Ghrelin as a Potential Anti-Obesity Target

Tamas L. Horvath, Tamara Castañeda, Mads Tang-Christensen, Uberto Pagotto and Matthias H. Tschöp

Depts. of Ob/Gyn and Neurobiology, Yale University School of Medicine, New Haven CT, USA, Dept. of Pharmacology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany, RheoScience A/S, Copenhagen Denmark and Dept. of Endocrinology, S. Orsola-Malpighi Hospital, Bologna, Italy

Abstract: In order to develop an effective pharmacological treatment for obesity, an endogenous factor that promotes a positive energy balance by increasing appetite and decreasing fat oxidation could represent the drug target scientists have been looking for. The recently discovered gastric endocrine agent ghrelin, which appears to be the only potent hunger-inducing factor to naturally circulate in our blood stream, was discovered in 1999. Since then the acylated peptide hormone ghrelin has evolved from an endogenous growth hormone secretagogue to a regulator of energy balance to a pleiotropic hormone with multiple sources, numerous target tissues and most likely several physiological functions. Although neither the exact mechanism of action by which ghrelin increases food intake and adiposity is known, nor the putatively differential effects of brain-derived and stomach-derived ghrelin on energy homeostasis have been determined, blocking or neutralizing ghrelin action still seems one of the more reasonable pharmacological approaches to reverse a chronically positive energy balance. However, based on growing experience with compounds targeting the neuroendocrine regulation of energy balance, it is quite possible that a ghrelin antagonist will either fail to cure obesity due to the existence of compensatory mechanisms or undesired effects might reveal the true biological function of ghrelin (e.g. cardiovascular mechanisms, anti-proliferative effects, reproduction).

INTRODUCTION

The discovery that a novel receptor and its endogenous ligand are controlling several essential physiological functions naturally sparks the interest of the scientific community. Thus, the discovery of the novel hormone ghrelin in 1999 [1], much like leptin in 1994 [2], triggered an enormous amount of research studies focussing on this new endocrine player. The discovery of ghrelin has often been referred to as a classical example of reverse pharmacology, since synthetic ghrelin analogues (growth hormone secretagogues, GHS) were described first, while the natural receptor (GHS-R1a), its endogenous ligand (ghrelin) and the physiological role of ghrelin in energy balance regulation have been discovered stepwise over the last 25 years [1,3,4]. The pharmacological applicability of ghrelin’s endogenous counterpart leptin has been somewhat dissapointing, at least with respect to the treatment of diet induced human obesity [5]. The ghrelin pathway could now offer new therapeutic perspectives as an anti-obesity target, since ghrelin effectively induces hunger in humans and is the only known peripheral orexigenic signal counterbalancing a variety of pancreatic, gastrointestinal and adipocyte derived satiety factors via its appetite-stimulating effects [6]. This review attempts to comprehensively evaluate the potential of ghrelin and its receptor as a drug target, focussing on its role in appetite regulation and energy homeostasis, but also shortly discussing a potential role for ghrelin, ghrelin mimetics or ghrelin antagonists in specific disease entities such as the Prader-Willi Syndrome.

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 [7]. 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 posttranslationally added n-octanoylic acid already impair receptor binding or activation [8]. 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 [9]. 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 [10].

*Address correspondence to this author at the Dept. of Pharmacology, German Institute of Human Nutrition, Arthur-Scheunert-Allee 114-116, 14558 Bergh.-Rehbrücke, Germany; Tel: +49.33200.88.372, Fax: +49.33200.88.334; E-mail: tschoep@mail.dife.de
DETERMINANTS OF GHRELIN ACTION

While an enormous amount of data on ghrelin biology and physiology is currently emerging, it should not be overlooked that additional ghrelin receptors (in addition to the growth hormone secretagogue receptor type 1, GHS-R1a/b), as well as other endogenous ligands of the GHS-R1a, might exist and could play a relevant role [11]. The vast majority of currently available data are either focussing on the GHS-R1a, or are extrapolating from changes of the overall concentration of circulating (bio-active and bio-inactive) ghrelin peptide, that for now is the best available, easily accessible, surrogate parameter for the activity level of the ghrelin pathway. While the detection of relative differences in total circulating ghrelin levels (predominantly representing bio-inactive peptide) between disease states or in response to physiological challenges can be regarded as useful, one should be careful not to over-interpret these data. Once more sophisticated methods to monitor plasma concentrations of active ghrelin as well as expression levels of GHS-R are available, substantial parts of the current view on ghrelin (patho-)physiology might have to be adjusted, if not corrected. The extent and magnitude of ghrelin action must involve multiple regulatory levels, which might, at least in part, be independent from each other. Relevant mechanisms include the (A) regulation of transcription and translation of the ghrelin gene, (B) the level of enzymatic activity of the putative acyl transferase that is responsible for the posttranslational octanoylation of the ghrelin molecule, (C) secretion rates of bioactive ghrelin, (D) putative enzymatic processes de-activating circulating ghrelin, (E) possible influence of ghrelin binding proteins on the hormones bioactivity (e.g. binding of HDL), (F) variable accessibility of target tissue (i.e. blood brain barrier transport), (G) clearance or degradation of ghrelin by kidney or liver passage, (H) circulating concentration of additional endogenous ligands or other possibly crossreacting hormones (I) the amount of expression of ghrelin receptor(s) in target tissue and (K) their sensitivity at the level of intracellular signaling mechanisms.

GASTRIC AND HYPOTHALAMIC GHRELIN

Ghrelin is primarily expressed in the stomach and the upper intestinal tract [11]. However, recent studies using PCR amplification techniques have revealed the potential localization of ghrelin mRNA in several other tissues, such as the kidneys, immuno-competent blood cells, placenta, testicles, ovaries, pancreas, pituitary, hypothalamus and other tissues [12]. In respect to ghrelin’s putative role in the regulation of energy homeostasis, a localisation in the hypothalamus seems to be particularly interesting. Using antibodies as well as RT-PCR, a uniquely distributed hypothalamic group of mostly bipolar neurons has recently been identified as producing small amounts of ghrelin [13]. These neurons are not co-localized with any known centrally expressed hormone or neuropeptide, but they intriguingly do project directly to several previously identified hypothalamic appetite control centers. Ghrelin receptor expression and binding can furthermore be localized in multiple hypothalamic areas in direct neighbourhood to Neuropeptide Y (NPY), Agouti related protein (AGRP), Proopiomelanocortin (POMC), GABA and other neuropeptides and neurotransmitters substantially involved in appetite control. Ghrelin expression can be found in neurons closely situated to, but not identical with, the previously mentioned ones. These neuroanatomical findings, complemented by electrophysiological studies, provided evidence for the existence of a central circuit regulating appetite that involves ghrelin as a relevant modulator [13].

The main source of circulating endogenous ghrelin however is the stomach [14]. Several possibilities regarding the role of central versus peripheral ghrelin in the regulation of food intake are thinkable, but have to be proven or disproven in the future, possibly by using sophisticated approaches such as gastrectomized mice or mice with tissue-specific disruption of the ghrelin gene:

1. Gastroenterally derived circulating ghrelin could be co-modulating central networks regulating energy balance together with hypothalamically derived ghrelin after crossing the blood brain barrier or via neural projection from areas that are not protected by the blood brain barrier (circumventricular organs e.g. median-eminence)

2. Gastroenterally derived circulating ghrelin could be responsible for peripheral effects of ghrelin including i.e. direct effects on endocrine axes at the pituitary level, cardiovascular, anti-proliferative or adipocyte specific effects, while centrally derived ghrelin may mainly modulate energy balance control circuits

3. Gastroenterally derived ghrelin co-modulates the central regulation of energy balance at the gastric level via the vagal nervous system and the brainstem.

There is no reason why ghrelin could not act in parallel via paracrine mechanisms in the brain, via endocrine mechanisms at circumventricular organs or after crossing the blood brain barrier and via the parasympathetic nervous system at the gastrointestinal level to regulate energy balance [15].

Ghrelin administration induces adiposity [16,17], raises the respiratory quotient (reflecting reduced fat utilization) [18] and suppresses spontaneous locomotor activity in rodents (Tang-Christensen & Tschöp, unpublished observations). Neutralization studies with polyclonal ghrelin antibodies yielded encouraging results, showing decreased food intake in rodents after intracerebroventricular injection [19]. These data were confirmed by a transgenic rat model overexpressing antisense-oligonucleotides against the ghrelin receptor GHS-R1a, where decreased food intake and lower body fat were observed as a consequence [20]. There is clear evidence that, in spite of a relatively short half life, ghrelin administration in physiologically relevant doses is inducing a positive energy balance [21]. Ghrelin affects body weight and food intake more than 1000-fold more potently following central administration, strongly supporting the hypothesis that ghrelin influences energy homeostasis predominantly via the modulation of central mechanisms [22]. Regardless where the decisive endogenous amount of ghrelin that regulates energy balance is mainly derived from, blocking its endogenous actions acutely as well as chronically will at
least teach a valuable physiology lesson, if not pave the way for the development of a new drug.

REGULATION OF GASTRIC GHRELIN EXPRESSION AND SECRETION

Mainly secreted by gastric A/X-like cells within the oxyntic glands, the half life of ghrelin is relatively short (5-15min) and less than 20% of the circulating immunoreactive ghrelin appears to be octanoylated and therefore bioactive [23, 24]. Gastrointestinal X/A-like cells represent about a quarter of all endocrine cells in the oxyntic mucosa, while other cells within these glands, such as histamine-rich enterochromaffin-like (ECL) cells (ca. 70%) and D-(somatostatin) cells (10%), are ghrelin-negative [25,26]. From the stomach to the colon ghrelin is found with caudally decreasing expression [26]. Ghrelin containing enteroendocrine cells mostly have no continuity with the lumen, probably respond to physical and/or chemical stimuli from the baso-lateral side, and are closely associated with the capillary network running through the lamina propria. Ghrelin secreting cells occur as open- and close-type cells (open or closed towards the stomach lumen) with the number of open type cells gradually increasing from the stomach to the lower gastrointestinal tract [25,26]. A closer look at the structural and functional relationship between ghrelin and its receptor and the structure of motilin and its receptor suggests that a larger family of peptide hormones is co-modulating gastrointestinal motility, appetite, secretion of pituitary hormones and other physiological processes [27]. An extended peripheral endocrine network most likely also includes gastrointestinal hormones such as cholecystokinin (CCK), peptide YY (PYY1-36, PYY3-36), glucagon-like peptide 1 (GLP-1), gastric inhibitory peptide (GIP) and others [28]. Both ghrelin peptide secretion and ghrelin mRNA expression are adjusted according to metabolic challenges [21]. Acute as well as chronic periods of food deprivation (e.g. fasting) increase ghrelin peptide levels and ghrelin mRNA concentration, whereas re-feeding reduces ghrelin peptide secretion as well as ghrelin mRNA expression [29].

![Ghrelin Structure](image)

**Fig. (1). Ghrelin structure.** Two de-acylated forms of ghrelin are present in stomach tissue: ghrelin [1-28] and ghrelin [1-27] (with the last residue then being proline instead of arginine). Bio-inactive ghrelin peptides are acylated in a post-translational process that is catalysed by a still unidentified enzyme, which attaches n-octanoic acid or n-decanoic acid to amino residue Ser3 of ghrelin. The predominant active form of ghrelin is n-octanoylated ghrelin [1-28], [1,9].
OTHER SOURCES OF GHR ELIN

While a classical endocrine role for ghrelin as a peptide hormone that is secreted into a capillary network is evident, local, paracrine activities of ghrelin might play an additional role. Largely decreased ghrelin levels following the surgical removal of the stomach or the acid-producing part of the stomach indicate that the stomach is the main source of this endogenous GHS-receptor ligand [14]. That total plasma ghrelin is hardly detectable following gastric bypass surgery, Cummings and coworkers interpreted as a “shutdown” of gastric ghrelin secretion due to complete lack of contact with ingested nutrients [30,31]. More recent observations appear to partially contradict these results, describing that plasma ghrelin increases after gastric bypass surgery in patients experiencing active weight loss [32].

Ghrelin mRNA and peptide have also been detected in rat and human placenta [33]. Here, ghrelin is expressed predominantly in cytotrophoblast cells and very sporadic in syncytiotrophoblast cells. A pregnancy related time-course, represented by an early rise of ghrelin expression in the third week and decreasing levels in the latest stages of gestation, as well as still detectable presence of ghrelin at term, was found in rats. In human placenta, ghrelin is mainly expressed in the first half of pregnancy and is not detectable at term, while a putative involvement of ghrelin in fetal-maternal interaction via autocrine, paracrine, or endocrine mechanisms still remains to be shown. Small concentrations of ghrelin are found in the pancreas, where ghrelin immunoreactivity has been localized in a sub-group of endocrine cells that are also immuno-positive for pancreostatin. Ongoing, partially contradictory studies suggest that ghrelin positive cells are identical or overlapping with pancreatic A-cells [15], pancreatic B-cells [34], or pancreatic non-A-non-B-cells [35].

In normal pituitary cells as well as in pituitary tumors ghrelin mRNA expression and ghrelin immunopositive cells were detected in addition to the known presence of GHS receptors in pituitary cells. This suggests a possible autocrine or paracrine role for hypophyseal ghrelin, although only ca. 5% of the detected ghrelin peptide derived from the pituitary has been found to be octanoylated [36]. Using Real-Time PCR methodology, small amounts of ghrelin were detected in the adrenal glands, esophagus, adipocytes, gall bladder, muscle, myocardium, ovary, prostate, skin, spleen thyroids blood vessels and liver [37]. Prepro-ghrelin production was shown in rat mesangial cells and mouse podocytes, indicating the production of ghrelin in kidney, glomerulus and renal cells and suggesting possible paracrine roles for ghrelin in the kidney [38]. Human ghrelin as well as GHS receptor mRNA-expression was shown by Real Time-PCR and confirmed by DNA-sequencing in human T-lymphocytes, B-lymphocytes and neutrophils from venous blood of healthy volunteers. Cell type and maturity of the cells did not seem to have an influence on ghrelin production in immune cells [39]. Interestingly, it has recently been shown that small molecule GHS have a considerable immune-enhancing effect [40]. In summary, secreted ghrelin is expressed in its majority by the stomach, followed by still substantial concentrations deriving from lower parts of the gastrointestinal tract [41]. While its physiological significance as a paracrine factor in extra-gastrointestinal tissue is subject of ongoing studies, a classical endocrine role for extra-gastrointestinal ghrelin appears to be unlikely since ghrelin expression levels in other organs are relatively low in comparison. Published studies on the regulation of ghrelin expression were therefore primarily focussing on gastric ghrelin. As a cautionary note it should be added however that studies on ghrelin expression or secretion in rodents are however not necessarily relevant for the physiological regulation of ghrelin in humans.

REGULATION OF CIRCULATING GHR ELIN LEVELS

A very intriguing series of clinical studies indicates that each daily meal is followed by decreases of circulating ghrelin levels, most likely reflecting acutely reduced ghrelin secretion from the gastrointestinal tract [42,43]. The authors speculate in addition that an observed pre-meal rise of circulating human ghrelin levels might reveal a role for ghrelin in meal initiation, which fits well with the observation that ghrelin administration in healthy volunteers causes hunger sensations [21,42]. Ghrelin might also reflect the acute state of energy balance, signalling to the CNS in times of food deprivation when increased energy intake and an energy-preserving metabolic state are desirable [21].

Only few determinants of circulating ghrelin concentration have been identified to date, including insulin, glucose, somatostatin and possibly growth hormone, leptin, melatonin and the parasympathetic nervous system tone [11,15]. In several species (e.g. rodents, cows, goldfish and humans) ghrelin mRNA-expression levels or circulating ghrelin levels have been shown to be increased by food deprivation and to be decreased postprandially [16,42]. This phenomenon further supports the concept of ghrelin as an endogenous regulator of energy homeostasis that has apparently been preserved throughout species during evolution [12]. Rat ghrelin expression can also be stimulated by insulin-induced hypoglycemia [29], leptin administration [29] and central leptin gene therapy [45]. Ingestion of sugar suppresses ghrelin secretion in rats in vivo, indicating a possible direct inhibitory effect of glucose/caloric intake on ghrelin containing X/A-like cells in the oxyntic mucosa of the rat stomach but not excluding an additional insulin-mediated effect [16]. That insulin is an independent determinant of the circulating ghrelin concentration has recently been shown by several study groups using hyperinsulinemic euglycemic studies in humans [46-50]. These findings add further evidence for a connecting role of ghrelin between mechanisms governing energy balance and the regulation of glucose homeostasis. It remains however to be shown if postprandially occurring insulin peaks are sufficient to decrease circulating ghrelin levels, since hyperinsulinemic-euglycemic clamp studies causing decreased ghrelin secretion involve either supraphysiological or markedly prolonged (e.g. >120 min) periods of hyperinsulinemia [51]. Further insight into the complex mechanisms regulating ghrelin secretion is based on studies showing an increase of circulating ghrelin levels in rats following surgical interventions such as vagotomy and hypophysectomy. Human growth hormone (GH) deficiency, however, does not seem to exhibit increased plasma ghrelin levels [52]. On the other hand, administration of synthetic
GH in rats decreases circulating ghrelin levels [53] and patients with acromegaly show low endogenous ghrelin levels [54]. Contradictory observations may be explained by species-specific differences between rodents and humans. Another explanation would be that an acute, but not a chronic, change of GH levels modulates ghrelin concentration. A more exotic pathophysiological mechanism responsible for high circulating ghrelin levels is the production of ghrelin by endocrine tumors of the stomach and the intestine such as carcinoids [11].

In summary, ghrelin expression as well as ghrelin secretion are predominantly influenced by changes in energy balance and glucose homeostasis, followed by alterations of endocrine axes (e.g. increasing GH concentrations). Based on the currently available data, ghrelin therefore mainly seems to represent a molecular regulatory interface between energy homeostasis, glucose metabolism and physiological processes regulated by the classical endocrine axes such as growth and reproduction. One particular biological purpose of these multiple roles of ghrelin might be to ensure the provision of calories that GH requires for growth and repair.

**GHRELIN AND OBESITY**

Differing from earlier models expecting the endogenous ligand of the GHS receptor to govern GH secretion [1], ghrelin is currently believed to have its main physiological role in the regulation of energy balance [21]. As the only potent circulating orexigenic agent known to date, ghrelin triggers appetite and nutrient intake [16,42,43]. Ghrelin might even represent the first “meal initiation factor” known [42]. However, conclusive evidence that meal-related...
circadian changes of plasma ghrelin concentrations are responsible for the initiation of nutrient intake rather than representing an epiphenomenon of trained meal patterns is still missing. Based on clinical investigations of meal related changes of plasma ghrelin levels and data generated by insulin- and glucose-clamp studies, plasma insulin as well as blood glucose levels are very likely to be involved in the general regulation of ghrelin secretion [42,43,46-50]. Although hyperinsulinemic-euglycemic clamps have been repeatedly shown to decrease circulating ghrelin, it remains unclear, if experimental conditions during clamp studies are comparable with the lower maximum peaks and the shorter duration of postprandial insulin levels [51]. It can not be excluded that additional blood derived factors are responsible for meal-related changes in ghrelin concentrations or that gastrointestinal nutrient sensors are modulating ghrelin expression and secretion rates.

Although counterintuitive, low circulating ghrelin concentrations in obesity not only mirror earlier observations of hyperleptinemia in obesity, but may be explained by compensatory mechanisms aiming to communicate to central regulatory centres that energy stores are sufficiently filled [21]. While it is not clear which signal communicates increased adipocyte size to ghrelin secreting cells (leptin, IL-6 or adiponectin would be candidates), it has to be carefully investigated if other phenomena and symptoms that are frequently occurring in obesity (such as a filled stomach or presence of insulin resistance) contribute to hypoghlerninemia. Ghrelin gene polymorphisms have been described by several groups, linkage analysis studies however failed to prove a solid association between ghrelin and obesity [55,56,57]. While diet-induced human obesity, as well as polygenic (e.g. Pima Indians) or monogenic (e.g. MC4-R defect) causes of human obesity all present with low plasma ghrelin levels, there is one group of severely obese patients where markedly increased plasma ghrelin levels have been observed. In patients with Prader-Willi syndrome, an impressive hunger syndrome along with morbid obesity and numerous other symptoms is caused by a missing part of the short arm of chromosome 15 and accompanied by 3-5 fold higher circulating ghrelin levels when compared to healthy controls [58,59,60]. While the overlap between symptoms of Prader-Willi syndrome and effects of ghrelin administration is impressive, only treatment with a potent, but safe, ghrelin antagonist compound will show if ghrelin is part of the pathogenesis in Prader-Willi syndrome [58,59,60]. There is an ongoing discussion if increased plasma ghrelin levels are only a consequence of severe caloric restrictions which are a central part of treatment strategies for patients with Prader-Willi syndrome in an attempt to control their energy balance. The only other population where comparably high ghrelin levels have been reported are patients with cachexia or anorexia nervosa. In these pathophysiological states high ghrelin levels are believed to reflect a physiological compensation effort in response to either a chronically empty stomach or a markedly decreased fat mass [61,62]. While circulating ghrelin levels are significantly lower in obese individuals [63], their ghrelin levels are still very substantial, when compared to nearly undetectable ghrelin concentrations in patients after gastric bypass surgery [30]. The superior effectiveness of this bariatric procedure is discussed as partially due to a “knock-down” of endogenous ghrelin secretion caused by the lack of stimulation of gastric cells by incoming nutrients (30). On the other hand there is a chance that an increase of endogenous ghrelin in response to diet-induced weight loss contributes to the high probability of obesity recidivism. Carefully conducted clinical studies are mandatory to validate this intriguing hypothesis.

**GHRELIN INDUCES ADIPOITY IN RODENTS**

Ghrelin administration in rodents causes weight gain [16]. This effect would not be as surprising if it was merely reflected by longitudinal growth or at least by an increase in lean mass, effects that one would expect after stimulation of GH secretion. However, a still growing body of data generated in rodents clearly shows that ghrelin-induced weight gain is based on accretion of fat mass without changes in longitudinal skeletal growth and with a tendency toward a decrease, rather than an increase, of lean (muscle) mass [16,17,18]. These findings have not only been confirmed by several groups but, have also been repeated using synthetic GHS receptor agonists NNC 26-0161 (ipamorelin) [16], or GHRP-2 [18] and GHRP-6 [17]. Changes in body weight induced by ghrelin administration become significant in rodents after no more than 48 h and are clearly visible at the end of two weeks. Changes in fat mass induced by GHS have been quantified using Dual Energy X-ray Absorptiometry measurements specifically adapted for analysis of rodent body composition and have also been confirmed using chemical carcass analysis [53], or by measuring the weight of omental and retroperitoneal fat pads. Energy balance is achieved when energy intake is equal to energy expenditure. A positive energy balance, leading to weight gain, occurs when calories ingested, digested and re-absorbed exceed calories expended. Like leptin, but in an opposite manner, ghrelin influences both energy intake and metabolism to change body fat and body weight in rodents [11].

Ghrelin’s orexigenic action is comparable to that of neuropeptide Y (NPY) when administered centrally and is more effective than any other orexigenic agent [22,64]. While peripherally injected GHS or ghrelin does have less impressive, predominantly acute and transient orexigenic effects, continuously administered ghrelin (3rd ventricle) causes potent and constant stimulation of appetite in rats [16]. However, further studies (i.e. involving mice with tissue-specific disruption of the ghrelin gene) will have to prove the existence of an endogenous ghrelin-tone that supports ghrelin’s putative relevance for physiological appetite regulation and metabolic control. Synthetic GHS (ghrelin receptor agonists) that have been shown to have orexigenic activity so far include GHRP-2, ipamorelin, GHRP-6, hexarelin, EP 50885, EP 40904 and EP92632 [18,64]. Apart from an increase of food intake, other mechanisms can contribute to an increase in fat mass, such as a decrease in energy expenditure or reduced cellular fat oxidation. No significant changes of 24-hour total energy expenditure have been observed in rodents after ghrelin administration [16]. Improved methodology to quantify and monitor energy expenditure in small animals are needed to clarify discrepancies in energy balance characterization,
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since lack of sufficient sensitivity could be one reason, why small (but significant when chronically occurring) changes in energy expenditure are currently overlooked. One effect however that was detected by indirect calorimetry is an increase of the respiratory quotient after ghrelin administration in rodents [16]. It remains to be shown that this increase is independent from an increase in food intake. A raised respiratory quotient is interpreted as a shift from fat utilization to carbohydrate oxidation and has also been referred to as indicating changes in “nutrient partitioning” [65].

The network of neurons, neuropeptides, neurotransmitters and receptors controlling energy balance is an extremely complex, multi-centered system [66]. Based on early surgical and chemical deletion studies in rodents, the hypothalamus has long been recognized as a crucial interface between afferent peripheral signals, other regulatory centers in the CNS and efferent pathways regulating energy balance in concert [67]. In addition, the brainstem and other brain areas are emerging as equally important regulatory centres of energy homeostasis control [68]. Ghrelin binds predominantly at NPY- and AGRP co-expressing neurons in the arcuate nucleus, which are also known to express the GHS-R1a [13,69]. Ghrelin has an increasing effect on both orexigenic neuropeptides, along with an indirect suppressing effect on POMC and CART (cocaine-amphetamine regulated transcript) expressing neurons in the same area via reduced inhibitory tone of the NPY/AGRP neurons and a possible contribution of activity changes in orexin/hypocretin expressing neurons in the lateral hypothalamus [13,70]. Several recent reviews give excellent overviews on these circuits, their connectivity, as well as current hypotheses and open questions in this rapidly advancing field of central appetite and body weight control [66-68,71-73].

In rat adipocytes it has been observed that the GHS-R expression increases with age and during adipogenesis. According to very recently reported data, ghrelin in vitro stimulates the differentiation of preadipocytes and antagonizes lipolysis. Ghrelin may therefore play a role in the direct peripheral control of adipogenesis [74]. Other effects of ghrelin at the adipocyte level are the suppression of adiponectin expression, and a stimulating influence on mitogen-activated protein kinase phosphorylation [75].
investigating these open questions was conducted by Wren et al. who showed that i.v. administration of physiologically occurring concentrations of ghrelin effectively triggers appetite and increases food intake in humans [82]. Chronic studies investigating the effects of ghrelin and ghrelin receptor agonists are ongoing and will deliver data on long-term effects of ghrelin on body composition in humans. Independent from the outcome of these studies, a large number of additional experimental as well as clinical studies will be necessary to determine if a ghrelin antagonist, once it becomes available, may represent an effective and safe treatment for obesity.

One of the most crucial questions will be, which side effects might be the ones to watch during studies attempting to decrease nutrient intake and fat mass by blocking endogenous ghrelin action. Certainly there must be concern to create GH deficiency, but researchers are even more worried about the antiproliferative, cardiovascular or sleep regulating effects of ghrelin that have been described recently. Possible effects on cardiac rhythm and heart contractility or the possible occurrence of malignant diseases in various tissues have to be monitored closely in toxicology studies, once a ghrelin receptor antagonist will be found that has effects on energy balance in rodents. For more extensive information on historical facts, as well as on the biology and the physiology of GHSs and ghrelin, several comprehensive review articles can be recommended [83,84].

Several strategies can be employed to diminish or abolish ghrelin action. Apart from a classical pharmacological approach of antagonizing ghrelin at the GHS-R1a (which heavily relies on the notion that this is the only existing or at least the crucial ghrelin receptor), binding or neutralizing ghrelin (e.g. using synthetic antibody fragments) or blocking ghrelin transcription or translation (antisense oligonucleotides) possible. A very elegant way of inhibiting ghrelin action would be to block the posttranslational acylation process by inactivating the responsible enzyme. Multiple questions have to be clarified on the way toward an effective drug using any of the above mentioned strategies. Does the putative drug cross the blood brain barrier or does it even have to? Will it make a difference to further decrease circulating ghrelin levels when obesity is present or will there be resistance to ghrelin antagonists in obesity?

It has become a popular hypothesis, that evolution has shaped an endogenous control system governing body weight and appetite that is based on genetic redundancy to prevent a negative energy balance and to ensure survival, while current environmental conditions in westernised civilisations turn these defense mechanisms into a health hazard (“thrifty gene hypothesis”) [85]. Although there is no definite proof for this concept, the hypothesis seems intriguing and would make it very unlikely that a ghrelin receptor antagonist alone would cause sustained fat loss, since numerous adjustments of endogenous factors controlling energy balance would occur immediately to keep body weight stable.

On the other hand, there seem to be examples where pharmacological manipulation of circulating hormone levels work well in “cheating” the brain regarding information on physiological functions in the periphery (e.g. oral contracep-

GHRELIN RECEPTOR AGONISTS AND ANTAGONISTS

Recent studies underline the importance to find an anti-obesity agent, since increased adiposity and its consequences have been identified as major killers in western civilizations [76]. While one can only speculate based on the currently known scientific facts, if a ghrelin antagonist may represent a useful therapeutic agent against increased body fat mass, several recent findings may provide arguments for and against a blockade of the ghrelin pathway [68]. Apart from possibly representing a general anti-obesity drug, a ghrelin-antagonist will help to answer a variety of open questions regarding ghrelin physiology, such as a possible role in meal initiation or its putative involvement in the etiology of Prader-Willi Syndrome (PWS) ([59]. As an exception to the generally observed negative correlation between body fat mass and plasma ghrelin concentration, patients with Prader-Willi Syndrome (PWS) have been identified as the only population of individuals with increased fat mass and several-fold increased plasma ghrelin levels [58]. PWS is the most frequent known cause of genetically induced obesity and is associated with a defect on the short arm of chromosome 15, while the exact pathogenetic mechanisms leading to the obesity syndrome in PWS remain unclear [77]. Apart from their adiposity, patients with PWS are suffering from a hunger syndrome (which in severe cases might even lead to the ingestion of non-food items), they exhibit decreased spontaneous locomotor activity, impaired growth hormone secretion, sleepiness and relative hypoinsulinemia [78,79]. Ghrelin on the other hand is known to increase fat mass, increase hunger, promote sleep, decrease locomotor activity, control GH-secretion and is suppressed by insulin [11]. Although it appears intriguing that hyperghrelinemia in PWS might be responsible, at least in part, for the majority of symptoms characterizing this disease, a genetic link can only be explained via indirect influences (e.g. genetic imprinting) since the gene encoding ghrelin is located on chromosome 3 [80]. A compound with the ability to neutralize or antagonize ghrelin action could not only prove if ghrelin is causally involved in the pathogenesis of PWS, but also represent the first causal therapeutic approach for patients with PWS. However, no ghrelin receptor antagonists or ghrelin neutralizing agents are available until now.

The possibly most pressing question concerns the transferability and validity of the above described findings on the role of ghrelin in energy balance regulation from rodents to humans. Several recent clinical studies on the effects of ghrelin on GH-secretion in humans have reported hunger sensations as the only noticeable side effect in up to 80% of the treated individuals [81]. Prospective clinical studies focusing on all aspects of energy balance using contemporary methods for the analysis of body composition, energy expenditure, metabolic and endocrine changes can help to clarify these issues. A first clinical study investigating these open questions was conducted by Wren et al.
tives) which are of a comparably essential character for species survival as a stable energy balance (e.g. reproduction). An additional option would be to combine two or three agents to fight obesity. A combination of drugs increasing resting energy expenditure and motivation for locomotor activity as well as decreasing pre-prandial appetite and increasing post-prandial satiety, might have a higher chance of achieving sustained weight loss, however, such a concept will require elaborate and costly studies, while it would markedly increase the risk for side effects. Alternating several drug treatments to avoid resistance (as historically done e.g. to cure tuberculosis) is another possibility. A more modern strategy may rely on substantial advances in the characterization and diagnosis of specific subtypes of the disease obesity: Based on the hypothesis that there are several molecular reasons to be susceptible to diet-induced obesity (increased ghrelin production or sensitivity, decreased leptin production or sensitivity, decreased MC-4R expression etc.), effective anti obesity drugs may have to be tailored to meet the specific molecular defects of these subgroups of patients. This may become possible since both the understanding of mechanisms governing energy balance as well as the clinical approaches and genetic tools to more specifically diagnose obesity phenotypes and genotypes are rapidly improving [86].

This concept would in addition have the advantage that one has to worry less about possible side effects of a compound since one would correct a specific defect rather than trying to force physiologically balanced systems toward an unnatural metabolic state. For example, if hyperghrelinemia in PWS patients turns out to be involved in the pathogenesis of the disease, a ghrelin antagonist could theoretically offer a safe and effective treatment option.

**POTENTIAL SIDE EFFECTS**

In patients with diet-induced obesity, where plasma ghrelin levels are already relatively low and presumably ghrelin does not represent the reason for the disease, one can at this point not exclude the possibility that ghrelin antagonists might do more harm than good. Since ghrelin apparently displays beneficial hemodynamic effects through both GH-dependent and independent mechanisms, its inactivation could be detrimental for cardiac function. In particular, ghrelin has been shown to reduce cardiac afterload and to increase cardiac output [87,88], as well as to prevent apoptosis in cardiomyocytes [89], while it is speculated that these effects are not mediated by the GHS-R1a, but by the fatty acid scavenger CD36 or other receptors or receptor subtypes [90]. As long as the mechanisms and magnitude of ghrelin induced changes in heart function are poorly understood, there remains the risk of cardiovascular side effects.

The same is the case for proliferative or (anti-) proliferative effects of acylated and non-acylated ghrelin,
which have been shown in vitro using several tumor cell lines [91]. It has been speculated, that these effects were based on the activation of an until now unknown GHS receptor [92]. In contrast, other findings seem to support the concept that at least in some organs such as prostate and adrenal gland, ghrelin may represent a proliferative stimulus through activation of the GHS-R1a [93]. Furthermore, ghrelin has been shown to be a sleep promoting factor [94] and ghrelin receptor agonists increase bone mineral density [18]. A putative ghrelin antagonist may therefore impair sleep and have a negative influence on bone formation and remodeling. It is difficult to predict the complete spectrum of effects of a new compound with ghrelinergic action or antagonistic properties before its affinity and specificity to putatively existing subtypes of ghrelin receptors are known. Possible oncogenic, cardiovascular, sleep-related and bone-density affecting side effects should be monitored carefully among others, when these compounds are tested.

While obesity clearly represents a rapidly spreading disease with an enormous market potential due to what will be a life long drug treatment, ghrelin receptor agonists are not only already available as orally active compounds, but also could be more effective as a drug for cachexia than ghrelin receptor antagonists for obesity, due to a less potent defensive system protecting against body weight increase. Ghrelin receptor agonists or ghrelin itself might therefore offer drug treatment for diseases such as cancer cachexia, HIV-wasting syndrome, cardiac cachexia or even anorexia nervosa. Comparable with leptin resistance in obesity, cachectic patients might be resistant against treatment with ghrelin since their endogenous ghrelin levels are markedly increased. However, preliminary results in rodents bearing melanoma cells however show encouraging results [95]. Again, side effects might occur: In particular GH-mediated stimulation of IGF-1 might be an undesired effects in malignant diseases presenting with cachexia [96]. In addition, while ghrelin or its receptor agonists may promote appetite and food intake, it remains yet to be shown that these agents can increase body weight and fat mass in humans. In rodents adipogenic effects are most potent during the first 14 days of (peripheral) treatment and may disappear due to desensitisation, adaptation or compensation after a few weeks.

SUMMARY

In summary, ghrelin represents a recently discovered gastric hormone that induces hunger and increases fat deposition via central and possibly peripheral mechanisms in response to a negative energy balance. Ghrelin is one of the most potent and the only known peripheral orexigenic agent, it possibly also represents the first meal initiation factor. Although plasma ghrelin concentrations are negatively correlated with fat mass, substantial levels are still secreted in the vast majority of obese individuals, while in patients with a gastric bypass weight loss occurs along with loss of circulating ghrelin. Apart from a possible effectiveness of a ghrelin antagonist for the general prophylaxis and treatment of adiposity, the blockade of ghrelin could be the first specific pharmacotherapeutic approach to successfully treat patients with Prader-Willi syndrome. However, various additional effects of ghrelin on physiological processes and organ systems implicate not only therapeutic perspectives, but make unwanted cardiovascular, gastrointestinal or proliferative effects a likely consequence of blocked endogenous ghrelin action.

REFERENCES

References 97-99 are related articles recently published in Current Pharmaceutical Design.


Ghrelin as a Potential Anti-obesity Target


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