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Review

Ghrelin and appetite control in humans—Potential application in the treatment of obesity

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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 administration 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.

Contents

1. Introduction

The prevalence of obesity is rapidly increasing in the developed world and is a major drain on healthcare resources. It is associated with many serious medical conditions including diabetes, coronary heart disease, hypertension, osteoarthritis and several cancers [17,33]. Recommendations to diet and exercise are failing control the rising obesity levels. At present pharmaceutical based therapies are very limited and the only truly effective treatment is obesity surgery. Although effective, surgery is impractical as a wide spread treatment; therefore there is an urgent need for more effective pharmaceutical based therapies. Circulating hormones are known to play an important role in the regulation of appetite and energy expenditure. These hormonal systems represent valuable targets for the development of anti-obesity therapies. While there are many hormones that reduce appetite, ghrelin is the only known orexigenic circulating hormone.

Ghrelin is a 28 amino acid peptide hormone first identified as a growth hormone (GH) stimulating peptide which is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) [30]. Ghrelin has a unique acylation on its serine 3 residue which is required for activation of GHS-R1a [31]. Both ghrelin and des-acylated (lacks the serine 3 acylation) are found in circulation. Shortly after its discovery ghrelin administration was

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demonstrated to acutely stimulate feeding and chronically induce adiposity in rodents [38,46,51]. These effects were thought to be mediated via hypothalamic neuropeptide Y (NPY)/agouti related protein (AgRP) neurones [29,38]; a population of neurones known to stimulate feeding. In humans it was shown that circulating ghrelin levels rise before a meal and are suppressed post-prandially [12,47]. Subsequently the first study investigating ghrelin administration in man reported a potent stimulation of appetite [50]; findings that have since been replicated many times. Together these data led to the hypothesis that ghrelin acts as a hunger signal initiating the onset of meals. Antagonism of the ghrelin system therefore appeared an attractive target for anti-obesity therapies. This led to much research and investment aimed at developing anti-obesity drugs designed to antagonize the ghrelin system. Despite this early optimism a ghrelin based treatment for obesity has yet to emerge. This review analyzes the role of the ghrelin system in appetite regulation in humans and assesses its potential as a target for obesity therapies.

2. Ghrelin in lean and obese humans

2.1. Ghrelin regulation

Ghrelin is expressed in many tissues including the stomach, pancrea, brain, testis, kidney, intestine, pituitary and heart [19]. The highest ghrelin levels are found in the stomach which is believed to be the source of circulating ghrelin [1]. Following gastrectomy ghrelin levels are dramatically reduced [1]. Circulating ghrelin levels correlate with hunger [11], are high before a meal and are suppressed post-prandially [12,47]. In general the suppression in circulating ghrelin levels is greater following larger, higher calorie meals [8]. However, the magnitude of the suppression in circulating ghrelin levels is also influenced by the macronutrient makeup of the meal. Carbohydrates are the most potent macronutrient suppressors of ghrelin; while fats and proteins suppress ghrelin levels to a lesser extent [32]. The precise mechanisms behind the postprandial suppression is yet to be fully determined. However, we do know that ghrelin release is unlikely to be suppressed by the detection of nutrients in the stomach as in rodents gastric emptying is required for ghrelin suppression [49]. Neuronal or hormonal signaling is more likely responsible for regulating ghrelin secretion. There is evidence that the post-prandial release of insulin [18,34,37,42] and possibly gut hormones (Peptide YY, cholecystokinin, glucagon-like peptide and oxyntomodulin) [3,6,9] contributes to the inhibition of ghrelin release.

The obese have increased hunger and take longer to reach satiety [13]. Ghrelin is thought act as a hunger signal. Consequently, it was originally hypothesized the obese may have increased concentrations of circulating ghrelin. In fact the contrary is true, ghrelin levels correlate negatively with body mass index (BMI) [44,48] and increase following weight loss [22]. Ghrelin is therefore unlikely to affect the development of obesity. The only exception is Prader–Willi syndrome, a rare genetic cause of hyperphagia and severe obesity. Ghrelin levels are dramatically elevated in Prader–Willi syndrome [10], levels are approximately 4.5 times higher than obese controls. However, currently it is still unclear whether this is a consequence or cause of their obesity. Prader–Willi sufferer's excluded; the ghrelin system appears to be down regulated in the obese. Therefore, further pharmacological suppression of ghrelin may not prove an effective obesity therapy.

However, in addition to lower circulating ghrelin levels, an attenuated suppression of ghrelin in the obese has been reported [35]. A greater number of calories are required before a significant suppression of fasting ghrelin levels are observed in the obese, when compared to the lean [35]. The magnitude of ghrelin suppression is also proportionally smaller in the obese [35]. It has been hypothesized attenuated ghrelin suppression in the obese may contribute to a lack of satiety following smaller meals. If this hypothesis is correct then antagonizing the ghrelin system may still prove effective at suppressing appetite in the obese.

2.2. The effects of ghrelin administration on appetite

The original studies reporting the appetite stimulating properties of ghrelin were performed in lean or normal weight volunteers [50]. To determine whether the obese retained sensitivity to ghrelin and it may therefore represent a potential therapeutic target. It was important to establish whether ghrelin administration to overweight and obese humans stimulated appetite. If the obese were resistant to ghrelin then antagonizing the ghrelin system would not be effective for the treatment of obesity. Druce et al. [15] compared the effects of ghrelin administration in the lean and obese. The study reported both groups were sensitive to the appetite stimulating effects of ghrelin. In fact, in these studies effects of ghrelin on voluntary energy intake were greater in the obese although no formal comparison was not made. If the obese are more sensitive to ghrelin than the lean, then despite their reduced circulating levels, ghrelin antagonism may still represent an effective treatment for obesity. However, the mean BMI of the obese subjects studied by Druce et al. was only 31.9 kg/m^2 which represents mild obesity. The effects of the GHS-R1a agonist, GHRP-2, have also been compared in the lean and obese [34]. In this study GHRP-2 stimulated feeding with similar magnitude in the lean and obese; again the group studied were only mildly obese with a mean BMI of 31.4 kg/m^2. Together these studies suggest the mildly obese are at least as sensitive to ghrelin as the lean. However, ghrelin sensitivity is still to be confirmed in more severe forms of obesity.

3. Approaches targeting the inhibition of the ghrelin system

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

3.1. Antagonizing the ghrelin receptor (GHS-R1a)

The ghrelin receptor, GHS-R1a, was first identified as the receptor through which growth hormone secretagogues (GHSs) act [28]. 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 [16,41]. Acute administration of their antagonists improved glucose tolerance[16,41]. 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 [21]. 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. GHR-R1a 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 piperazine-bisamide based GHS-R1a antagonists. They state the compound is now being used for *in vivo* proof of concept studies [53]. 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 [26]. It has been reported GHS-R1a signaling can be at half maximum levels in the absence of ghrelin [24,25]. The importance of this is highlighted by the identification of human mutations which cause a loss of GHS-R1a constitutive activity. These mutations cause short stature due to growth hormone deficiency [39]. The use of inverse agonists to reduce basal GHS-R1a signaling even when circulating ghrelin is low may therefore prove effective in the treatment of obesity.

When administered centrally for 6 days, the GHS-R1a inverse agonist, (d-Arg [1], D-Phe [5], D-Trp [7,9],Leu [11])- substance *P* significantly reduced cumulative food intake and body weight gain [40]. In addition, they reported an associated reduction in NPY mRNA, which is in line with reduced appetite and ghrelin signaling. This study highlights the potential inverse agonist as an obesity treatment. However, 6 days is a relatively short treatment period, it will be critical to establish whether efficacy of GHS-R1a inverse agonists is maintained over longer treatment periods. (d-Arg [1], D-Phe [5], D-Trp [7,9],Leu [11])- substance *P* is also an antagonist of the ghrelin receptor at higher concentrations. However, the concentrations used in this study should have only acted as an inverse agonist. Efforts have been made to develop better ghrelin inverse agonists; however currently we are unaware of any *in vivo* results from compounds that show significant potential as anti-obesity agents.

3.2. Neutralizing circulating ghrelin

Neutralizing circulating ghrelin aims to reduce/prevent ghrelin reaching target receptors in the hypothalamus and/or the brainstem. This could decrease the hunger signal associated with high ghrelin levels observed prior to a meal. Two main methods have been used to neutralize circulating ghrelin and suppress appetite. The first is development of an anti-ghrelin vaccine where antibodies against ghrelin are used to neutralize circulation peptide. The second is the use of novel Spiegelmer technology which relies on specifically designed oligonucleotides binding and neutralizing ghrelin.

Using the vaccine approach Zorrilla et al. [54] demonstrated reduced weight gain in rodents. Following immunization with a rat ghrelin hapten, rats developed antibodies against their own ghrelin. Rats with a high antibody titer had reduced weight gain compared to those with a lower titer. Interestingly there was no associated reduction in feeding. The weight difference was attributed to reduced feed efficiency. Chronic ghrelin administration has been reported to increased body weight without significant feeding effects [46]; thus this is a plausible explanation.

Cyto biotechnology tested the vaccine approach in humans. Their vaccine, named CYT009-GhrQb underwent a Phase I/II trial. The trial was unsuccessful, with equal weight loss in both control and treatment groups. The reason for this was not clear. Either insufficient anti-ghrelin antibodies were produced, or neutralizing circulating ghrelin in humans does not have a sufficient effect to alter body weight. An additional major concern with this approach is that should neutralization of ghrelin affect long term health adversely, the vaccine treatment is not reversible. Until we have a better knowledge of all ghrelin's physiological roles and data has shown long term inhibition of ghrelin has no unwanted side effects this approach may be judged too risky. An alternative approach of injecting monoclonal anti-ghrelin antibodies has been trialed by Amgen. Although some acute effects were observed, the lack of a long term effect on body weight led to the conclusion that antagonizing peripheral ghrelin using the antibodies they developed would not prove to be an effective treatment for obesity [36].

Spiegelmers are single stranded mirror image oligonucleotides designed to bind and inhibit specific proteins. Instead of the natural L-ribose sugar moiety they have a D-ribose which is more resistant to breakdown increasing their half life. In 2004 NOXXON Pharma reported the development of a ghrelin binding Spieglmer [4]. Intravenous administration of a polyethylene glycol modified version their compound L-NOX-B11 supressed the growth hormone inducing effects of ghrelin in rats [23]. Subsequent studies reported inhibition of the acute effects of ghrelin on food intake, and continuous administration reduced obesity in diet induce obese (DIO) mice [43]. However, food intake was only reduced for the first 8 days of the 13 days period tested and appeared to be converging in the latter half of the study. This suggests treatment of obesity with ghrelin Spiegelmers may not be successful long term. In addition it has been speculated the cost of synthesizing compounds such as LNOX-B11 may be high [27]. Less data relating to ghrelin Spieglmers has emerged recently and we are not aware of the results of any trials involving humans.

3.3. Preventing the production of acylated ghrelin through the inhibition of ghrelin O-acyltransferase (GOAT)

To bind to its receptor, GHS-R1a, ghrelin requires a unique acylation of its third serine residue. Almost a decade after the discovery of ghrelin, the enzyme that catalyzes this acylation was identified [20,52]. Ghrelin O-acyltransferase (GOAT) is a member of the membrane bound O-acyltransferase (MOAT) family and is also known as MOAT4. GOAT is highly conserved throughout different species. High levels of GOAT mRNA are found in the stomach, pancreas and intestine [20,52]. Disruption of the GOAT gene in mice results in a complete absence of acylated ghrelin. Since GOAT is essential for the production of acylated ghrelin, inhibition of the GOAT enzyme represents a novel way of supressing the ghrelin system. Recently Barnett and colleagues reported the development of a peptide based bisubstrate analog that inhibited GOAT [2]. This inhibitor, GO-CoA-Tat, successfully reduced circulating acyl ghrelin, and when administered over a one month period caused an impressive reduction in weight gain and fat mass. Acute administration improved glucose tolerance and increased glucose stimulated insulin release. Thus therapies based on inhibition of GOAT may be useful as a combined treatment for obesity and type 2 diabetes. Although these early results are promising GOAT inhibition may still not prove an effective strategy for obesity treatment. It is important to note that although the mice in these studies were maintained on a high fat diet they did not have pre-existing obesity at the start of the treatment. Obese humans or rodents may respond less well to GOAT inhibition as the ghrelin system is already supressed.

4. Conclusions

The discovery of ghrelin and subsequent characterization as an appetite stimulating hormone was met with much excitement. Inhibition of the only known orexigenic hormone in circulation may have led to the development of effective obesity therapies. Over a decade later we are unaware of any ghrelin based obesity therapies close to reaching the market. The most likely reasons are either, ghrelin based therapies are ineffective because the ghrelin system is already suppressed in obesity, or simply ghrelin is not as influential in the regulation of appetite as originally believed. Although antagonizing the ghrelin system can reduce body weight in the short term, compensatory mechanisms may override these effects in the long term. Interestingly, we have recently reported chronically elevating ghrelin through models of overexpression or implantation of infusion pumps fails to increase feeding or bodyweight in mice when they are fed a high fat diet (10). This data suggests when a high fat diet is consumed, as is common in the obese, ghrelin is not an important initiator of feeding. This may represent a novel why reason antagonizing the ghrelin system might not be an effective obesity treatment. Antagonizing the ghrelin system may prove more effective in the treatment of type two diabetes. In transgenic models either ablating or overexpessing ghrelin effects on glucose homeostasis were much more pronounced than effects on bodyweight [5,45].

Although results have not been encouraging, it may be premature to completely dismiss the possibility of a ghrelin based anti-obesity therapy. It is possible we are just yet to deliver inhibitors with sufficient potency or have not targeted the system at the correct level. This is especially true in light of the promising early results from the GOAT inhibition studies. If findings from these studies can be successfully translated into obese humans, inhibition of the ghrelin system may yet prove a successful treatment for both obesity and type II diabetes.

Disclosure statement

Authors have nothing to disclose.

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