R1a antagonism may prove a successful obesity therapy.

A complicating factor is that the GHS-R1a is reported to have high ligand independent constitutive, signaling [106]. It has been reported GHS-R1a signaling can be at half maximum levels in the absence of ghrelin [107,108]. The importance of this is highlighted by the identification of human mutations which cause a loss of GHSR1a constitutive activity. These mutations cause short stature due to growth hormone deficiency [109].

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