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Ghrelin receptor signaling: a promising therapeutic target for metabolic syndrome and cognitive dysfunction

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Abstract

The neuroendocrine hormone ghrelin is an octanoylated 28-residue peptide that exerts numerous physiological functions. Ghrelin exerts its effects on the body mainly through a highly conserved G protein-coupled receptor known as the growth hormone secretagogue receptor subtype 1a (GHS-R1a). Ghrelin and GHS-R1a are widely expressed in both peripheral and central tissues/organs, and ghrelin signaling plays a critical role in maintaining energy balance and neuronal health. The multiple orexigenic effects of ghrelin and its receptor have been studied in great detail, and GHS-R1a-mediated ghrelin signaling has long been a promising target for the treatment of metabolic disorders, such as obesity. In addition to its well-characterized metabolic effects, there is also mounting evidence that ghrelin-mediated GHS-R1a signaling exerts neuroprotective effects on the brain. In this review, we will summarize some of the effects of ghrelin-mediated GHS-R1a signaling on peripheral energy balance and cognitive function. We will also discuss the potential pharmacotherapeutic role of GHS-R1a-mediated ghrelin signaling for the treatment of complex neuroendocrine disorders.

Keywords

Cognitive function; energy balance; ghrelin; growth hormone secretagogue receptor; memory; metabolic disorders; neuroprotection; obesity

INTRODUCTION

Ghrelin is a peptide secreted mainly by the stomach and is an endogenous ligand for the growth hormone secretagogue receptor 1a (GHS-R1a) [1]. GHS-R1a is a G protein-coupled receptor that is widely expressed in peripheral tissues and also in various regions of the brain such as the pituitary gland, hypothalamus, thalamus, cortex, and hippocampus [2,3]. For many years, the ghrelin receptor has been a much-researched therapeutic target for obesity and metabolic syndrome. Obesity is a rapidly spreading epidemic in the majority of developed nations. Within the United States, the percentage of the population aged 20–74 considered to be overweight or

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obese (BMI >25) has increased from 45.8% in the early 1960s to over 72% in the year 2006, and this number continues to rise rapidly [4]. Obesity adversely affects health by increasing the risk for various associated conditions including metabolic syndrome, type 2 diabetes, coronary artery disease, and hypertension, all of which are associated with increased mortality [5]. The economic ramifications of this consistent rise in obesity were estimated to be \$75 billion in 2003, of which roughly half was subsidized by Medicare and Medicaid [6]. Cognitive dysfunction and dementia have recently been shown to be common complications of metabolic syndrome and type 2 diabetes. Similar to obesity and metabolic syndrome, cognitive dysfunction represents another serious health problem, and it is increasing in prevalence worldwide, especially amongst elderly people [7].

The GHS-R1a is a promising drug target for complex neuroendocrine disorders that involve both the central nervous system (CNS) and the endocrine system, such as cognitive dysfunction, Alzheimer's disease, and stroke. In this review, we will summarize some of the energy-regulating peripheral and neuroprotective actions of ghrelin and its receptor and discuss the therapeutic potential of ghrelin-mediated GHS-R1a signaling in neurological disorders with underlying metabolic dysfunction.

GHRELIN IS EXPRESSED IN MULTIPLE TISSUES

Ghrelin is a 28 amino-acid peptide that was initially purified from rat stomach and was later found to be specifically expressed by endocrine α -cells in the mucosa layer of the stomach [8]. The ghrelin gene encodes a 117 amino-acid preproghrelin that shares 82.9% homology between rodents and humans, although the functional 28 amino acid protein only differs by 2 amino acids between the two species. The expression of ghrelin has been demonstrated in various tissues, such as the stomach [9], ovary [10], placenta [11], kidney [12], pituitary gland [13], pancreas [14], and brain. The main brain site of ghrelin synthesis is the hypothalamus, although overall peptide levels are much lower than in the stomach [15]. Using various immunocytochemical techniques, it has also been demonstrated that ghrelin expression is present in the internuclear space between the lateral hypothalamus, the arcuate nucleus, the ventromedial nucleus (VMN), the dorsomedial nucleus (DMN), the paraventricular nucleus (PVN), and the ependymal layer of the third ventricle [16]. Outside of the hypothalamus, ghrelin-immunopositive staining has been shown to be present in the pyramidal neurons of layer V in the sensorimotor area and in the cingulate gyrus of the cerebral cortex. Additionally, ghrelin mRNA expression has also been reported in the sensorimotor cortex and in the dorsal vagal complex (DVC) of the medulla oblongata [3].

THE GHRELIN RECEPTOR: GROWTH HORMONE SECRETAGOGUE RECEPTOR (GHS-R1a)

Ghrelin was initially identified in 1999 as the endogenous ligand for the orphan receptor growth-hormone secretagogue (GHS-R) and hailed for its potent growth hormone stimulating capabilities [1]. Prior to the identification of ghrelin, stimulation of the GHS-R was achieved using synthetic peptides, including growth hormone-releasing peptide-6 (GHRP-6), which as the name implies, was also regarded for its ability to induce growth hormone secretion [8]. The GHS-R was first identified in 1996 as a seven transmembrane domain peptide totaling 366 amino acids. It is a G protein-coupled receptor (GPCR) that is linked to both G_q and G_s signaling pathways. It generates intracellular signaling through its $G_{\alpha 11}$ subunit, although the specific intracellular pathways elicited by this receptor are dependent on the tissue type in which it is expressed [17] (see Figure 1 for an overview). The gene encoding GHS-R resides on chromosome 3q26.2 [18] and presents sequence homology with only two other identified protein receptors: the motilin receptor, with 52% sequence identity [19], and the neurotensin receptor. The functional ghrelin receptor GHS-R type 1a (GHS-R1a) is one of the transcripts

produced by the GHS-R gene. Two isoforms of the GHS-R have been described (GHS-R1a and GHS-R1b), but only the GHS-R1a isoform appears to functionally transduce ghrelin signaling [20] and is the most widely studied isoform of the GHS-R.

ENERGY REGULATORY ACTIONS OF GHRELIN

It is clear that ghrelin and GHS-R1a are widely expressed in numerous tissues of both the peripheral and central nervous systems, indicating that ghrelin has many functions. Ghrelin and GHS-R1a are known to have important biological actions in many physiological processes, such as the central regulation of food intake and energy homeostasis [21], regulation of cardiovascular functions [22,23], stimulation of gastric acid secretion and motility [24], modulation of cell proliferation and survival [25], and inhibition of inflammation and regulation of immune function [26,27]. Next, we will summarize some of the energy regulatory actions of ghrelin-mediated GHS-R1a signaling.

I. GHRELIN AND APPETITE CONTROL

In rodents, circulating levels of ghrelin increase significantly with fasting and return to baseline following re-feeding [28,29]. Both peripheral and intracerebroventricular (i.c.v.) administration of ghrelin have been shown to lead to an increase in food intake in rodents, with chronic treatments contributing to body weight gain due to excessive adiposity [21]. Likewise, peripheral ghrelin administration has also been shown to increase food consumption in human subjects [30]. Circulating ghrelin levels rise throughout the day in humans (reflecting circadian entrained feeding times) and decrease after feeding (postprandially) [31]. The arcuate nucleus of the hypothalamus (Arc) is a central player in regulating food intake and satiety. Two main populations of neurons exist here: the anorexigenic proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) expressing neurons and the orexigenic NPY/AgRP expressing neurons [32]. The mechanisms underlying ghrelin's ability to control appetite seem to involve the direct interaction of ghrelin with neuropeptide Y (NPY) and agouti-related peptide (AgRP)-expressing neurons in the hypothalamic Arc region. For example, Nakazato and colleagues demonstrated that following ghrelin administration and consequential increases in feeding behavior, Fos protein expression was upregulated specifically in the NPY and AgRP-expressing neurons of the Arc [21]. Additionally, using NPY/AgRP double knock-out mice, Chen and colleagues showed that both of these peptides were necessary to mediate the appetite-stimulating effects of ghrelin, as these mice failed to increase feeding following ghrelin administration [33]. Thus, the stimulation of NPY/AgRP neurons facilitated the orexigenic effects of ghrelin. The orexin-expressing neurons of the lateral hypothalamus have also been shown to play a role in appetite stimulation by ghrelin. It is thought that the effects of ghrelin on the orexin neurons is independent of upstream NPY activation, as anti-NPY IgG pre-treatment failed to attenuate ghrelin-induced Fos expression in the orexin neurons [34]. Additionally, several recent studies have shown that the endocannabinoid system might also mediate the orexigenic effects of ghrelin. It has been proposed that ghrelin increases endogenous cannabinoid expression, which subsequently mediates the release of appetite stimulating hormones such as NPY [35].

II. GHRELIN AND INSULIN-MEDIATED GLUCOSE REGULATION

Immunohistochemical analyses have demonstrated that ghrelin is endogenously expressed in pancreatic islets in the α -, β -, and δ -cells, while the GHS-R1a is expressed primarily on pancreatic α - and β -cells [36]. These findings indicate that the regulation of ghrelin and insulin is tightly linked. In addition to obesity, low plasma ghrelin levels have been associated with major hallmarks of metabolic syndrome, such as hyperinsulinemia and insulin resistance [37]. Ghrelin's ability to regulate glucose metabolism via insulin is another essential mechanism through which it mediates energy homeostasis. In both humans and rodents, acute

ghrelin administration has been shown to induce hyperglycemia and reduce insulin secretion [38]. In rodents, this effect was shown to be completely lost with the administration of a GHS-R antagonist, demonstrating the importance of the ghrelin receptor in mediating this effect [39]. It is thought that stomach derived ghrelin acts distally in the brain to regulate food intake, while pancreatic ghrelin acts locally to influence insulin release as a paracrine hormone.

III. GHRELIN AND METABOLIC DYSFUNCTION

The ability of ghrelin to stimulate food intake in rodents and humans undoubtedly suggests a role in the etiology of obesity. In healthy individuals, ghrelin is upregulated under conditions of negative energy balance and decreases once balance is restored [31,40]. However, it is likely that over-eating, potentially leading to an obese phenotype, could ensue if ghrelin is upregulated when an organism is not in a state of negative energy balance or if ghrelin levels fail to fall postprandially. Interestingly, plasma ghrelin levels are decreased in obese patients compared to healthy controls [41,42], suggesting that ghrelin levels may adapt to reduce further orexigenic stimulation in a state of extreme energy dysregulation [43]. It is likely, however, that this adaptation only occurs once the organism has reached a chronic state of positive energy balance and that heightened ghrelin levels may in fact play a role in reaching the obese state. Indeed, the circulating ghrelin level of obese patients was found to rise following diet-induced weight loss [44], a phenomenon that may impair weight loss maintenance [45].

Further evidence of ghrelin's role in obesity and energy homeostasis comes from the study of individuals suffering from various binge-eating disorders. For example, both bulimia nervosa (BN) and Prader-Willi Syndrome (PWS) are disorders that are associated with habitual and extreme overeating (hyperphagia). Studies have shown that each of these conditions is associated with significantly increased circulating ghrelin levels in affected patients compared to healthy controls [46,47]. Furthermore, patients with BN exhibit a blunted reduction in ghrelin levels following food intake [48,49]. Spontaneous or genetically induced increases in circulating levels of ghrelin may put individuals at risk for hyperphagia and thus obesity. Indeed, obese patients participate in many of the same "at risk" eating behaviors as people with binge-eating disorders, including binge eating, snacking, night eating, and eating in the absence of hunger [50–53]. Interestingly, it has been shown that i.c.v. administration of ghrelin led rats to preferentially choose a high-fat diet over a high-carbohydrate diet, suggesting that ghrelin may play a role in unhealthy food choices as well [54]. Patients with anorexia nervosa (AN), a condition characterized by extreme fasting, also have significantly increased levels of circulating ghrelin. Despite having a heightened level of orexigenic signaling, these patients fail to increase feeding, suggesting a decreased sensitivity or resistance to ghrelin [8,40,55, 56]. Gueorguiev and colleagues recently investigated ghrelin and GHS-R gene polymorphisms that could be associated with obesity, eating behaviors, or glucose metabolism. While other studies have found associations between leptin or leptin receptor polymorphisms and eating behaviors [57], the Gueorguiev group failed to identify any ghrelin or ghrelin-receptor based single-nucleotide polymorphisms (SNPs) that could be considered major contributors to the obese phenotype [58]. However, their data did suggest an association between ghrelin SNPs and altered insulin sensitivity. More specifically, their results suggested that the rs27647 variant of the ghrelin gene could play a role in increasing the risk of developing insulin resistance and obesity [58].

EFFECTS OF GHRELIN SIGNALING ON BRAIN FUNCTION

Based on widespread GHS-R1a expression in the CNS, it is reasonable to hypothesize that ghrelin signaling may also play a role in other aspects of normal brain functioning in addition to those associated with appetite and energy homeostasis. There is mounting evidence that demonstrates that ghrelin-mediated GHS-R1a signaling also plays an important role in mediating cognitive function. Specifically, ghrelin is thought to play a role in memory retention

for the spatial localization of food sources. The ability to locate food sources, remember those locations, and recall whether all available food has been consumed are evolutionarily important skills for survival. In the following sections, we will discuss some of the brain functions associated with ghrelin-mediated GHS-R1a signaling.

I. GHRELIN SIGNALING AND MEMORY FUNCTION

The hippocampus, amygdala, and dorsal raphe nucleus are some of the main brain regions responsible for facilitating learning and memory. Synaptic plasticity has traditionally been associated with higher brain functions, including learning and memory processing. In particular, various forms of neuronal plasticity within the hippocampus are thought to underlie spatial learning and memory development. One of the first reports showing that ghrelin could affect cognition was by Carlini and colleagues, which demonstrated that i.c.v. injections of ghrelin increased memory retention [59]. In order to more precisely define the exact site of ghrelin action, the group then repeated the experiment and used intraparenchymal injections of increasing ghrelin concentrations in the hippocampus, amygdala, and dorsal raphe nucleus [60]. Following behavioral testing with rats, they concluded that ghrelin potentiates a dose-dependent increase in memory retention, with the maximal effect occurring in the hippocampus [60]. More recently, another group showed that peripherally administered ghrelin could enter the CNS via a passive uptake mechanism and could bind to hippocampal neurons, promoting dendritic spine formation and generation of long-term potentiation (LTP). The electrophysiological and synaptic morphological changes observed in these studies were also associated with enhanced performance in spatial learning and memory tasks [61]. Moreover, in line with this data, it was also found that ghrelin knock-out animals had a smaller number of spine synapses in the stratum radiatum, and they underperformed compared to their wild-type litter mates in novel object recognition tests. Ghrelin administration, however, was found to rapidly restore this impairment in the ghrelin knock-out mice, indicating that this stomach hormone governs neuronal morphology of brain areas that play an important role in learning performance and memory formation [61]. It is interesting to note that both ghrelin and GHS-R knock-out mice do not show any obvious metabolic phenotype while maintained on a standard rodent lab chow [62,63], although they do show resistance to diet-induced obesity. This suggests that ghrelin-mediated GHS-R1a signaling may only play a limited role in homeostatic energy balance and that ghrelin may play a larger role in regulating higher brain functions that are dependent upon metabolic status rather than solely controlling peripheral energy balance.

For several years, there has been considerable effort and research funds directed at the discovery and development of selective ghrelin receptor agonists. Atcha and colleagues recently investigated the efficacy of two structurally-related, non-peptide ghrelin receptor agonists (GSK894490A and CP-464709-18) on rat (male Lister hooded rat) cognition [64]. The effects of the two test compounds on rat cognitive performance was determined using the novel object recognition test, a water maze paradigm, and a scopolamine-induced deficit in cued fear conditioning. It was found that both novel compounds significantly improved performance in the novel object preference test and water maze test, but they were unable to attenuate a scopolamine deficit in the cued fear conditioning paradigm. These results have demonstrated that these two small-molecule ghrelin receptor agonists were able to cross the blood-brain-barrier and elicit pro-cognitive effects in recognition and spatial memory tests [64].

GHS-R1a is expressed in hippocampal progenitor cells, a finding that also suggests an involvement of ghrelin-mediated GHS-R1a signaling in adult hippocampal neurogenesis [65]. Moon and colleagues recently demonstrated that peripheral administration of ghrelin stimulated the proliferation and differentiation of hippocampal neuronal progenitor cells in the

subgranular zone (SGZ) of adult mice. This proliferative effect of ghrelin is characterized by the observation that the number of 5-bromo-2'-deoxyuridine (BrdU) positive cells was significantly increased in animals that were treated with ghrelin for 8 days. Furthermore, an immunohistochemical study using doublecortin (DCX) showed that the number of newly generated neurons in the SGZ increased significantly. Additionally, this study also demonstrated that even endogenous ghrelin-mediated GHS-R1a signaling plays an important role in neurogenesis, as immuno-neutralization of ghrelin inhibited BrdU incorporation and decreased DCX-positive cells in the SGZ [65]. The finding that ghrelin-mediated GHS-R1a signaling plays a role in mediating proliferation and early neuronal differentiation of adult hippocampal progenitor cells could pave the way for the development of ghrelin receptor agonists that could be used for the treatment of impaired learning and memory processing.

II. GHRELIN SIGNALING AND NEUROPROTECTION

Ghrelin-mediated GHS-R1a signaling has been shown to exert protective effects in mouse models of Parkinson's disease (PD) and focal ischemia/reperfusion. PD is a neurological disorder which is clinically characterized by resting tremor, rigidity and bradykinesia. The major neuropathological feature of PD is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SN). Several cellular mechanisms have been shown to be involved in the pathogenesis of dopaminergic nigrostriatal neurons in PD patients, such as apoptosis, oxidative stress, increased iron content, and mitochondrial dysfunction [66–69]. These interrelated events form a complex cascade that ultimately lead to neuronal death by apoptosis [66,67,70–72]. Recently, it was shown that ghrelin mediated GHS-R1a signaling protected dopaminergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in C57BL/6 mice [73]. I.c.v. injections of ghrelin at a low dose (3 nmol/kg) administered daily for 8 days was shown to produce a dramatic rescue of the dopaminergic neuronal death and an effective prevention of dopamine depletion in the striatum induced by MPTP injection. Additionally, Jiang and colleagues further demonstrated that ghrelin amplified dopamine signaling through the formation of GHS-R/DR1 heterodimers [74]. These results suggest a potential crosstalk between ghrelin signaling and the dopamine signaling pathway. It has also been demonstrated that ghrelin promotes and protects nigrostriatal dopamine function via an uncoupling protein 2 (UCP2)-dependent mitochondrial mechanism. Recent evidence indicates that the ablation of ghrelin or the ghrelin receptor reduces UCP2, indicating that decreased endogenous ghrelin signaling affects UCP2. As a consequence, reduced UCP2 activity increases the susceptibility of SN neurons toward oxidative stress [75].

In addition to protecting nigrostriatal dopamine function, the anti-apoptotic and protective effects of ghrelin-mediated GHS-R1a signaling on neurons have also been observed in ischemia/reperfusion injury models [76,77]. One study by Miao and coworkers showed that ghrelin protected rat hippocampal neurons after ischemia/reperfusion injury by inducing an increase in cell survival [78]. Moreover, ghrelin-treated primary cortical neurons were also protected from apoptosis induced by lipopolysaccharide, glutamate, N-methyl-D-aspartic acid (NMDA) and H₂O₂. The mechanisms behind this neuroprotective effect are thought to involve the up-regulation of Bcl-2 and heat-shock protein 70 (HSP70) and the inhibition of caspases 8, 9, and 3 upon binding to GHS-R1a [78]. Similarly, ghrelin was also reported to protect hippocampal neurons from pilocarpine-induced seizure by regulating the phosphatidylinositol-3-kinase and Akt pathway in rats [79].

CONCLUSIONS

Ghrelin and its receptor, GHS-R1a, are widely expressed throughout the body and play important roles in an extensive range of biological functions. It is becoming apparent that

peripheral metabolic signals, such as ghrelin-mediated GHS-R1a signaling (which has predominately been associated with metabolic and endocrine regulation thus far), may also have a profound influence on higher brain functions. In this review, we have discussed ghrelin's role in regulating appetite, glucose homeostasis, and metabolic dysfunction. In addition, we have provided an overview of some of the putative roles of ghrelin and ghrelin-mediated GHS-R1a signaling in aspects of higher brain functioning, such as learning and memory and neuroprotection. Our understanding of neurodegenerative disorders has evolved to a point in which we should no longer isolate central neuronal health from whole somatic health, *i.e.*, "mens sana in corpore sano". Therefore, contribution of both metabolic health and glycemic health seems to be critical for the maintenance of properly functioning nervous tissue [80, 81]. Thus, with further careful research, selective ghrelin receptor signaling could serve as an efficacious therapeutic target for the treatment of impaired cognitive function, which frequently occurs in association with metabolic syndrome, type 2 diabetes, aging, and neurological disorders such as Parkinson's disease, Alzheimer's disease, and stroke.

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ABBREVIATIONS

AgRP	Agouti related peptide
CNS	Central nervous system
GHS-R	Growth hormone secretagogue receptor
NPY	Neuropeptide Y
PD	Parkinson's disease
SGZ	Subgranular zone

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Figure 1. An overview of some ghrelin and GHS-R1a down-stream signaling pathways

These signaling pathways include the dominant growth hormone secretagogue-related PLC/ PKC/IP3 pathway, hypothalamic ghrelin activation of NPY-containing neurons and inhibition of POMC neurons, and ghrelin augmentation of dopamine signaling, involving GHS-R1a/D1R heterodimerization, leading to a synergistic activation of AC2 via G α s (D1Rcoupling) and $\beta\gamma$ -subunits derived from G α i (GHS-R1a coupling). DAG = diacylglycerol, IP3 =Inositol (1,4,5)triphosphate, POMC = pro-opiomelanocortin, D1R = dopamine receptor 1, AC2 = adenylyl cyclase-2