Review

Current Understanding of the Hypothalamic Ghrelin Pathways Inducing Appetite and Adiposity

Omar Al Massadi,[1,2,*] Miguel López,[1,2] Matthias Tschöp,[3,4] Carlos Diéguez,[1,2] and Ruben Nogueiras[1,2,*]

Ghrelin is a multifaceted regulator of metabolism. Ghrelin regulates energy balance in the short term via induction of appetite and in the long term via increased body weight and adiposity. Recently, several central pathways modulating the metabolic actions of ghrelin were unmasked, and it was shown to act through different hypothalamic nuclei to induce feeding. Ghrelin also modulates glucose homeostasis, but the central mechanisms responsible for this action have not been studied in detail. Although ghrelin also acts through extrahypothalamic areas to promote feeding, this review specifically dissects hypothalamic control of ghrelin's orexigenic and adipogenic actions and presents current understanding of the intracellular ghrelin orexigenic pathways, including their dependence on other relevant systems implicated in energy balance.

Ghrelin: Precursor, Products, and Metabolic Actions

The hypothalamus, a central structure composed of anatomically distinct nuclei interconnected via axonal projections, integrates central and peripheral information to regulate energy balance [1]. Among these nuclei, the arcuate (ARC), ventromedial (VMH), dorsomedial (DMH), paraventricular (PVH), and the lateral hypothalamic area (LHA) regions play major roles in the modulation of energy metabolism.

Ghrelin was discovered as the endogenous ligand of the growth hormone (GH) secretagogue receptor 1a (thereafter called ghrelin receptor) [2]. Ghrelin is primarily produced in the stomach, and first described as a potent inducer of GH secretion [2–4]. However, soon after its discovery, its ability to induce food intake and adiposity was reported [5,6]. The ghrelin gene generates a peptide of 117 amino acids called preproghrelin, which encodes different subproducts, the most abundant being the acylated ghrelin (AG) and des-acyl ghrelin (DAG) forms of the peptide, both composed of 28 amino acids [2]. The AG form represents 10% of the total amount of ghrelin and is acylated with an octanoic acid in the Ser3, a process that is mediated by the ghrelin O-acyl transferase [7,8]. Ghrelin modulates feeding behavior, adiposity, and glucose homeostasis through the ghrelin receptor, which is abundantly expressed in the hypothalamus and in extrahypothalamic areas. This review focuses on the actions of ghrelin in the hypothalamus and the resultant effects on appetite, adiposity, and glucose metabolism. We will discuss the relevance of hypothalamic ghrelin receptor, the physiological relevance of the ghrelin system at the central level, and the factors constituting the different hypothalamic ghrelin signaling pathways that control feeding, such

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The lack of ghrelin in adulthood has no effect on feeding or body weight. Ghrelin- or ghrelin receptor-deficient mice fed a high-fat diet after weaning and neuronal deletion of ghrelin receptor are diet-induced resistant. Ghrelin inhibition before weaning caused increased adiposity and feeding.

Energy sensors controlling neuronal function and plasticity are located in the hypothalamus and ghrelin acts through these energy sensors to modulate feeding.

The orexigenic but not the adipogenic action of ghrelin is impaired in obese animals.

Mutations in the ghrelin receptor that prevented its binding to beta-arrestin did not influence ghrelin orexigenic action but increased its effects on adiposity and insulin resistance.

Ghrelin's actions on energy and glucose homeostasis are of clinical relevance: ghrelin agonists show beneficial effects in patients with cancer cachexia; and an agonist of des-acyl ghrelin improves insulin sensitivity in humans.

[1]Department of Physiology, School of Medicine-CiMUS, Instituto de Investigación Sanitaria (IDIS), University of Santiago de Compostela, Av de Barcelona s/n Santiago de Compostela (A Coruña), 15782, Spain

as neuropeptides in the ARC, energy and nutrient sensors, fatty acid metabolism, neuro-peptides in the LHA, and its interaction with the dopamine, opioid, cannabinoid, and serotonin systems. Finally, the main feeding-independent actions of central ghrelin system on adiposity and glucose metabolism will be discussed.

Ghrelin Receptor Forms Dimers and Heterodimers with Other G-Protein-Coupled Receptors to Regulate Feeding

The ghrelin receptor gene generates two isoforms, the functional ghrelin receptor (ghrelin receptor subtype 1a) and another truncated isoform termed ghrelin receptor subtype 1b. The ghrelin receptor is a G-protein-coupled receptor (GPCR) that can form heterodimers with other key components of body weight regulation, for example, with melanocortin receptor 3 (MC3 receptor). Both receptors are co-localized in ARC neurons [9] and the interaction stimulates MC3 receptor signaling while simultaneously inhibiting ghrelin receptor signaling [9]. Ghrelin receptor also interacts with GPR83, a rhodopsin-like or family A orphan GPCR [10]. GPR83 co-localizes with ghrelin receptor in the ARC, and its hetero-dimerization represses the activity of ghrelin receptor [10]. Thus, ghrelin-induced food intake and adiposity are potentiated in GPR83-null animals [10].

Ghrelin receptor also heterodimerizes with dopamine receptor 2 (D_2 receptor), another GPCR. These two receptors are co-expressed in subsets of neurons in the hypothalamus as well as in the striatum and in the hippocampus [11]. In opposition to wild-type (WT) mice, the treatment of ghrelin receptor-null mice with a D_2 receptor agonist does not induce anorexia, and a ghrelin antagonist inhibits D_2 receptor agonist signaling *in vivo* and *in vitro*, indicating that these two receptors interact by allosteric mechanisms [11]. Interestingly, a similar interaction was also proposed between ghrelin receptor and dopamine receptor 1 (D_1 receptor) in extrahypothalamic areas [12].

Finally, ghrelin receptor can form heterodimers with serotonin receptor 2c (5-HT$_{2c}$ receptor) [13,14]. These two receptors are co-localized in primary hypothalamic and hippocampal rat neurons [14] and 5-HT$_{2c}$ receptor attenuates ghrelin signaling by reducing Ca^{2+} release (see the text in the following section describing ghrelin and the serotonin system) [14].

Lessons from Animal Models: The Physiological Relevance of Central Ghrelin on Energy Balance and Glucose Metabolism

The endogenous role of the ghrelin system has been studied comprehensively (reviewed in [15,16]). The adult-onset ablation of ghrelin-producing cells has no effect on feeding or body weight [17]. However, ghrelin- or ghrelin receptor-deficient mice given early exposure to a high-fat diet showed reduced weight and adiposity, features explained by a decrease in fuel efficiency and an increase in fat oxidation [18,19]. This high fat diet-resistance is mediated by the central nervous system (CNS), as neuronal deletion of ghrelin receptor almost completely prevented diet-induced obesity by enhancing thermogenesis in brown adipose tissue and the induction of browning in subcutaneous adipose tissue, thereby stimulating energy expenditure but not affecting energy intake [20].

Ghrelin-deficient mice fed with a high-fat diet also showed improved glucose disposal and insulin sensitivity compared to controls [18] and the double lack of ghrelin and leptin enhanced glucose tolerance and insulin sensitivity [21]. These effects are likely regulated by the CNS, since deletion of ghrelin receptor exclusively in the neurons yielded the same improvement in insulin sensitivity as observed in whole-body-deficient mice [20]. More specifically, neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons are essential, since in mice deficient for ghrelin receptor, the re-expression of physiological levels of ghrelin receptor in NPY/AgRP neurons in adulthood recovered the lowered blood glucose levels observed upon caloric

[2]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Spain
[3]Helmholtz Diabetes Center, Helmholtz Zentrum München and German Center for Diabetes Research (DZD), Neuherberg, Germany
[4]Division of Metabolic Diseases, Technische Universität München, Munich, Germany

*Correspondence:
omar.al-massadi@usc.es
(O. Al Massadi) and
ruben.nogueiras@usc.es
(R. Nogueiras).

restriction without affecting body weight [22]. Thus, it seems that ghrelin signaling in the brain plays a key physiological role in the control of energy expenditure, fatty acid metabolism, and insulin sensitivity.

Inhibiting ghrelin with NOX-B11-2, an antighrelin compound that specifically binds and inactivates acyl ghrelin during the preweaning period (from postnatal Day 4 to Day 18), gives different results than those observed when ghrelin was inhibited in adult animals. Specifically, inhibiting ghrelin before weaning promoted higher body weight, adiposity, and food intake; increased glucose and leptin levels; and altered leptin sensitivity when the mice became adults (Figure 1) [23]. In addition, the inhibition of ghrelin during the neonatal period induced an increase in the subsequent density of ARC NPY/AgRP and α-melanocyte stimulating hormone neuronal fiber projections to the PVH, DMH, and LHA in adulthood (conversely, no changes in these projections were detected when ghrelin was inhibited in adult mice) [23]. This suggests that ghrelin plays an inhibitory role in the development of hypothalamic neural circuits and that its expression during neonatal life is pivotal for lifelong metabolic regulation. In line with this, circulating ghrelin levels were reduced in early postnatal life but not in adulthood in a rodent model of neonatal overnutrition [24]. However, ghrelin treatment in these mice did not rescue the obesogenic phenotype, although restored normal fasting glucose levels, indicating that neonatal overnutrition causes ghrelin resistance [24]. The explanation for this resistance is a defective transport of ghrelin into the hypothalamus.
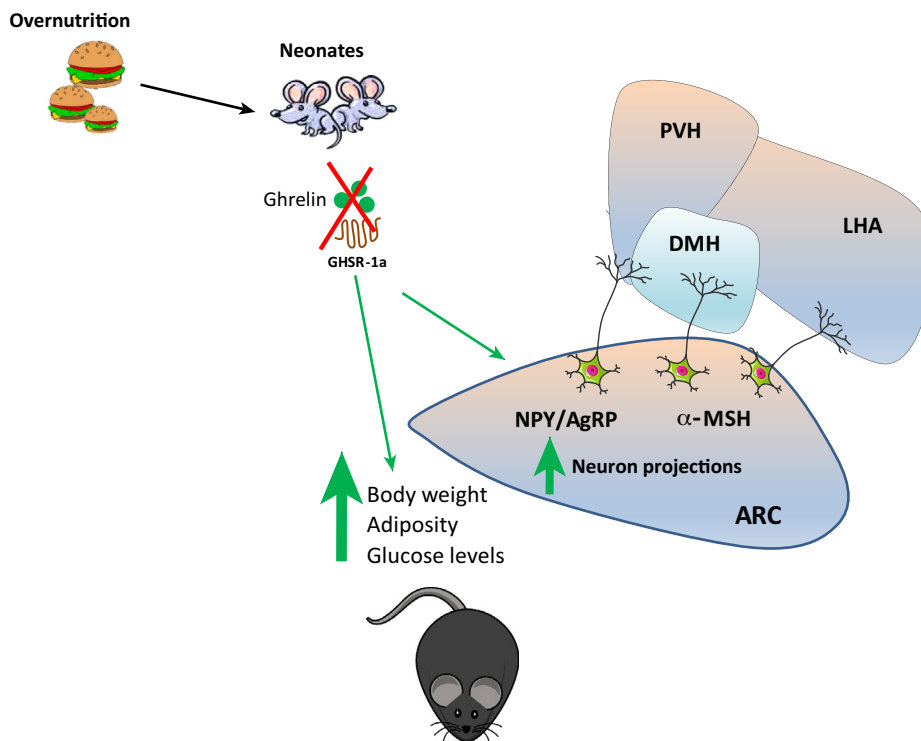
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Figure 1. Influence of Neonatal Overnutrition on Ghrelin's Actions. Ghrelin inhibition during the neonatal period promotes higher body weight, adiposity, food intake, and hyperglycemia. This inhibition increased the density of hypothalamic arcuate (ARC) neuropeptide Y/agouti-related peptide (NPY/AgRP), and α-melanocyte stimulating hormone (α-MSH) neuronal fiber projections to the paraventricular (PVH), dorsomedial (DMH), and lateral hypothalamic area (LHA) in adulthood. GHSR, growth hormone secretagogue receptor.

Ghrelin and NPY/AgRP

Ghrelin receptor is expressed in the ARC [25] predominantly in NPY/AgRP neurons [26] and genetic deletion of both NPY and AgRP completely blunted ghrelin-induced feeding, being partially recovered in ghrelin receptor knockout mice with the selective rescue of ghrelin receptor in NPY/AgRP neurons [22]. Using an *in vivo* imaging approach it was shown that circulating fluorescent-labeled ghrelin (F-ghrelin) reached the hypothalamus by passive diffusion through the fenestrated capillaries of the median eminence, which project to the ventromedial part of the ARC, and finally targeted NPY/AgRP neurons to develop a metabolic response [27]. Similarly, other studies using a F-ghrelin showed that the peripheral injection of F-ghrelin increases the fluorescence signal mainly in the hypothalamic ARC. However, a central injection of F-ghrelin showed a wide distribution of the tracer in the hypothalamus and also in extrahypothalamic areas [28,29]. Within the ARC, the number of ghrelin-labeled neurons was dynamically regulated by nutritional status, because the number of NPY/AgRP ghrelin-labeled neurons was increased after fasting, while no changes were found in pro-opiomelanocortin (POMC) ghrelin-labeled neurons [27].

NPY/AgRP neurons are depolarized and activated by ghrelin, whereas POMC neurons are hyperpolarized, by the increment of gamma-aminobutyric acid (GABA) inhibitory postsynaptic currents. In addition, ghrelin inhibits the secretion of the anorexigenic peptide α-melanocyte stimulating hormone (the cleaved product of POMC) [30] by the upregulation of prolyl carboxypeptidase [31], which represents another mechanism of ghrelin to inhibit melanocortin signaling and enabling even greater orexigenic impulse. Ghrelin failed to stimulate feeding when NPY/AgRP neurons were chemically [6] or genetically [32–35] inhibited. Of note, the NPY/AgRP dependence of ghrelin to induce food intake is restricted to mice fed a chow diet, but these neurons are dispensable when a palatable diet is used [36], suggesting that ARC NPY/AgRP neurons are only essential for the homeostatic, but not hedonic, action of ghrelin, as explained in the following section.

Another important player in the crosstalk between ghrelin and NPY/AgRP neurons is astrocytes. Genetic activation of astrocytes in the mediobasal hypothalamus of mice blunted ghrelin-induced food intake, while their inhibition maximized the orexigenic action of ghrelin [37]. This mechanism implies the direct reduction of NPY/AgRP firing after activation of astrocytes and by the increase of extracellular adenosine levels. For instance, the administration of an adenosine 1 receptor antagonist in the ARC reduces adenosine levels and amplified ghrelin orexigenic action [37]. The ghrelin-stimulated NPY/AgRP expression involves nuclear transcription events. Brain-specific homeobox domain, Forkhead box 1, and phosphorylated cAMP response element-binding protein modulate NPY/AgRP expression and are upregulated after ghrelin administration (reviewed in [38]).

The results found in rodents can be translated to humans, where ghrelin also enhances both GH secretion and food intake [3,4]. Although the knowledge of the mechanisms in the human brain is obviously very limited, one study using functional neuroimaging showed that infusion of ghrelin inhibited the activation of the CNS induced by the intragastric fatty acid (dodecanoate, C12) delivery [39]. The areas controlled by ghrelin were very diverse and included the hypothalamus, the midbrain, the brainstem, and the cerebellum among others [39]. Further studies using imaging techniques will help to clarify other aspects relevant for the neuronal response to ghrelin.

Ghrelin Orexigenic Pathways

Ghrelin depends not only on the most prominent energy/nutrient sensors known to date, such as AMP-activated protein kinase (AMPK), sirtuin1 (SIRT1), or mammalian target of rapamycin (mTOR), to increase food intake and modulate transcription factors controlling the expression
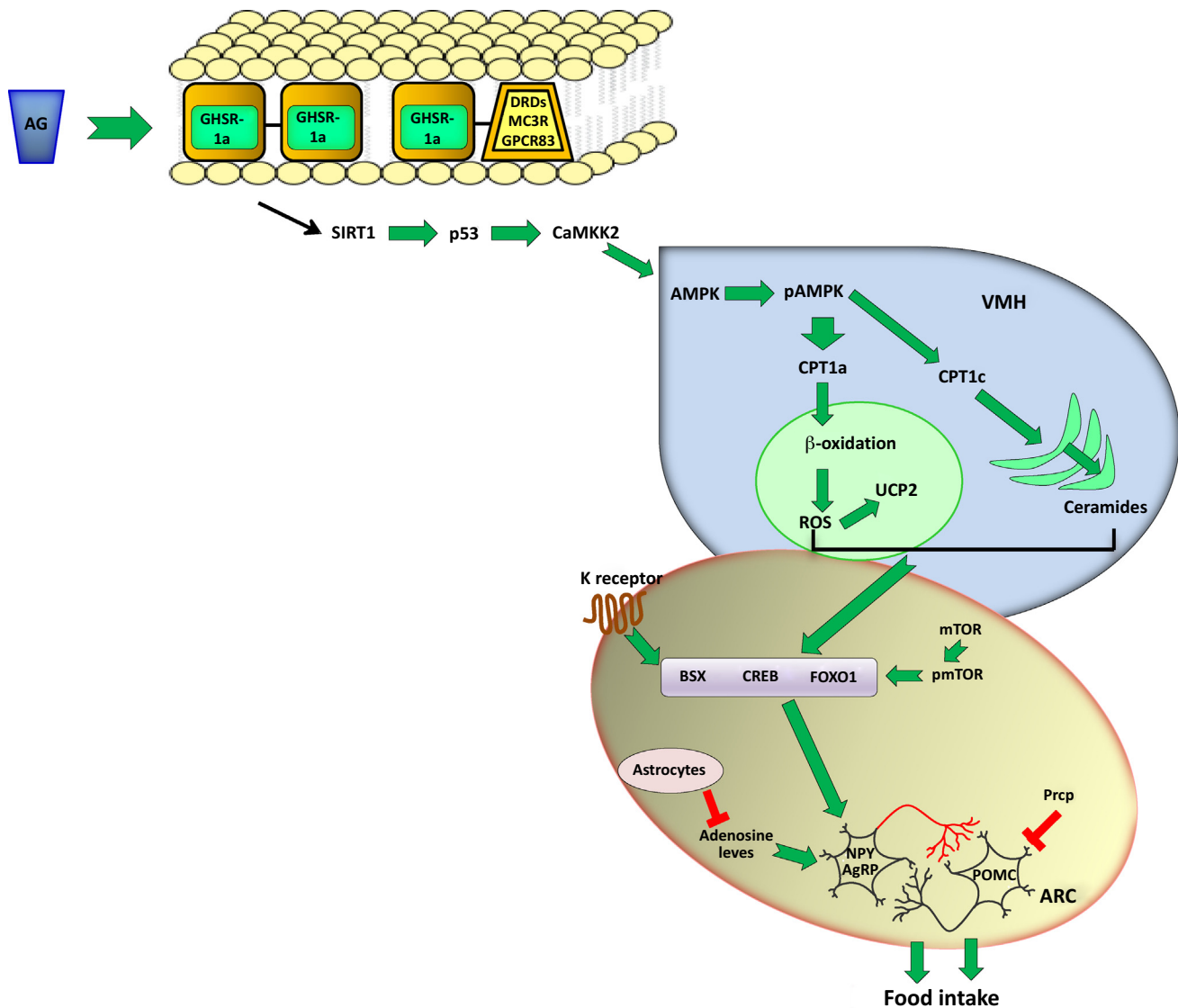
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Figure 2. Ghrelin Signal Transduction in the Hypothalamic Arcuate (ARC) and Ventromedial (VMH) Nucleus. Acylated ghrelin (AG) targets growth hormone secretagogue receptor 1a (ghrelin receptor) and triggers several pathways leading to the stimulation of food intake. These pathways include the interaction with some key nutrient sensors such as AMPK in the VMH and mTOR in the ARC and the opioid system (more specifically the kappa opioid receptor) in the ARC. These pathways will then act through three transcription factors (BSX, CREB, and FOXO1) and finally increase neuropeptide Y/agouti-related peptide (NPY/AgRP) expression to stimulate food intake. AMPK, AMP-activated protein kinase; BSX, Brain-specific homeobox domain; CaMKK2, calcium/calmodulin-dependent protein kinase kinase 2; CPT, carnitine palmitoyltransferase; CREB, cAMP response element-binding protein; FOXO1, forkhead box 1; GPCR, G-protein-coupled receptor; KOR, kappa opioid receptor; MC3R, melanocortin receptor 3; mTOR, mammalian target of rapamycin; pAMPK, phosphorylated AMP-activated protein kinase; p-mTOR, phosphorylated mammalian target of rapamycin; ROS, reactive oxygen species; SIRT1, sirtuin1; UCP2, uncoupling protein 2.

and activity of different neuropeptides (Figure 2), but also interacts with other signals such as dopamine, cannabinoids, opioids, or serotonin to induce appetite.

Ghrelin and SIRT1/p53/AMPK

Once the role of ARC NPY/AgRP neurons was established, the question of their regulation and signaling pathway activated by the ghrelin receptors arose. Since the effect of ghrelin on these two neuropeptides was markedly influenced by feeding/fasting, the involvement of energy/nutrient sensors was hypothesized. SIRT1 is an energy sensor with actions on energy

balance and glucose homeostasis [40]. Ghrelin increases SIRT1 activity, and the pharmacological inhibition of SIRT1, or the deletion of p53, blunted the orexigenic action of ghrelin. The lack of effect of ghrelin in p53-deficient mice was consistent with the inability of ghrelin to phosphorylate hypothalamic AMPK when compared with WT animals [41,42].

AMPK is a ubiquitous energy sensor that maintains energy homeostasis at both the cellular and whole body levels [43]. Central ghrelin administration increases AMPK phosphorylation [44,45] and the orexigenic action of ghrelin is blunted after the pharmacological or genetic inhibition of AMPK [45]. The interaction between ghrelin and AMPK, exerted through a rise in intracellular Ca^{2+} and activation of calcium/calmodulin-dependent protein kinase kinase 2, occurs in the VMH, since local administration of an adenovirus encoding a dominant negative isoform of AMPK into the VMH is able to block ghrelin-induced food intake [45–47]. These changes underlie, at least in part, the rise in the orexigenic NPY/AgRP neuropeptides in the ARC. The interesting feature of this mechanism is the fact that AMPK and its downstream enzymes on one side, and the orexigenic neuropeptides on the other side, are located in different neuronal populations. Therefore, it is likely that NPY/AgRP neurons in the ARC, may be presynaptically [48] modulated by AMPK neurons in the VMH [45].

Interestingly, it was proposed that AMPK can induce food intake through the stimulation of autophagy [49]. For instance, genetic or pharmacological inhibition of autophagy abolishes the effect of AMPK on NPY/AgRP and POMC neuropeptides *in vitro*, and the knockdown of AMPK in mice diminishes autophagy and ultimately affects feeding [49]. In this sense, both exogenous ghrelin and a caloric restriction mimetic cell culture medium stimulate autophagy in rat cortical neurons, an effect that is blocked by ghrelin receptor antagonists [50]. Whether the ghrelin/AMPK pathway stimulates feeding by changes in hypothalamic autophagy remains unknown but deserves further investigation.

AMPK might also mediate other metabolic actions of ghrelin such as the maintenance of hypoglycemia in states of severe calorie restriction [51–53]. Levels of both ghrelin and hypothalamic AMPK are increased after calorie restriction (hypoglycemic state) [43,49,54–57]. Thus, it is tempting to speculate that ghrelin-induced activation of hypothalamic AMPK might prevent hypoglycemia.

The interaction between ghrelin and AMPK is not only relevant for the control of food intake or glucose levels, but also for neuroprotection. One of the metabolic ways to counteract neurodegeneration is induction of calorie restriction, a state in which circulating ghrelin levels are elevated. In a model of Parkinson's disease, caloric restriction protects against the loss of tyrosine hydroxylase labeling (a marker of dopamine neurons), tyrosine hydroxylase neural volume, and dopamine content in the striatum in WT animals but not in ghrelin or ghrelin receptor knockout mice [58]. The mechanism underlying this effect is dependent on AMPK activation in dopamine neurons of the substantia nigra, since the deletion of AMPKβ1 and β2 subunits in dopaminergic neurons abolished the neuroprotective effects of ghrelin [58]. Therefore, it was suggested that ghrelin/AMPK signaling is a response mechanism activated in states of negative energy balance to maintain proper physiological and neurological function as reflected in its mediation of the beneficial effects of calorie restriction.

Ghrelin and Hypothalamic Fatty Acid Metabolism/Mitochondrial Respiration

Uncovering the role of AMPK led us to postulate the involvement of hypothalamic fatty acid metabolism, also known to be involved in the regulation of feeding, in ghrelin action [43,59]. The *de novo* biosynthesis pathways of fatty acids comprise key enzymes such as acetyl Co-A carboxylase (ACC), fatty acid synthase (FAS), and malonyl-CoA. Notably, ghrelin-induced AMPK phosphorylation decreased FAS and ACC levels, with a concomitant decrease in

malonyl-CoA and an increase in CPT-1A activity [45], which induces mitochondrial lipid oxidation [45]. This stimulated increase in mitochondrial lipid oxidation increases reactive oxygen species and triggers uncoupling protein 2 (UCP2) activation and production [34]. UCP2 is highly expressed in ARC NPY/AgRP neurons and activates GABAergic signaling onto POMC neurons (via NPY/AgRP neurons), ultimately leading to a subsequent increase in feeding [34]. Consistent with the aforementioned finding, ghrelin failed to induce feeding in UCP2-null mice, as it did not induce transcriptional activation of NPY/AgRP or carnitine palmitoyltransferase 1A (CPT1A) [34].

By contrast, carnitine palmitoyltransferase 1C (CPT1C) is a brain-specific CPT1 isoform that, in contrast to mitochondrial CPT1A, localizes in the endoplasmic reticulum of neurons. CPT1C is a sensor of malonyl-CoA levels in hypothalamic neurons and is involved in the control of energy homeostasis [60]. In mice lacking CPT1C, ghrelin failed to induce food intake due to its inability to stimulate NPY/AgRP neurons [61]. This effect was AMPK independent but was mediated by ceramide levels, since the pharmacological inhibition of ceramide synthesis in WT mice blunted the orexigenic effect of ghrelin [61].

Ghrelin and mTOR

mTOR is an intracellular nutrient sensor that plays a key role in cellular energy homeostasis [62]. Pioneering studies have shown that it is involved in the central control of energy balance [63]. mTOR is expressed in NPY/AgRP neurons, and central ghrelin increases the levels of phosphorylated mTOR (active form) and its downstream targets pS6K1 and pS6 in the ARC [64,65]. Furthermore, central inhibition of mTOR signaling with rapamycin decreased ghrelin's orexigenic action and normalized the mRNA expression of NPY/AgRP [64]. Finally, pS6K1-null mice were irresponsive to ghrelin orexigenic action [65].

Physiological Relevance of Ghrelin Interactions with Different Orexigenic Pathways at the Hypothalamic Level

Data gleaned over the last few years have conclusively shown that ghrelin exerts most of its metabolic effects acting in the CNS within different hypothalamic nuclei. These hypothalamic effects of ghrelin are to be added to other well-characterized neuroendocrine effects including the regulation of the hypothalamic–GH–insulin-like growth factor axis, the hypothalamic–pituitary–gonadal axis, and the hypothalamic–pituitary–adrenal axis. Through these effects, ghrelin emerged as a regulator of different biological processes such as growth, reproduction, and stress. The interesting feature of all these processes is that they require energy. This energy demand can be provided/denied by the hypothalamic effects of ghrelin in food intake, lipid mobilization, or glucose homeostasis. This raises the question of how hypothalamic neurons can sense energy availability. Recent evidence indicates that this could be mediated via changes in the expression of different energy (AMPK) and nutrient (SIRT1, mTOR) sensors, which are regulated in hypothalamic neurons in response to energy availability. The fact that these nutrient sensors are involved in the signaling cascade by which ghrelin exerts many of its neuroendocrine effects may offer an explanation of the tight inter-relationship between energy availability and function of the different hypothalamic–pituitary axes.

Ghrelin and the LHA: Interactions with Orexin

The LHA presents a large number of projections to several hypothalamic and extrahypothalamic brain areas. The LHA also receives direct input from neurons of the ventral temporal subregion of the hippocampus (vHP). Although the levels of GHSR-1a in the LHA are low, ghrelin directly injected into the vHP stimulated food intake, an effect that is blocked after N-methyl-d-aspartate-induced lesions in the LHA (Figure 3) [66]. Within the LHA, two main orexigenic neuronal populations coexist, one expressing melanin concentrating hormone and another expressing orexin [67]. The injection of ghrelin into the vHP activated around
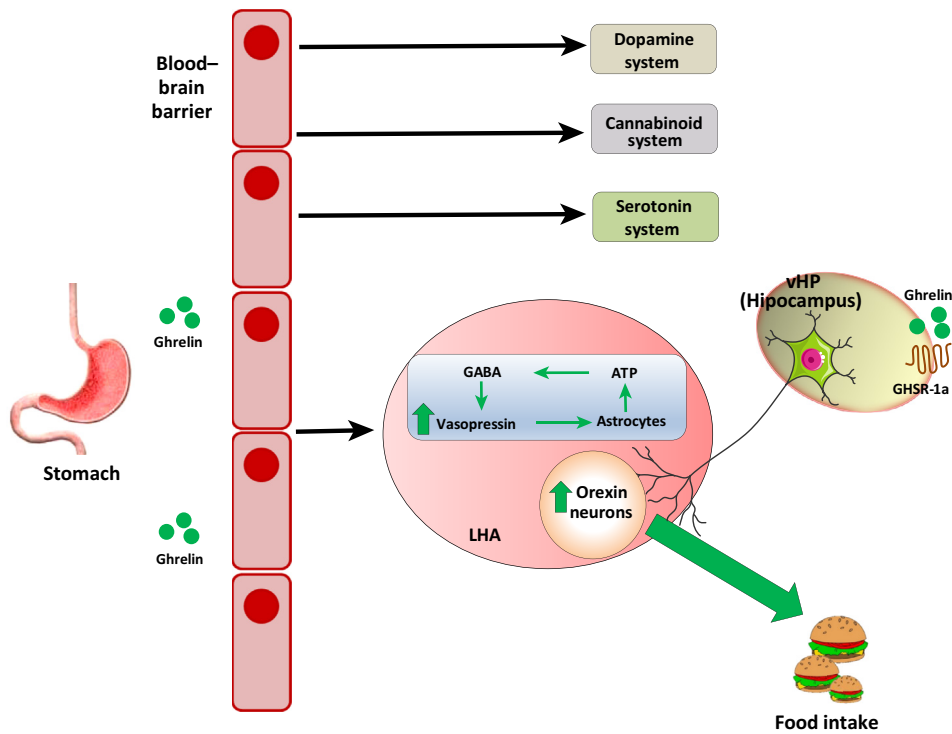
Figure 3. Ghrelin Signal Transduction in Other Hypothalamic Areas, Including the Lateral Hypothalamic Area (LHA). After crossing the blood–brain barrier, ghrelin is also able to stimulate food intake through its actions on alternative brain systems and different hypothalamic nuclei. Those alternative pathways include the dopamin, serotonin, and cannabinoid systems (it is not clear where in the hypothalamus this interaction occurs). Within the LHA, ghrelin stimulates orexin neurons via its actions on the ventral temporal subregion of the hippocampus (vHP). By contrast, ghrelin triggers a retrograde neuronal–glial autoregulatory circuit involving vasopressin that also modulates feeding behavior. GHSR, growth hormone secretagogue receptor.

70% of orexin neurons. Importantly, ghrelin did not induce food intake when rats were pre-treated centrally with an orexin antagonist [66]. Although in this study ghrelin directly injected in the LHA did not stimulate feeding, another report indicated that (i) ghrelin-immunoreactive axonal terminals made direct synaptic contacts with orexin-producing neurons, (ii) centrally administered ghrelin induced Fos expression in orexin-producing neurons, and (iii) pretreatment with anti-orexin IgG attenuated ghrelin-induced feeding [68]. In line with this, the blockade of orexin receptors in the ventral tegmental area also attenuated food intake induced by ghrelin [69]. In addition, ghrelin-labeled neurons receive direct synaptic input from the suprachiasmatic nucleus, the central circadian timekeeper of the brain, and lateral geniculate nucleus, a visual center, and project synaptically to the LHA, suggesting that ghrelin-labeled neurons may mediate circadian and visual cues in the hypothalamus [70]. Therefore, these functional studies demonstrate that the orexin pathway mediates the orexigenic action of ghrelin. Notably, this effect seems to be independent of changes in orexin gene expression, which is not affected after ghrelin administration [71]. Overall, these results suggest that the actions of ghrelin in the LHA are indirect, and that ghrelin acts first in other brain areas, like the hippocampus, and that neuronal projections from these extrahypothalamic sites then stimulate orexin neurons to induce feeding.

Central administration of ghrelin also increased plasma vasopressin levels [72]. The mechanism underlying this effect involves an increase in the spontaneous postsynaptic current inputs to vasopressin neurons, specifically inputs originating in the LHA [73]. This stimulation occurs

because ghrelin triggers the dendritic release of vasopressin, which activates astrocytes to release ATP, stimulating an excitatory GABAergic input back to the vasopressin neurons, thus completing a retrograde neuronal–glial autoregulatory circuit [73].

Ghrelin Interacts with Other Brain Systems to Induce Appetite

The orexigenic effect of ghrelin is dependent on other central networks implicated in energy balance, such as the dopamine system [11,81,82], the cannabinoid system [74], the opioid system [75], or the serotonin system [76–78] (Figure 3), illustrating the complexity of the ghrelin pathways underlying feeding control.

Ghrelin and the Dopamine System

Apart from the hypothalamus, the ghrelin receptor is expressed in dopaminergic neurons, mostly in the ventral tegmental area, where ghrelin receptors colocalize with the dopamine receptors (D receptors) D_1 [79] and D_2 receptors [11]. The interaction between the dopamine system and ghrelin to modulate reward and motivation (extensively reviewed in [80]), and the ability of ghrelin to modulate activity and synaptic input of midbrain dopamine neurons, leads to promotion of appetite [81]. Furthermore, as we noted earlier, ghrelin receptor and dopamine receptors interact to form heterodimers [11,79], and the central dopamine system modulates the acute orexigenic action of ghrelin [82]. Specifically, both activation and blockade of D_1, D_2, or D_3 receptors blunted the increased feeding normally seen after an intracerebroventricular injection of ghrelin, without affecting malaise or locomotor activity [82]. The fact that both pharmacological activation and blockade of dopamine receptors interfere with ghrelin signaling seems to be paradoxical and certainly deserves further investigation. However, another elegant study supported the functional interaction between both systems by showing that while NPY/AgRP-ablated mice fed a standard diet did not respond to ghrelin, diet sensitivity to ghrelin was restored when these mice were fed a high fructose/high sucrose diet [36]. These results suggest that ghrelin-induced feeding in the absence of NPY/AgRP neurons is dependent on food palatability. In other words, when NPY/AgRP neuron activity is impaired, neural circuits sensitive to emotion and stress are engaged via dopamine signaling [36]. These findings highlight the complexity of feeding behavior with regard to the different sites and mechanisms of action, as well as the importance of palatable diets.

Ghrelin and Opioids

The endogenous opioid system is an important regulator of appetite and metabolism [83]. The stimulation of the three opioid receptors, mu, kappa (κ receptor), and delta, increases food intake while their antagonists decrease food intake. κ receptor signaling plays an important role in controlling the orexigenic effect of ghrelin [75]. This effect is specific to the ARC, where the ghrelin and κ receptors are co-expressed in ARC neurons. Prodynorphin (the precursor of dynorphin, the endogenous ligand of κ receptor) expression in the ARC increases after the injection of ghrelin, and pharmacological or genetic inhibition of ARC κ receptor blunts ghrelin-induced food intake [75]. The interaction between κ receptor and ghrelin is independent of AMPK but is dependent on the transcription factors BSX and phosphorylated cAMP response element-binding protein [75].

Ghrelin and Cannabinoids

The cannabinoid system, which stimulates food intake and affects body weight, also modulates the orexigenic effect of ghrelin. Ghrelin increased the concentration of hypothalamic endo-cannabinoids and stimulated feeding in WT mice but not in cannabinoid receptor 1 (CB1)-null mice and WT mice pretreated with the CB1 antagonist rimonabant [74]. Cannabinoids also modulate ghrelin-induced AMPK activity and GH release [84–86], since the ghrelin-induced changes in AMPK activity and lipid metabolism in peripheral tissues, such as white adipose tissue and liver, were abolished in CB1-null mice and WT animals pretreated with rimonabant

[84]. However, the dependence of the cannabinoid system in the GH release induced by ghrelin is not totally depicted, because only the pharmacological but not the genetic inhibition of the cannabinoid system is able to abolish ghrelin-induced GH release *in vivo* [84,86].

Ghrelin and the Serotonin System

Serotonin is a neuropeptide involved in reward-related behaviors [87], but also controls satiety and energy metabolism [88,89]. Extensive evidence demonstrates an interaction between the serotonin and ghrelin signaling in the homeostatic or the hedonic control of feeding [76–78]. For instance, ghrelin reduces the serotonin release in rat hypothalamic synaptosomes [78]. Furthermore, the pharmacological administration of serotonin or the use of $5\text{-}HT_{2a}$ or $5\text{-}HT_{2c}$ receptor agonists blunts the fasting increase in plasma acyl ghrelin [76] and ghrelin-induced feeding and elevation in respiratory quotient [14,77].

In light of the interaction of ghrelin with a wide number of different systems implicated in energy balance regulation, it seems that ghrelin uses redundant hypothalamic mechanisms known to respond to many different inputs including hormones, peptides, nutrients, etc. This redundancy might offer an evolutionary advantage inherited to avoid death in states of famine, involving multiple metabolic systems that are interconnected to preserve a proper energy balance.

Central Ghrelin Controls Adiposity and Glucose Metabolism Independent of Feeding

Another pivotal action of central ghrelin signaling is the induction of adiposity, independent of food intake or GH secretion [5,6,42,45,90,91]. Indeed, central ghrelin affects nutrient partitioning, triggering the utilization of carbohydrates, reducing fat oxidation, and stimulating lipid synthesis [5]. Specifically, chronic central infusion of ghrelin increased triglyceride and glucose uptake and the expression of fat storage promoting enzymes such as lipoprotein lipase, ACC, FAS, and SCD1; and reduced CPT1A that induces β-oxidation in the white adipose tissue of ghrelin-treated rats independent of food intake [91,92]. These effects were not reproduced in mice lacking β1, β2, and β3 adrenoreceptors, a model reflecting absence of sympathetic nervous system signaling [92]. Interestingly, in ghrelin-deficient mice there was a decrease in lipoprotein lipase and SCD1 expression compared with WT animals despite no differences in body weight [92], fat mass, or food intake, indicating a physiological action of ghrelin on nutrient partitioning. Moreover, chronic central infusion of ghrelin also increased total plasma cholesterol due to higher high-density lipoprotein cholesterol [93]. Consistently, mice lacking both ghrelin and ghrelin receptor have lower cholesterol levels [93].

DAG ghrelin also has some metabolic effects, but here the results are controversial. For instance, ghrelin transgenic (Tg) mice had higher circulating levels of DAG without differences in AG (compared with WT mice) [94–96]. These Tg mice showed a lean phenotype with an improvement in body weight, adiposity, and glucose homeostasis [94–96], suggesting that DAG has metabolic actions opposite to AG. Pharmacological experiments have not clarified this point, since DAG exhibited both orexigenic [97] and anorexigenic actions [95,98]. However, at the central level, both AG and DAG have a stimulatory action on plasma insulin levels and increase adiposity independent of the hyperphagic effect (Figure 4) [99].

The fact that ghrelin-induced adiposity is independent of its orexigenic action suggests the existence of different neuronal pathways controlling feeding and adiposity. In line with this, the knockdown of ghrelin receptor specifically in the PVH decreased body weight but not food intake [100]. Further evidence pointing toward different neuronal pathways for food intake and adiposity is the fact that the orexigenic effect of ghrelin is impaired in obese animals but its adipogenic effect persists [101–104]. The lowered orexigenic action of ghrelin in diet-induced
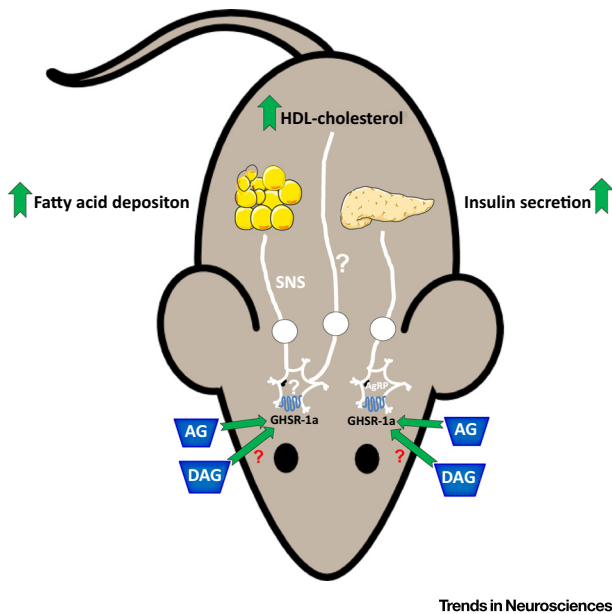
Figure 4. Central Ghrelin and Peripheral Metabolism. Both acylated-ghrelin (AG) and des-acyl ghrelin (DAG) stimulate insulin secretion and adiposity in a feeding-independent manner. Ghrelin receptor in neuropeptide Y/agouti-related peptide neurons is essential for maintaining blood glucose levels, but the mechanism linking the central nervous system with the pancreas remains unknown. By contrast, central ghrelin promotes fatty acid deposition via the sympathetic nervous system and also increases plasma high-density lipoprotein (HDL)–cholesterol levels; however, the neuronal populations targeted by ghrelin for these actions have not been unmasked. GHSR, growth hormone secretagogue receptor; SNS, sympathetic nervous system.

obesity rodents is due to the reduced activation and plasticity of NPY/AgRP neurons [102] and has been reviewed in detail elsewhere [104]. However, the relevance of most of the ghrelin-controlled neuronal pathways in obese models has not been investigated in detail.

Concluding Remarks and Future Directions

Ghrelin may act through different brain areas but the hypothalamus is the main site for most of ghrelin's metabolic actions, as it is a homeostatic center integrating peripheral and central signals. The initial findings describing ghrelin's induction of c-Fos expression in NPY/AgRP neurons and the concomitant rise in NPY/AgRP mRNA expression in the ARC have been largely expanded in recent years.

The ghrelin receptor signaling leading to food intake is mediated by the SIRT1/p53/AMPK pathway, which controls fatty acid metabolism in the VMH and mTOR signaling in the ARC. As a downstream regulator of fatty acid metabolism, ghrelin produces changes in hypothalamic mitochondrial metabolism by increasing UCP2 and subsequent generation of reactive oxygen species in the VMH. Parallel to this action, ghrelin activates CPT1C and the synthesis of ceramides to induce food intake. However, ghrelin also stimulates feeding by other pathways such as the cannabinoid, opioid, serotonin, and dopamine systems.

The characterization of the hypothalamic pathways triggered by ghrelin controlling not only food intake and satiety/hunger perception but also peripheral metabolism is pivotal to the potential development of new compounds antagonizing/blocking the ghrelin receptor in specific hypothalamic sites. Finally, but no less important, we cannot forget that the effects of ghrelin are specifically modulated by nutritional status, and the precise response of each ghrelin-mediated pathway to different nutrients will help to enhance our knowledge of the etiology of obesity-related pathologies (see Outstanding Questions).

Conflicts of interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

Outstanding Questions

The effects of 'alternative' components of the ghrelin system
• What are the precise metabolic actions of des acyl ghrelin when acting through the hypothalamus?
• Hippocampal synaptic plasticity mediated by the D_1 receptor is dependent on ghrelin receptor. Is a similar mechanism taking place in the hypothalamus?

The physiological role of ghrelin within the hypothalamus
• How do all the jigsaw pieces on energy sensing/brain pathways interact to modulate the physiological actions of ghrelin?
• Why is the physiological role of ghrelin so different depending on the developmental stage at which the system is disrupted?
• If ghrelin is critical for the development of hypothalamic neural circuits during neonatal life, does endogenous ghrelin affect multiple biological actions in addition to energy and glucose metabolism?
• Does the interaction between ghrelin and orexin neurons mediate the stimulation of food intake induced by reduced sleep duration?

The effects of diets on ghrelin's actions
• Why does ghrelin maintain its ability to induce adiposity but not food intake in diet-induced obesity?
• How does diet composition (nutrients) engage an alternative ghrelin-induced hedonic pathway that bypasses homeostatic mechanisms – namely NPY/AgRP neurons – to induce appetite?

The interaction of ghrelin with other brain systems
• Does the strong interaction between the ghrelin system and dopamine receptors suggest that its role in Parkinson's diseases is as relevant as for the metabolic syndrome?

The central effects of ghrelin on peripheral metabolism
• Which are the specific neural mechanisms by which the ghrelin system controls lipid, cholesterol, and glucose metabolism at the hypothalamic level?
• Administration of ghrelin receptor agonists in aged animals showed clear functional benefits in terms of lean mass, bone density, and immune function. Are they exerted at the hypothalamic level? If so, through which signaling pathway?

Acknowledgments

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