Review Article

Ghrelin and the central regulation of feeding and energy balance

Alfonso Abizaid, Tamas L. Horvath[1]

Department of Neuroscience, Carlton University, Ottawa, ON, Canada, [1]Program in Integrative Cell Signaling and Neurobiology of Metabolism, Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT, USA

ABSTRACT

Ghrelin was discovered in 1999 as growth hormone secretagouge released from the gut. Soon after it was recognized that ghrelin is a fundamental driver of appetite in rodents and humans and that its mode of action requires alteration of hypothalamic circuit function. Here we review aspects of ghrelin's action that revolve around the central nervous system with the goal to highlight these pathways in integrative physiology of metabolism regulation including ghrelin's cross-talk with the action of the adipose hormone, leptin.

Key words: Food intake, ghrelin, hypothalamus, neuropeptide Y, proopiomelanocortin

Introduction

The discovery of ghrelin, a 28-aminoacid peptide hormone, has generated a substantial amount of attention for a number of reasons. Initially, ghrelin was heralded as the long-sought endogenous ligand for the orphan growth hormone secretagogue receptors (GHS-Rs). Indeed, like growth hormone secretagogues (GHS), ghrelin targeted these receptors to potently increase the release of growth hormone (GH) both *in vitro* and *in vivo*. Soon, however, it became evident that ghrelin was implicated in a variety of physiological processes that include cell proliferation, metabolism, cell protection, reproduction, etc. Of these, the effects of ghrelin on food intake and metabolism have had the biggest impact; unlike other peripheral signals associated with energy balance, ghrelin increases appetite and leads to the accumulation of body fat. Indeed, the stimulatory effects of ghrelin on food intake and its apparent opposite relation to the anorectic hormone, leptin, have been proposed as the yin-yang model for hormonal regulation of energy balance. Nevertheless, the more is known about ghrelin, the more it becomes obvious that ghrelin produces its metabolic effects via a multitude of central and peripheral mechanisms that work in parallel to modulate the effects of ghrelin in energy regulation. This chapter will review the literature regarding the effects of ghrelin on energy balance. Energy balance implies the regulation of both food intake and energy expenditure; therefore, we will discuss both topics in relation to ghrelin. A description of the possible central routes of ghrelin's actions on energy balance within the brain will follow. Finally, we will present additional data suggesting that ghrelin targets various brain circuits besides those in the mediobasal hypothalamus, involved in energy homeostasis. The effects of ghrelin on other physiological processes can be found in several excellent recent reviews.[1-3]

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.105580

Ghrelin: The Endogenous Growth Hormone Secretagogues

Much of the work that culminated in the unveiling of the ghrelin protein began with descriptions of peptides that were cleaved from metenkephalin and that increased the secretion of GH, but were devoid of opioidergic effects.[4,5] Because of this, these compounds were named growth hormone secretagogues. For years, efforts were focused on designing more effective GHS, leading to the production of peptide and non-peptide compounds including growth hormone

Corresponding Author: Prof. Tamas L. Horvath, BML 330, 310 Cedar St., New Haven CT USA, 06520. E-mail: tamas.horvath@yale.edu

releasing peptide-6 (GHRP-6), GHRP-2, hexarelin, L-692, 429, and MK-0677, to name a few.[6-8]

The latter compound was used by Howard *et al.* in studies leading to the cloning and characterization of the G-coupled protein receptor named the growth hormone secretagogue receptor (GHS-R).[9,10] To date, two subtypes of this receptor have been identified: GHS-R 1a and GHS-R 1b. Most of the current work on the biology of these receptors has focused on GHS-R 1a because GHS-R 1b is devoid of any known ligand-specific responses.[10] Interestingly, GHS-R 1a and 1b localization is ubiquitous, but high concentrations of these receptors are found in the pituitary and hypothalamus.[9,10] The presence of a receptor that bound GHS suggested that organisms produced an endogenous substance with biological properties similar to those of these compounds.

The identification of the GHS-R was soon followed by the first description of ghrelin by Kojima *et al.*[11] In their studies, they identified a protein product of oxyntic cells in the stomach that bound to GHS-R and increased the secretion of GH in a manner that suggested it was the endogenous GHS. The structure of the 28-aminoacid ghrelin protein was peculiar in that its biological activity depended upon the acylation of the hydroxyl group of the serine-3 residue by *n*-octanoic acid. Several splice variants of ghrelin have been identified, some of which produce similar effects to those of ghrelin (i.e. Des-Gln14-ghrelin),[12] some which have no known effect (non-acylated ghrelin),[3] and some which have opposite effects to those of ghrelin (Obestatin).[13] In their seminal paper, Kojima *et al.* also showed that the stomach was the main source of circulating ghrelin, although other organs including the brain produced ghrelin. Within the brain, it appeared that ghrelin was secreted by a subset of neurons in the lateral portion of the hypothalamic arcuate nucleus.[11] Further studies have confirmed the presence of ghrelin in the hypothalamus, and a detailed neuroanatomical distribution of the central ghrelin system has been described.[14] The relative contribution of peripheral versus central ghrelin to the regulation of energy balance remains a topic of discussion.

Ghrelin and Food Intake

Evidence for the orexigenic effects of ghrelin was predicted by several reports showing that GHS stimulated food intake independently from its effects on GH secretion.[15-18] The identification of the GHS-R and its localization within hypothalamic sites heavily implicated in the regulation of energy balance provided a second clue to the regulatory role of GHS and of ghrelin.[19] Finally, studies showing that GHS increased the expression of Fos immunoreactivity, an index of increased transcriptional activation, in the same hypothalamic sites where the GHS-R was located, were final indicators that the endogenous GHS was a potent regulator of energy balance.[20-25]

The effects of ghrelin on food intake were first established in three very influential papers.[26-28] These papers demonstrated that unlike other orexigenic peptides such as neuropeptide Y (NPY), peripherally delivered ghrelin acted in the brain to stimulate food intake and adiposity. Wren *et al.* determined that acute ghrelin treatment delivered either peripherally or into the cerebral ventricles [intracerebroventricular (ICV)] produced a potent increase in food intake that was similar to that obtained with equimolar concentrations of NPY.[26] Soon after, Nakazato and associates showed similar orexigenic effects of central ghrelin administration, and further, they demonstrated that the hypothalamic arcuate nucleus (ARC) and particularly neurons producing NPY/agouti related peptide (AgRP) were implicated in the food intake effects of ghrelin.[28] Finally, Tschop and colleagues determined that chronic central ghrelin treatment, in addition to potently stimulating food intake, also decreased the utilization of fat as fuel, leading ultimately to an increase in the accumulation of body fat.[27] Furthermore, Tschop's and Nakazato's groups demonstrated that ghrelin stimulated food intake in GH-deficient mice, showing that the orexigenic effects of ghrelin are not mediated indirectly by increases in GH secretion.[27,28] Several things are evident from these studies. The first is that ghrelin increases food intake dramatically especially when infused directly into the brain. The second is that even though the effects of chronic peripheral ghrelin on food intake subside, they do not decrease when ghrelin is administered chronically into the brain. Finally, these studies show the hypothalamus is sensitive to ghrelin stimulation, as reflected by increases in Fos immunoreactivity in the ARC following ghrelin treatment. In all, these data demonstrated that ghrelin targets cells within the ARC to increase food intake and produce metabolic changes geared to accumulate body fat.

In agreement with these data, circulating levels of ghrelin fluctuate in response to changes in energy status. In rodents, plasma ghrelin concentrations rise just before the onset of the dark phase of the light/dark cycle, the time of day when these animals consume most of their food.[29] Moreover, fasting increases circulating plasma levels of ghrelin in both mice and rats.[27,30-32] Rats treated with streptozotocin, a drug that destroys pancreatic beta cells and that results in a diabetic state, also have high levels of circulating ghrelin levels that correlate with increased food intake.[33-35] In humans, ghrelin concentrations rise prior to scheduled meals and are usually elevated after

fasting periods.[36] Interestingly, in both laboratory animals and human subjects, ghrelin levels decrease throughout the course of a meal.[29,36-38] Therefore, it is not surprising that hormones that regulate metabolic function such as gonadal and adrenal steroids, and those that are related to short-term regulation of food intake such as glucagon also appear to regulate plasma ghrelin levels.[30,39-44]

In addition to circulating ghrelin levels being increased, sensitivity to ghrelin may also rise in states of negative energy balance. Fasting, for example, leads to changes in the expression of GHS-R in the hypothalamus and pituitary of rats and mice.[45-47] Within the hypothalamus, increases in the message for GHS-R have been reported in NPY/AgRP/gamma-amino butyric acid (GABA) cells, but it is likely that this also occurs in cells within other brain regions where the GHS-R is present.[45,48,49] Importantly, immunoneutralization of ghrelin, or pharmacological blockade of GHS-R using GHS-R antagonists both result in decreases in food intake and adiposity in normal rats and mice, in streptozotocin-treated rats, and in leptin-deficient ($^{Lep}/^{Lep}$) mice.[28,33,50] Similar results are seen in rats that overexpress an antisense for the GHS-R in the brain under the activity of the tyrosine hydroxylase promoter.[51]

As mentioned above, ghrelin and its analogs also produce increases in adiposity. Ghrelin appears to do this by a variety of mechanisms. One is by altering metabolic function in order to preferentially utilize energy derived from carbohydrates, thus decreasing the use of fat as a fuel.[27] A second mechanism is the one in which ghrelin preferentially increases the ingestion of calories derived from fat.[50,52] A third mechanism is the one where ghrelin decreases metabolic function by lowering the resting metabolic rate and heat production.[53] A final mechanism identified thus far is the one where ghrelin decreases energy expenditure by decreasing spontaneous locomotor activity.[54] These ghrelin-induced changes are of particular importance, especially if ghrelin and its related compounds are to be considered as pharmacological tools to control metabolic function. These changes may also provide clues as to why ghrelin-treated animals are susceptible to accumulate more fat under a high-fat diet regimen than saline-treated animals, and provide insights for the possible causes that lead to metabolic dysfunction in obese individuals.

The role of ghrelin in the regulation of food intake and energy balance has been questioned because initial studies using mice with genetic deletions of either the ghrelin or the *GHS-R* gene showed no observable phenotypical differences from their wild-type littermates.[55-57] Contrary to what was expected, ghrelin ($^{ghrl/ghrl}$) and GHS-R ($^{ghsr/ghsr}$) deficient mice ate and weighed the same as wild-type mice, showed no differential feeding in response to fasting, and were still susceptible to diet-induced obesity when fed a high-fat diet. Nevertheless, recent studies show that both $^{ghrl/ghrl}$ and $^{ghsr/ghsr}$ mice are resistant to diet-induced obesity if they are placed on the diet regimen at a relative young age (18 weeks).[58,59] Moreover, these mice showed altered metabolic parameters such as increased energy expenditure (in $^{ghrl/ghrl}$), decreased respiratory quotient (in $^{ghsr/ghsr}$), and better glycemic regulation, indicating that ghrelin may play an important role in the development of obesity and metabolic aberrations associated with this disorder.[58,59] These results are echoed in recent studies where diet-induced obese mice lost weight after treatment with a mirror (*Spiegel*) l-oligonucleotide that antagonizes the activity of the active form of ghrelin on the GHS-R.[60-62]

Ghrelin Receptors: Role in Energy Balance

The above-described reports suggest that the GHS-R 1a is crucial for ghrelin to have an effect on energy balance. The GHS-R 1a is a G-coupled protein receptor that, upon activation, increases the activity of intracellular calcium and protein kinase C (PKC) by increasing the activity of inositol 1,4,5-triphosphate (IP_3)/phopholipase C (PLC) and diacylglycerol.[63,64] This is a similar signaling cascade activated by GHS.[10,11] In any event, ghrelin increases the activity of other intracellular pathways via alternate G-coupled protein complexes. Of these, ghrelin-induced increases in the activity of 5'-AMP-activated protein kinase (AMPK), and the cyclic GMP-related release of nitric oxide (NO) are thought to play a role in the hypothalamic regulation of energy balance.[65,66] However, complete characterization of these signaling pathways in relation to ghrelin activation of GHS-R 1a and GHS-R 1b pathways is needed. In addition, it appears that GHS-Rs have a high level of constitutive activity, and can be activated by other ligands such as cortistatin and adenosine.[67-71] The physiological relevance of these phenomena is not yet understood. Furthermore, several studies have suggested the existence of additional GHS-Rs that have not yet been characterized.[64,72,73]

Early descriptions of the localization of GHS-R showed that high concentrations were detected in various endocrine organs. Of these, the hypothalamus and pituitary stood out as potential mediators for the effects of ghrelin on GH secretion.[10] *In situ* hybridization studies revealed that the hypothalamic ARC and the ventromedial hypothalamus (VMH) of rats and primates strongly expressed GHS-R mRNA.[19,74] These reports additionally showed that the message for the receptor was also localized in hypothalamic and extrahypothalamic brain regions not directly implicated in

the regulation of energy balance. Among these, the preoptic area (POA), suprachiasmatic nucleus (SCN), hippocampus, ventral tegmental area (VTA), substantia nigra (SN), and dorsal raphe nucleus (DRN) showed relatively high concentrations of GHS-R mRNA expression.[19,74] A recent and more complete study describing the neuroanatomical localization of the GHS-R transcript has confirmed these earlier data and extended them to mice. In addition, they have demonstrated that the receptor is located in brain stem regions also associated with the regulation of food intake, such as the parabrachial nucleus, area postrema, and the nucleus of the solitary tract.[75]

The number of GHS-Rs that are available for binding is modulated by ghrelin itself and by other hormones. Ghrelin has been shown to produce GHS-R internalization into vesicles *in vitro* via endosomal trafficking, where it gets recycled back to the membrane without being degraded.[76] The duration of the cycle of internalization/recycling of the GHS-R corresponds well with the pulsatile nature of ghrelin release.[76] In contrast, *in vivo* ghrelin treatment leads to a rapid increase in the expression of GHS-R mRNA in the ARC of rats, whereas leptin treatment results in decreases in GHS-R mRNA in the same region. These effects are specific to the ARC and, the effects of ghrelin at least are dependant on the presence of GH, given that GH-deficient rats do not show a ghrelin-induced increase in GHS-R.[45] Conversely, GH-deficient rats (*dw/dw* dwarf rats) do have significantly higher GHS-R mRNA expression in the hypothalamus than control rats, and the expression of GHS-R is decreased with GH treatment.[45] The idea that leptin may also play a role is suggested by the fact that genetically obese Zucker rats, and fasted animals also show increased levels of GHS-R mRNA expression in the ARC.[45]

Ghrelin and the Hypothalamic Regulation of Energy Balance

The localization of high levels of GHS-R transcripts in the ARC, as well as the increases in the expression of transcription factors in the ARC following GHS or ghrelin treatment quickly highlight to the ARC as the most relevant hypothalamic site for the regulation of ghrelin's effects on food intake and adiposity.[77] Because the ARC lies outside of the blood–brain barrier, it is placed in a position where it can monitor circulating levels of a variety of hormones, including ghrelin. Not surprisingly, the ARC contains first-order sensory neurons that have receptors for most, if not all, hormones associated with energy balance.[78-82] Orexigenic peptides like NPY and AgRP, and anorexigenic peptides like α-melanocyte stimulating hormone (α-MSH) and the cocaine–amphetamine regulated trancript (CART) are both produced by cells in the ARC. Among these, the GHS-R is reportedly expressed in NPY/AgRP/GABA secreting cells, but it is likely to be also present in α-MSH/CART secreting cells. Because both of these cell groups are also affected by leptin, and represent the cornerstone for the melanocortin hypothesis of body weight regulation, it has been suggested that ghrelin targets the hypothalamus to oppose the anorectic activity of leptin.[81-85]

Nevertheless, substantial concentrations of GHS-R are present in other hypothalamic nuclei heavily implicated in energy balance, including the VMH, paraventricular nucleus (PVN), dorsomedial hypothalamus (DMH), and suprachiasmatic nucleus (SCN).[19,74,75] In addition, central infusions of ghrelin into the lateral hypothalamus increase food intake and the activity of cells that produce hypocretin/orexin, a hypothalamic peptide implicated in arousal and food intake.[86-88] Infusions of ghrelin into the PVN are also effective in increasing food intake, and modulating neuronal activity in PVN cells at picomolar concentrations.[86,89] While no data is yet available on the effects of direct ghrelin infusions into the DMH, SCN, and VMH on food intake, they are all activated by ICV administration of ghrelin as demonstrated by increases in the expression of c-fos in these regions.[28,72] In contrast to these data, increases in the expression of c-fos following peripheral ghrelin treatment are seldom seen outside of the ARC and other regions outside the blood–brain barrier (see[90] for exception).

Ghrelin appears to modulate the activity of hypothalamic cells, and particularly cells in the ARC, by modulating their threshold of activation by inhibitory and excitatory neurotransmitters.[14,85,91] Like estrogen and leptin, ghrelin is capable of reorganizing synaptic inputs onto neurons within the ARC.[91] In contrast to estrogen or leptin, however, ghrelin increases the number of excitatory inputs and decreases inhibitory inputs onto NPY/AgRP/GABA cells, and decreases the number of excitatory inputs and increases the number of inhibitory inputs onto proopiomelanocortin (POMC, the precursor of α-MSH) secreting neurons.[91] This synaptic re-arrangement correlates with electrophysiological data (see below) and fits well with the stimulatory effects of ghrelin on food intake.[91] This, along with the fact that ghrelin appears to target regions of the brain that retain a high degree of plasticity in adulthood, like the hippocampus, SCN, and VTA, suggest that ghrelin produces its effects in the hypothalamus and elsewhere by mechanisms that involve synaptic changes.

Ghrelin and Appetite: Is it All Hypothalamic?

The relatively dense distribution of the GHS-R in the

ARC and other mediobasal hypothalamic nuclei has guided researchers to focus primarily on this region as mediating ghrlein's effects on energy regulation. In any event, the widespread distribution of the GHS-R throughout the CNS indicates that ghrelin may modulate a variety of systems associated with appetite.

The brain stem and food intake: The brain stem has been heavily implicated in the regulation of food intake and energy sensing.[92,93] Like the mediobasal hypothalamus, areas within the brain stem, such as the area postrema, lie outside the blood–brain barrier. Not surprisingly, the area postrema and the adjacent nucleus of the solitary tract have receptors for various metabolic hormones such as cholecystokinin (CCK), estrogen, leptin, and ghrelin.[75,92-94] Neurons in these regions also respond to changes in glucose and free fatty acid utilization, suggesting an important role in nutrient sensing.[95,96] In addition, the brain stem receives afferent signals from the gastrointestinal system through the ascending vagus nerve, and sends efferent signals to the gut via the descending vagus.[97-99] Vagal afferent signals target neurons in the nucleus of the solitary tract and the parabrachial nucleus, and from there they are transmitted to hypothalamic and forebrain structures for further processing.[99] Studies on animals whose brain stem is surgically isolated from the rest of the brain have demonstrated that this region is sufficient for normal responses to taste and meal size regulation in response to gastrointestinal cues.[92,93]

Studies show that ICV ghrelin infusions increase the expression of the transcription factor Fos in the area postrema and nucleus of the solitary tract.[100] These studies in addition to others show that ghrelin infused into the fourth ventricle or directly into dorsal vagal complex (DVC; a cluster of nuclei that include the area postrema, nucleus of the solitary tract, and others) increases food intake, suggesting that this area is sensitive to ghrelin stimulation.[101,102] Interestingly, ghrelin-induced food intake responses are attenuated by sub-diaphragmatic vagotomy in rodents and humans.[103,104] These data suggest that vagal signals from the gut to the brain and back play a significant role in the regulation of food intake by ghrelin.

Ghrelin and reward circuits: In addition to the hypothalamus and the brain stem, the GHS-R is localized within the VTA of the midbrain in relatively high concentrations.[19,75] The VTA contains primarily dopamine cells that play a key role in the neural system that underlie motivated behaviors. The activity of dopamine cells is correlated with increases in behaviors geared to obtain natural rewards like food or a receptive mate, and with behaviors directed toward obtaining drugs of abuse.[105,106] Double labeling studies have determined that about 40% of dopaminergic neurons in the VTA contain GHS-R 1a mRNA signal.[75] Ghrelin infusions into the VTA increase food intake, and they seem to do so independent of opioids.[107] Ghrelin, like food restriction, enhances the locomotor responses to cocaine, an indication that ghrelin modulates reward circuits.[108] In addition, ghrelin can increase food intake when infused into the nucleus accumbens, a major target for VTA dopaminergic cells and a structure implicated in the appetitive regulation of food intake.[107] Interestingly, ghrelin also increases hoarding and foraging behavior, both measures of appetitive behaviors with high motivational components in rodents.[109] In humans, ghrelin infusions not only provoke strong feeding responses, but also increase self-report scores on hunger measures and elicit vivid cravings for preferred foods.[110-112] In line with this, we found that ghrelin has a major impact on midbrain dopamine cell function and related behaviors, including feeding and responses to cocaine.[113,114] We also showed that the other midbrain dopamine system that is localized to the SN is also targeted by ghrelin action and plays a role in the protection of these cells in neurodegenerative processes resembling Parkinson's disease.[115]

Ghrelin and circadian rhythmicity: Food intake and most metabolic functions show fluctuations across the light/dark cycles, and these are regulated primarily by a central circadian pacemaker, which in mammals is located in the hypothalamic SCN.[113] It is therefore intriguing that the SCN contains dense concentrations of GHS-R.[74,75] While there are no data to date as to the role of these receptors in the regulation of circadian rhythms, GHS-R–deficient (but not ghrelin-deficient) mice show lower levels of locomotor activity in the early dark phase of the light/dark cycle, suggesting the possibility of altered circadian patterns.[59] In addition, scheduled meals can produce ghrelin peaks similar to pre-prandial ghrelin increases in the early dark phase of the light/dark cycle.[29,38,114] It could be speculated that ghrelin acts in the SCN to modulate behavioral rhythms according to the timing of food availability in the environment.

Brain Ghrelin?

A hypothesis that has generated a tremendous amount of interest and controversy is that ghrelin is synthesized directly in neurosecretory cells in the brain[14,115] and these are modified by energetic state.[116] Indeed, several independent groups have shown that hypothalamic cells secrete ghrelin and communicate with cell groups throughout the hypothalamic nuclei shown to contain ghrelin receptors.[14] Some papers suggest that the distribution of ghrelin producing neurons is restricted to the ventrolateral

portion of the ARC,[11,88,117,118] whereas others have shown an extensive distribution of ghrelin immunoreactive cells that literally envelops all major hypothalamic centers.[14] Projections from ghrelin cells make contact with NPY/AgRP cells in the ARC, hypocretin/orexin cells in the lateral hypothalamic region, and corticotropin releasing hormone (CRH) neurons in the PVN.[14,88,117] The electrical activity of these cell groups is modulated by ghrelin as shown using patch clamp electrophysiological studies where ghrelin stimulates the activity of NPY/AgRP and hypocretin/orexin cells, and decreases the activity of POMC and CRH cells.[85,86,91,119] This particular pattern of activity is associated with increased food intake and decreased energy expenditure. A caveat to this hypothesis is that the transcript for ghrelin in the hypothalamus can only be detected by amplifying mRNA signals using polymerase chain reaction (PCR), but not by *in situ* hybridization probes that work to detect the ghrelin transcript on stomach cells. Moreover, mice with the *lacZ* reporter gene inserted into the ghrelin promoter do not show LacZ enzyme activity in the hypothalamus,[57] although they do show LacZ mRNA.[55] Much work is needed to clarify these discrepancies, yet the widespread distribution of GHS-R in the central nervous system, coupled with the apparent difficulty of ghrelin to enter the brain still point to a central source of ghrelin.[120]

Ghrelin Signaling

For a long time, it was not known how intracellular events ensue after GHS-R activation to alter neuronal function in the brain. We addressed that question regarding ghrelin's effect on AgRP neuronal activation.[121] We found the following: (1) ghrelin induced a rapid increase of NPY/AgRP neuronal firing via activation of the ghrelin receptor, GHS-R. (2) Ghrelin-induced GHS-R activation resulted in activation of AMPK. (3) AMPK activation suppressed acetyl CoA carboxylase (ACC) activity, eliminating the inhibitory effect of malonyl-CoA on carnitine palmitoyl transferase 1 (CPT1) activity. (4) CPT1 activation enhances long chain fatty acid oxidation. (5) Fatty acid oxidation generated reactive oxygen species (ROS), which together with fatty acids promoted uncoupling protein 2 (UCP2) transcription and activity. (6) UCP2 activity neutralized ROS, allowing continuous CPT1-promoted fatty acid oxidation and transcription of genes promoting mitochondrial proliferation (such as *NRF1*) enabling continuous support of the bioenergetic needs of sustained firing of NPY/AgRP cells. (7) Sustained firing of NPY/AgRP neurons resulted in activity-dependent synaptic plasticity promoting an organization of increased inhibitory input onto POMC neurons.

Leptin Control of Ghrelin Secretion

As discussed above, the site of action of ghrelin overlaps that of leptin in the hypothalamus. Their effects on neuronal activation of the melanocortin system are opposing,[14,84,85] which in turn affects food intake, energy expenditure, and adiposity in opposing manners as well.[122] It is now well documented that leptin is the major endogenous inhibitory regulator of ghrelin secretion from oxyntic cells of the stomach and feeding at the level of NPY/AgRP/GABA neurons. This is supported by reciprocal relationship in minute-by-minute circulating levels of leptin and ghrelin during the day, pre- and post-prandially in normal and obese rodents and humans. However, there is also an apparent paradoxical regulatory relationship between exogenous leptin and ghrelin secretion in the periphery, where hypothalamic leptin gene therapy enhances peripheral *ghrelin secretion*.[123] This yin-yang relationship between leptin and ghrelin has important clinical implications.

Clinical Implications

Obesity is currently a primary target for drug development given the rapid increase in the incidence of this condition and the risk factors associated with it. Animal studies show that the antagonism of ghrelin leads to weight reduction and control of glucopenia in diet induced and in genetically obese animals, indicating that antagonists for ghrelin may provide for potential treatments for obesity, type II diabetes, and other metabolic disorders.[58,59] In addition, ghrelin-based treatments may be used to increase appetite in patients having wasting disorders such as cancer-induced cachexia or in those receiving chemotherapy.[124]

As we unravel the complexities of the multimodal actions of ghrelin on brain function, we may find that ghrelin and compounds associated with this hormone may be effective in modulating not only metabolism but also the appetitive and cognitive functions related to the human experience of "cravings," something that is key for the treatment of psychiatric conditions associated with eating disorders like anorexia nervosa and bulimia, and for the treatment of drug abuse.

Conclusion

The discovery of ghrelin and its well-established role in the regulation of feeding and energy balance has provoked an amount of attention that is almost comparable to that generated by leptin. Ghrelin appears to target different CNS regions to modify energy balance and food intake in a multimodal manner. Among these, only the effects of ghrelin

on hypothalamic function are somewhat understood, perhaps because we are only beginning to explain the hypothalamic circuits regulating energy homeostasis. Nevertheless, it is becoming more apparent that other systems interact with the hypothalamus to regulate energy balance, and most of these are also targeted by ghrelin as well as other metabolic signals such as leptin and insulin. Future studies will surely provide a clearer picture of the relative contribution of these regions to energy regulation and their responses to changes in metabolism. Gaining knowledge on these networks will surely advance basic understanding of energy regulation and perhaps generate alternative, more effective treatments for obesity.

References

1. Smith RG, Jiang H, Sun Y. Developments in ghrelin biology and potential clinical relevance. Trends Endocrinol Metab 2005;16:436-42.
2. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005;85:495-522.
3. van der Lely AJ, Tschop M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev 2004;25:426-57.
4. Bowers CY, Momany FA, Reynolds GA, Hong A. On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. Endocrinology 1984;114:1537-45.
5. Bowers CY, Reynolds GA, Momany FA. New advances on the regulation of growth hormone (GH) secretion. Int J Neurol 1984;18:188-205.
6. Bowers CY. GH releasing peptides—structure and kinetics. J Pediatr Endocrinol 1993;6:21-31.
7. Smith RG, Palyha OC, Feighner SD, Tan CP, McKee KK, Hreniuk DL, *et al.* Growth hormone releasing substances: Types and their receptors. Horm Res 1999;51(Suppl 3):1-8.
8. Smith RG. Development of growth hormone secretagogues. Endocr Rev 2005;26:346-60.
9. Davenport AP, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, *et al.* International Union of pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. Pharmacol Rev 2005;57:541-6.
10. McKee KK, Palyha OC, Feighner SD, Hreniuk DL, Tan CP, Phillips MS, *et al.* Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. Mol Endocrinol 1997;11:415-23.
11. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656-60.
12. Hosoda H, Kojima M, Matsuo H, Kangawa K. Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. J Biol Chem 2000;275:21995-2000.
13. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, *et al.* Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 2005;310:996-9.
14. Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, *et al.* The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 2003;37:649-61.
15. Torsello A, Luoni M, Schweiger F, Grilli R, Guidi M, Bresciani E, *et al.* Novel hexarelin analogs stimulate feeding in the rat through a mechanism not involving growth hormone release. Eur J Pharmacol 1998;360:123-9.
16. Torsello A, Locatelli V, Melis MR, Succu S, Spano MS, Deghenghi R, *et al.* Differential orexigenic effects of hexarelin and its analogs in the rat hypothalamus: Indication for multiple growth hormone secretagogue receptor subtypes. Neuroendocrinology 2000;72:327-32.
17. Locke W, Kirgis HD, Bowers CY, Abdoh AA. Intracerebroventricular growth-hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. Life Sci 1995;56:1347-52.
18. Lall S, Tung LY, Ohlsson C, Jansson JO, Dickson SL. Growth hormone (GH)-independent stimulation of adiposity by GH secretagogues. Biochem Biophys Res Commun 2001;280: 132-8.
19. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, *et al.* Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res 1997;48:23-9.
20. Dickson SL, Doutrelant-Viltart O, Leng G. GH-deficient dw/dw rats and lit/lit mice show increased Fos expression in the hypothalamic arcuate nucleus following systemic injection of GH-releasing peptide-6. J Endocrinol 1995;146:519-26.
21. Dickson SL, Leng G, Dyball RE, Smith RG. Central actions of peptide and non-peptide growth hormone secretagogues in the rat. Neuroendocrinology 1995;61:36-43.
22. Dickson SL, Doutrelant-Viltart O, Dyball RE, Leng G. Retrogradely labeled neurosecretory neurones of the rat hypothalamic arcuate nucleus express Fos protein following systemic injection of GH-releasing peptide-6. J Endocrinol 1996;151:323-31.
23. Dickson SL, Viltart O, Bailey AR, Leng G. Attenuation of the growth hormone secretagogue induction of Fos protein in the rat arcuate nucleus by central somatostatin action. Neuroendocrinology. 1997;66:188-94.
24. Dickson SL, Luckman SM. Induction of c-fos messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. Endocrinology 1997;138:771-7.
25. Dickson SL, Bailey AR, Leng G. Growth hormone (GH) secretagogues and neuroendocrine regulation of GH secretion. Growth Horm IGF Res1999;(9 Suppl A):89-91.
26. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, *et al.* The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000;141:4325-8.
27. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908-13.
28. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, *et al.* A role for ghrelin in the central regulation of feeding. Nature 2001;409:194-8.
29. Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, Woods SC. Effects of a fixed meal pattern on ghrelin secretion: Evidence for a learned response independent of nutrient status. Endocrinology 2006;147:23-30.
30. Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, *et al.* Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. Biochem Biophys Res Commun 2001;281:1220-5.
31. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, *et al.* Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 2001;86:4753-8.
32. Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA,

et al. Ghrelin causes hyperphagia and obesity in rats. Diabetes 2001;50:2540-7.
33. Ishii S, Kamegai J, Tamura H, Shimizu T, Sugihara H, Oikawa S. Role of ghrelin in streptozotocin-induced diabetic hyperphagia. Endocrinology 2002;143:4934-7.
34. Masaoka T, Suzuki H, Hosoda H, Ota T, Minegishi Y, Nagata H, *et al.* Enhanced plasma ghrelin levels in rats with streptozotocin-induced diabetes. FEBS Lett 2003;541:64-8.
35. Gelling RW, Overduin J, Morrison CD, Morton GJ, Frayo RS, Cummings DE, *et al.* Effect of uncontrolled diabetes on plasma ghrelin concentrations and ghrelin-induced feeding. Endocrinology 2004;145:4575-82.
36. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50:1714-9.
37. Tschop M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, *et al.* Post-prandial decrease of circulating human ghrelin levels. J Endocrinol Invest 2001;24:RC19-21.
38. Sugino T, Yamaura J, Yamagishi M, Ogura A, Hayashi R, Kurose Y, *et al.* A transient surge of ghrelin secretion before feeding is modified by different feeding regimens in sheep. Biochem Biophys Res Commun 2002;298:785-8.
39. Proulx K, Vahl TP, Drazen DL, Woods SC, Seeley RJ. The effect of adrenalectomy on ghrelin secretion and orexigenic action. J Neuroendocrinol 2005;17:445-51.
40. Otto B, Tschop M, Heldwein W, Pfeiffer AF, Diederich S. Endogenous and exogenous glucocorticoids decrease plasma ghrelin in humans. Eur J Endocrinol 2004;151:113-7.
41. Nakagawa E, Nagaya N, Okumura H, Enomoto M, Oya H, Ono F, *et al.* Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: Responses to the intravenous and oral administration of glucose. Clin Sci (Lond) 2002;103:325-8.
42. Matsubara M, Sakata I, Wada R, Yamazaki M, Inoue K, Sakai T. Estrogen modulates ghrelin expression in the female rat stomach. Peptides 2004;25:289-97.
43. Camina JP, Carreira MC, Micic D, Pombo M, Kelestimur F, Dieguez C, *et al.* Regulation of ghrelin secretion and action. Endocrine 2003;22:5-12.
44. Arafat MA, Otto B, Rochlitz H, Tschop M, Bahr V, Mohlig M, *et al.* Glucagon inhibits ghrelin secretion in humans. Eur J Endocrinol 2005;153:397-402.
45. Nogueiras R, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C, *et al.* Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. Diabetes 2004;53:2552-8.
46. Park S, Sohn S, Kineman RD. Fasting-induced changes in the hypothalamic-pituitary-GH axis in the absence of GH expression: Lessons from the spontaneous dwarf rat. J Endocrinol 2004;180: 369-78.
47. Kim MS, Yoon CY, Park KH, Shin CS, Park KS, Kim SY, *et al.* Changes in ghrelin and ghrelin receptor expression according to feeding status. Neuroreport 2003;14:1317-20.
48. Seoane LM, Lopez M, Tovar S, Casanueva FF, Senaris R, Dieguez C. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. Endocrinology 2003;144:544-51.
49. Traebert M, Riediger T, Whitebread S, Scharrer E, Schmid HA. Ghrelin acts on leptin-responsive neurones in the rat arcuate nucleus. J Neuroendocrinol 2002;14:580-6.
50. Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, *et al.* Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. Gut 2003;52:947-52.
51. Shuto Y, Shibasaki T, Otagiri A, Kuriyama H, Ohata H, Tamura H, *et al.* Hypothalamic growth hormone secretagogue receptor regulates growth hormone secretion, feeding, and adiposity. J Clin Invest 2002;109:1429-36.
52. Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H, *et al.* Central administration of ghrelin preferentially enhances fat ingestion. Neurosci Lett 2004;369:75-9.
53. St-Pierre DH, Karelis AD, Cianflone K, Conus F, Mignault D, Rabasa-Lhoret R, *et al.* Relationship between ghrelin and energy expenditure in healthy young women. J Clin Endocrinol Metab 2004;89:5993-7.
54. Tang-Christensen M, Vrang N, Ortmann S, Bidlingmaier M, Horvath TL, Tschop M. Central administration of ghrelin and agouti-related protein (83-132) increases food intake and decreases spontaneous locomotor activity in rats. Endocrinology 2004;145:4645-52.
55. Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. Mol Cell Biol 2003;23:7973-81.
56. Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc Natl Acad Sci U S A 2004;101:4679-84.
57. Wortley KE, Anderson KD, Garcia K, Murray JD, Malinova L, Liu R, *et al.* Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc Natl Acad Sci U S A 2004;101:8227-32.
58. Wortley KE, Del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO, *et al.* Absence of ghrelin protects against early-onset obesity. J Clin Invest 2005;115:3573-8.
59. Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, *et al.* Mice lacking ghrelin receptors resist the development of diet-induced obesity. J Clin Invest 2005;115:3564-72.
60. Helmling S, Maasch C, Eulberg D, Buchner K, Schroder W, Lange C, *et al.* Inhibition of ghrelin action *in vitro* and *in vivo* by an RNA-Spiegelmer. Proc Natl Acad Sci U S A 2004;101:13174-9.
61. Kobelt P, Helmling S, Stengel A, Wlotzka B, Andresen V, Klapp BF, *et al.* Anti-ghrelin Spiegelmer NOX-B11 inhibits neurostimulatory and orexigenic effects of peripheral ghrelin in rats. Gut 2006;55: 788-92.
62. Shearman LP, Wang SP, Helmling S, Stribling DS, Mazur P, Ge L, *et al.* Ghrelin neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice. Endocrinology 2006;147:1517-26.
63. Malagon MM, Luque RM, Ruiz-Guerrero E, Rodriguez-Pacheco F, Garcia-Navarro S, Casanueva FF, *et al.* Intracellular signaling mechanisms mediating ghrelin-stimulated growth hormone release in somatotropes. Endocrinology 2003;144:5372-80.
64. Camina JP. Cell biology of the ghrelin receptor. J Neuroendocrinol 2006;18:65-76.
65. Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, *et al.* AMP-activated protein kinase plays a role in the control of food intake. J Biol Chem 2004;279:12005-8.
66. Gaskin FS, Farr SA, Banks WA, Kumar VB, Morley JE. Ghrelin-induced feeding is dependent on nitric oxide. Peptides 2003;24:913-8.
67. Holst B, Schwartz TW. Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation. Trends Pharmacol Sci 2004;25:113-7.
68. Holst B, Cygankiewicz A, Jensen TH, Ankersen M, Schwartz TW. High constitutive signaling of the ghrelin receptor—identification of a potent inverse agonist. Mol Endocrinol 2003;17:2201-10.
69. Carreira MC, Camina JP, Smith RG, Casanueva FF. Agonist-specific coupling of growth hormone secretagogue receptor type 1a to different intracellular signaling systems. Role of adenosine.

Neuroendocrinology 2004;79:13-25.
70. Deghenghi R, Papotti M, Ghigo E, Muccioli G. Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GHS) receptors of human pituitary gland. J Endocrinol Invest 2001;24:RC1-3.
71. Broglio F, Arvat E, Benso A, Gottero C, Prodam F, Grottoli S, *et al.* Endocrine activities of cortistatin-14 and its interaction with GHRH and ghrelin in humans. J Clin Endocrinol Metab 2002;87:3783-90.
72. Halem HA, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, *et al.* A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. Neuroendocrinology 2005;81:339-49.
73. Halem HA, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, *et al.* Novel analogs of ghrelin: Physiological and clinical implications. Eur J Endocrinol. 2004;151(Suppl 1):S71-5.
74. Mitchell V, Bouret S, Beauvillain JC, Schilling A, Perret M, Kordon C, *et al.* Comparative distribution of mRNA encoding the growth hormone secretagogue-receptor (GHS-R) in *Microcebus murinus* (Primate, lemurian) and rat forebrain and pituitary. J Comp Neurol 2001;429:469-89.
75. Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. J Comp Neurol 2006;494:528-48.
76. Camina JP, Carreira MC, El Messari S, Llorens-Cortes C, Smith RG, Casanueva FF. Desensitization and endocytosis mechanisms of ghrelin-activated growth hormone secretagogue receptor 1a. Endocrinology 2004;145:930-40.
77. Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M. Minireview: Ghrelin and the regulation of energy balance—a hypothalamic perspective. Endocrinology 2001;142:4163-9.
78. Horvath TL, Diano S. The floating blueprint of hypothalamic feeding circuits. Nat Rev Neurosci 2004;5:662-7.
79. Elmquist JK, Maratos-Flier E, Saper CB, Flier JS. Unraveling the central nervous system pathways underlying responses to leptin. Nat Neurosci 1998;1:445-50.
80. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell 200123;104:531-43.
81. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. Food intake and the regulation of body weight. Annu Rev Psychol 2000;51:255-77.
82. Woods SC, Seeley RJ, Porte D, Jr., Schwartz MW. Signals that regulate food intake and energy homeostasis. Science. 1998;280:1378-83.
83. Tritos NA, Maratos-Flier E. Two important systems in energy homeostasis: Melanocortins and melanin-concentrating hormone. Neuropeptides 1999;33:339-49.
84. Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, Low MJ. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int J Obes Relat Metab Disord 2001;25(Suppl 5):S63-7.
85. Cowley MA. Hypothalamic melanocortin neurons integrate signals of energy state. Eur J Pharmacol 2003;480:3-11.
86. Chen X, Ge YL, Jiang ZY, Liu CQ, Depoortere I, Peeters TL. Effects of ghrelin on hypothalamic glucose responding neurons in rats. Brain Res 2005;1055:131-6.
87. Olszewski PK, Li D, Grace MK, Billington CJ, Kotz CM, Levine AS. Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. Peptides 2003;24:597-602.
88. Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, *et al.* Ghrelin-induced food intake is mediated via the orexin pathway. Endocrinology 2003;144:1506-12.
89. Olszewski PK, Grace MK, Billington CJ, Levine AS. Hypothalamic paraventricular injections of ghrelin: Effect on feeding and c-Fos immunoreactivity. Peptides 2003;24:919-23.
90. Ruter J, Kobelt P, Tebbe JJ, Avsar Y, Veh R, Wang L, *et al.* Intraperitoneal injection of ghrelin induces Fos expression in the paraventricular nucleus of the hypothalamus in rats. Brain Res 2003;991:26-33.
91. Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, *et al.* Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 2004;304:110-5.
92. Grill HJ, Kaplan JM. The neuroanatomical axis for control of energy balance. Front Neuroendocrinol 2002;23:2-40.
93. Grill HJ, Kaplan JM. Interoceptive and integrative contributions of forebrain and brainstem to energy balance control. Int J Obes Relat Metab Disord 2001;25(Suppl 5):S73-7.
94. Wade GN, Schneider JE. Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev 1992;16:235-72.
95. Ritter S, Dinh TT, Zhang Y. Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. Brain Res 2000;856:37-47.
96. Ritter S. Glucoprivation and the glucoprivic control of food intake. Orlando, Fla: Academic Press; 1986.
97. Sawchenko PE, Swanson LW. Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. Science 1981;214:685-7.
98. Sawchenko PE, Swanson LW. The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res 1982;257:275-325.
99. Swanson LW, Sawchenko PE. Paraventricular nucleus: A site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology 1980;31:410-7.
100. Lawrence CB, Snape AC, Baudoin FM, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology 2002;143:155-62.
101. Faulconbridge LF, Cummings DE, Kaplan JM, Grill HJ. Hyperphagic effects of brainstem ghrelin administration. Diabetes 2003;52:2260-5.
102. Faulconbridge LF, Grill HJ, Kaplan JM. Distinct forebrain and caudal brainstem contributions to the neuropeptide Y mediation of ghrelin hyperphagia. Diabetes 2005;54:1985-93.
103. Williams DL, Grill HJ, Cummings DE, Kaplan JM. Vagotomy dissociates short- and long-term controls of circulating ghrelin. Endocrinology 2003;144:5184-7.
104. le Roux CW, Neary NM, Halsey TJ, Small CJ, Martinez-Isla AM, Ghatei MA, *et al.* Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. J Clin Endocrinol Metab 2005;90:4521-4.
105. Berridge KC. Food reward: Brain substrates of wanting and liking. Neurosci Biobehav Rev 1996;20:1-25.
106. Wise RA. Brain reward circuitry: Insights from unsensed incentives. Neuron 2002;36:229-40.
107. Naleid AM, Grace MK, Cummings DE, Levine AS. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 2005;26:2274-9.
108. Wellman PJ, Davis KW, Nation JR. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. RegulPept 2005;125:151-4.
109. Keen-Rhinehart E, Bartness TJ. Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 2005;288:R716-22.
110. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, *et al.* Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86:5992.
111. Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. Am J Physiol Endocrinol Metab 2004;287:E297-304.

112. Schmid DA, Held K, Ising M, Uhr M, Weikel JC, Steiger A. Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. Neuropsychopharmacology. 2005;30:1187-92.
113. Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth J, *et al.* Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J Clin Invest 2006;116:3229-39.
114. Abizaid A, Mineur YS, Roth RH, Elsworth JD, Sleeman MW, Picciotto MR, *et al.* Reduced locomotor responses to cocaine in ghrelin deficient mice. Neuroscience 2011;192:500-6.
115. Andrews ZB, Erion D, Beiler R, Liu ZW, Abizaid A, Zigman J, *et al.* Ghrelin promotes and protects nigrostriatal dopamine function via a UCP2-dependent mitochondrial mechanism. J Neurosci 2009;29:14057-65.
116. Pando MP, Sassone-Corsi P. Signaling to the mammalian circadian clocks: In pursuit of the primary mammalian circadian photoreceptor. Sci STKE 2001;2001:RE16.
117. Sugino T, Hasegawa Y, Kikkawa Y, Yamaura J, Yamagishi M, Kurose Y, *et al.* A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. Biochem Biophys Res Commun 2002;295:255-60.
118. Mozid AM, Tringali G, Forsling ML, Hendricks MS, Ajodha S, Edwards R, *et al.* Ghrelin is released from rat hypothalamic explants and stimulates corticotrophin-releasing hormone and arginine-vasopressin. Horm Metab Res 2003;35:455-9.
119. Sato T, Fukue Y, Teranishi H, Yoshida Y, Kojima M. Molecular forms of hypothalamic ghrelin and its regulation by fasting and 2-deoxy-d-glucose administration. Endocrinology 2005;146:2510-6.
120. Lu S, Guan JL, Wang QP, Uehara K, Yamada S, Goto N, *et al.* Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. Neurosci Lett 2002;321:157-60.
121. Andrews ZB, Liu ZW, Wallingford N, Erion DM, Borok E, Friedman JM, *et al.* UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. Nature 454(7206):846-51.
122. Kalra SP, Ueno N, Kalra PS. Stimulation of appetite by ghrelin is regulated by leptin restraint: Peripheral and central sites of action. J Nutr 2005;135:1331-5.
123. Bagnasco M, Dube MG, Katz A, Kalra PS, Kalra SP. Leptin expression in hypothalamic PVN reverses dietary obesity and hyperinsulinemia but stimulates ghrelin. Obes Res 2003;11:1463-70.
124. Guan JL, Wang QP, Kageyama H, Takenoya F, Kita T, Matsuoka T, *et al.* Synaptic interactions between ghrelin- and neuropeptide Y-containing neurons in the rat arcuate nucleus. Peptides 2003;24:1921-8.

Cite this article as: Abizaid A, Horvath TL. Ghrelin and the central regulation of feeding and energy balance. Indian J Endocr Metab 2012;16:617-26.

Source of Support: Nil, **Conflict of Interest:** None declared