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Review

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The Homeostatic Force of Ghrelin

Shigehisa Yanagi,[1] Takahiro Sato,[2] Kenji Kangawa,[3] and Masamitsu Nakazato[1,4,*]
[1]Divisions of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki 889-1692, Japan
[2]Molecular Genetics, Institute of Life Science, Kurume University, Kurume 839-0864, Japan
[3]Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka 565-8565, Japan
[4]AMED-CREST, Japan Agency for Medical Research and Development, Chiyoda-ku, Tokyo 100-0004, Japan
*Correspondence: nakazato@med.miyazaki-u.ac.jp
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Ghrelin, a gastric-derived acylated peptide, regulates energy homeostasis by transmitting information about peripheral nutritional status to the brain, and is essential for protecting organisms against famine. Ghrelin operates brain circuits to regulate homeostatic and hedonic feeding. Recent research advances have shed new light on ghrelin's multifaceted roles in cellular homeostasis, which could maintain the internal environment and overcome metaflammation in metabolic organs. Here, we highlight our current understanding of the regulatory mechanisms of the ghrelin system in energy metabolism and cellular homeostasis and its clinical trials. Future studies of ghrelin will further elucidate how the stomach regulates systemic homeostasis.

Introduction

Regulation of whole-body energy homeostasis and body weight relies on an intricate balance between energy intake and expenditure, achieved by neural and hormonal controls deployed in the gut-brain axis. Obesity impairs this balance via diverse and complex mechanisms, including interactions between genetic predisposition and environmental factors. Under obesity, integration of metabolic stress (e.g., hypoxia, production of reactive oxygen species [ROS], disruption of mitochondrial function, and endoplasmic reticulum [ER] stress) and immune response pathways causes a low-grade chronic inflammatory state, referred to as ''metaflammation,'' which disrupts the functional flexibility of multiple metabolic organs (Hotamisligil, 2006, 2017; Reilly and Saltiel, 2017). In order to develop effective therapeutic strategies against metabolic diseases, the mechanisms regulating feeding, energy metabolism, and pathogenesis of metabolic organ dysfunctions must be understood.

Feeding behavior is regulated by homeostatic feeding, which is dependent on energy needs, as well as hedonic feeding, which is associated with reward-related eating based on pleasure. Elucidation of the circuital crosstalk between homeostatic and hedonic feeding will inspire new options for treatment of metabolic diseases. Biologically active peptides operate inter-organ, neural, and hormonal networks and play vital roles in the control of cell-to-cell communications. The discovery of novel peptides and the elucidation of their physiological roles have greatly contributed to our understanding of the homeostatic control system and the pathomechanisms of various diseases, and will eventually lead to the development of novel therapeutic strategies.

Our research group discovered ghrelin, a 28-amino acid peptide from the stomach, as an endogenous ligand of the growth hormone secretagogue receptor type 1a (GHS-R1a), which stimulates growth hormone (GH) secretion (Kojima et al., 1999). At present, ghrelin is the only orexigenic peptide hormone known to be produced in peripheral organs (Nakazato et al., 2001; Tschöp et al., 2000). Ghrelin has unique functions in the regulation of energy homeostasis, including the abilities to communicate current peripheral nutrition status with the hypothalamus and perform energy compensation (Müller et al., 2015). Over the 18 years since ghrelin's discovery, a large body of research has demonstrated that it has multifaceted peripheral and central biological activities. Innovative advances in neuroengineering and functional neuroanatomy have unveiled various neuronal functions of ghrelin beyond the hypothalamus. Furthermore, ghrelin's recently uncovered cellular homeostatic abilities suggest that it safeguards metabolically critical organs from metabolic stress and metaflammation.

Here, we provide a comprehensive overview of the ghrelin system based on recent molecular, biochemical, and physiological findings. We then highlight the regulatory mechanisms of ghrelin signaling in energy metabolism and feeding behavior. Finally, we describe ghrelin's role in cellular and tissue homeostasis.

Discovery, Structure, and Distribution of Ghrelin

The history of ghrelin research extends more than 40 years. In 1976, Bowers et al. demonstrated that some derivatives of methionine-enkephalin had a weak but assured activity of GH release from the anterior pituitary (Bowers et al., 1980). They designated these factors as GHSs. The discovery of GHSs led to the syntheses of novel GHS compounds, including GH-releasing peptide 6 (GHRP-6) (Bowers et al., 1984) and an orally active non-peptide GHS, L-163,191 (MK-0677) (Patchett et al., 1995). In 1996, GHS-R1a, a G-protein-coupled receptor (GPCR), was identified in the pituitary and hypothalamus as the cognate receptor for GHSs (Howard et al., 1996). By that time, extensive investigations aimed at identifying the endogenous ligand of GHS-R1a had been initiated by many groups.

In 1999, Kojima and Kangawa discovered a novel peptide, ghrelin, as an endogenous ligand of GHS-R1a in rat and human stomach (Kojima et al., 1999). They first established a stable Chinese hamster ovary (CHO) cell line expressing rat GHS-R1a (CHO-GHSR62 cells) to monitor changes in the intracellular Ca^{2+} levels after application of rat tissue extracts fractionated by several chromatographic steps. Unexpectedly, strong activity was detected in gastric extracts, but not in the hypothalamus or

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pituitary gland, where GHS-R1a is abundantly expressed. Ghrelin consists of 28 amino acids, which except for the third position could be identified (GSXFLSPEHQKAQQRKESKKPPAKLQPR; X, not determined). cDNA analysis of rat ghrelin indicated that the residue at position 3 should be serine. The molecular mass (M_r) of purified ghrelin was 3,315, 126 Da greater than the predicted value for the 28-amino acid peptide (M_r = 3,189), suggesting that Ser3 in natural ghrelin must be modified by addition of another moiety. The modification that best fit the observed size was addition of *n*-octanoyl fatty acid. Synthesized *O*-*n*-octanoyl-Ser3 peptide (M_r = 3,315) exhibited the same properties as natural ghrelin in biochemical experiments and increased the [Ca^{2+}] level in CHO-GHSR62 cells. Ghrelin stimulates GH secretion both *in vitro* and *in vivo*, and *O*-*n*-octanoylation at Ser3 is essential for binding of ghrelin to GHS-R1a. Ghrelin *O*-acyltransferase (GOAT) specifically acylates ghrelin at Ser3 with octanoic acid, as described below (Gutierrez et al., 2008; Yang et al., 2008).

The human ghrelin gene, *GHRL*, is localized on chromosome 3p25-26 (Kojima and Kangawa, 2005). Preproghrelin, which consists of 117 amino acids, is post-transcriptionally processed into five products, including ghrelin (Labarthe et al., 2014). The ghrelin sequence follows the signal peptide, and the cleavage sites for the signal peptide are common among all mammalian homologs (Kojima and Kangawa, 2010). Prohormone convertase (PC) 1/3 processes proghrelin to ghrelin (Zhu et al., 2006). The amino acid sequences of mammalian ghrelins are well conserved, and their N-terminal 10 amino acids are identical. The acyl-modification site of ghrelin is Ser3 in all investigated vertebrates except for bullfrog, which has Thr3.

Ghrelin mRNA is robustly expressed in gastric tissue, and is also present at low levels in the intestine, pancreas, kidneys, and placenta (Dornonville de la Cour et al., 2001; Gualillo et al., 2001; Kojima et al., 1999). Ghrelin-producing cells, termed X/A-like cells, are present from the neck to the base of oxyntic gland in the gastric fundus (Date et al., 2000). X/A-like cells, which account for ~20% of the endocrine cell population in oxyntic glands, contain round, compact, electron-dense granules (approximately 120 nm in diameter) that are filled with ghrelin.

Desacyl ghrelin accounts for 67% and 90% of total ghrelin immunoreactivity in rat stomach and blood, respectively (Hosoda et al., 2000). The rodent stomach has two ghrelin cell populations: closed-type round cells containing both ghrelin and desacyl ghrelin, and open-type cells containing only desacyl ghrelin (Mizutani et al., 2009) (Figure 1). Ghrelin is deoctanoylated by carboxylesterases and butyrylcholinesterase in humans and rats, respectively (De Vriese et al., 2004). Several lines of evidence have revealed that biological functions of desacyl ghrelin, discussed later in this review, are mediated in a GHS-R1a-independent manner. Given that desacyl ghrelin does not bind to GHS-R1a, desacyl ghrelin appears to have its own cognate receptor. However, the receptor(s) for desacyl ghrelin remains unidentified.

GHS-R1a

GHS-R1a, a 366-amino acid protein (M_r = 31,329), possesses the characteristic structures of a GPCR, including seven transmembrane (TM) domains (Howard et al., 1996). GHS-R1a belongs to the rhodopsin-like family of GPCRs. GHS-R1a interacts with $G\alpha_{q/11}$, which in turn recruits phospholipase C (PLC)-β to the membrane and induces the production of inositol triphosphate (IP_3) (Kohno et al., 2007). IP_3 stimulates Ca^{2+} release from the ER. Ghrelin also increases intracellular Ca^{2+} level by influx from the extracellular space through N-type Ca^{2+} channels (Kohno et al., 2003). The C-terminal tail of GHS-R1a is critical for ligand-GHS-R1a internalization, β-$arrestin_2$ recruitment, and subsequent termination of ligand-GHS-R1a signaling (Evron et al., 2014; Holliday et al., 2007). A truncated form of GHS-R, GHS-R1b, consists of 289 amino acids with five TM domains and does not bind ghrelin, but instead forms a heterodimer with GHS-R1a and attenuates its signaling (Leung et al., 2007).

GHS-R1a mRNA is expressed in the several nuclei of the hypothalamus, hippocampus, substantia nigra, ventral tegmental area (VTA), dorsal and median raphe nuclei, anterior pituitary gland, pancreatic islets, adrenal gland, thyroid, lung, liver, kidney, immune cells, intestine, adipose tissue, and myocardium (Guan et al., 1997; Hattori et al., 2001). The ghrelin-GHS-R1a axis participates in a variety of behaviors, including learning and memory, reward, impulsivity, anxiety, and vulnerability to stress (Anderberg et al., 2016; Chuang et al., 2011; Diano et al., 2006; Jerlhag et al., 2009; Lutter et al., 2008; Meyer et al., 2014). These diverse roles of ghrelin are partly explained by heterodimer formation of GHS-R1a with other GPCRs, including somatostatin receptor subtype 5 (SST5), dopamine receptor subtype 1 (D1R), serotonin 2C receptor, and melanocortin 3 receptor (Jiang et al., 2006; Park et al., 2012; Schellekens et al., 2013). When a D1R agonist binds the GHS-R1a:D1R heterodimer, it initiates non-canonical signal transduction via $G\alpha_q$-PLC-IP_3-Ca^{2+} at the expense of canonical D1R $G\alpha_s$ cyclic AMP (cAMP) signaling, resulting in calmodulin-dependent protein kinase II activation, glutamate receptor exocytosis, synaptic reorganization, and expression of early markers of hippocampal synaptic plasticity (Kern et al., 2015). Conversely, GHS-R1a inactivation inhibits D1R-mediated hippocampal behavior and memory. In a similar vein, GHS-R1a heterodimerizes with D2R in hypothalamic neurons, allosterically modifies canonical D2R dopamine signaling, induces $G\beta\gamma$-subunit-dependent Ca^{2+} mobilization, and is crucial for the anorexigenic effects of D2R agonism (Kern et al., 2012). Importantly, heterodimerization of GHS-R1a with D1R or D2R and modification of dopamine receptor signal transduction occur in the absence of ghrelin, suggesting that unliganded GHS-R1a regulates neuronal function through allosteric protein-protein interactions.

In contrast to most other GPCRs, which have extremely low constitutive activity, GHS-R1a exhibits strong, constitutive ligand-independent activity, resulting in high basal activation of $G\alpha_{q/11}$ (approximately 50% of its maximal capacity) (Damian et al., 2012; Holst et al., 2003). In the presence of $G\alpha_{q/11}$ and absence of ligand, GHS-R1a exhibits two distinct conformations: an inactive, preassembled complex with $G\alpha_{q/11}$ and an active complex (Damian et al., 2015). The occurrence of the active complex is responsible for the high constitutive activity of GHS-R1a. Ghrelin and other GHSs further potentiate GHS-R1a activity by stabilizing the active conformation of GHS-R1a. The inverse agonist (D-Arg^1-D-Phe^5-D-$Trp^{7,9}$-Leu^{11})-substance P stabilizes an additional inactive conformational state, dissociates the GHS-R1a:$G\alpha_{q/11}$ complex, and selectively decreases the constitutive activity of GHS-R1a without changing

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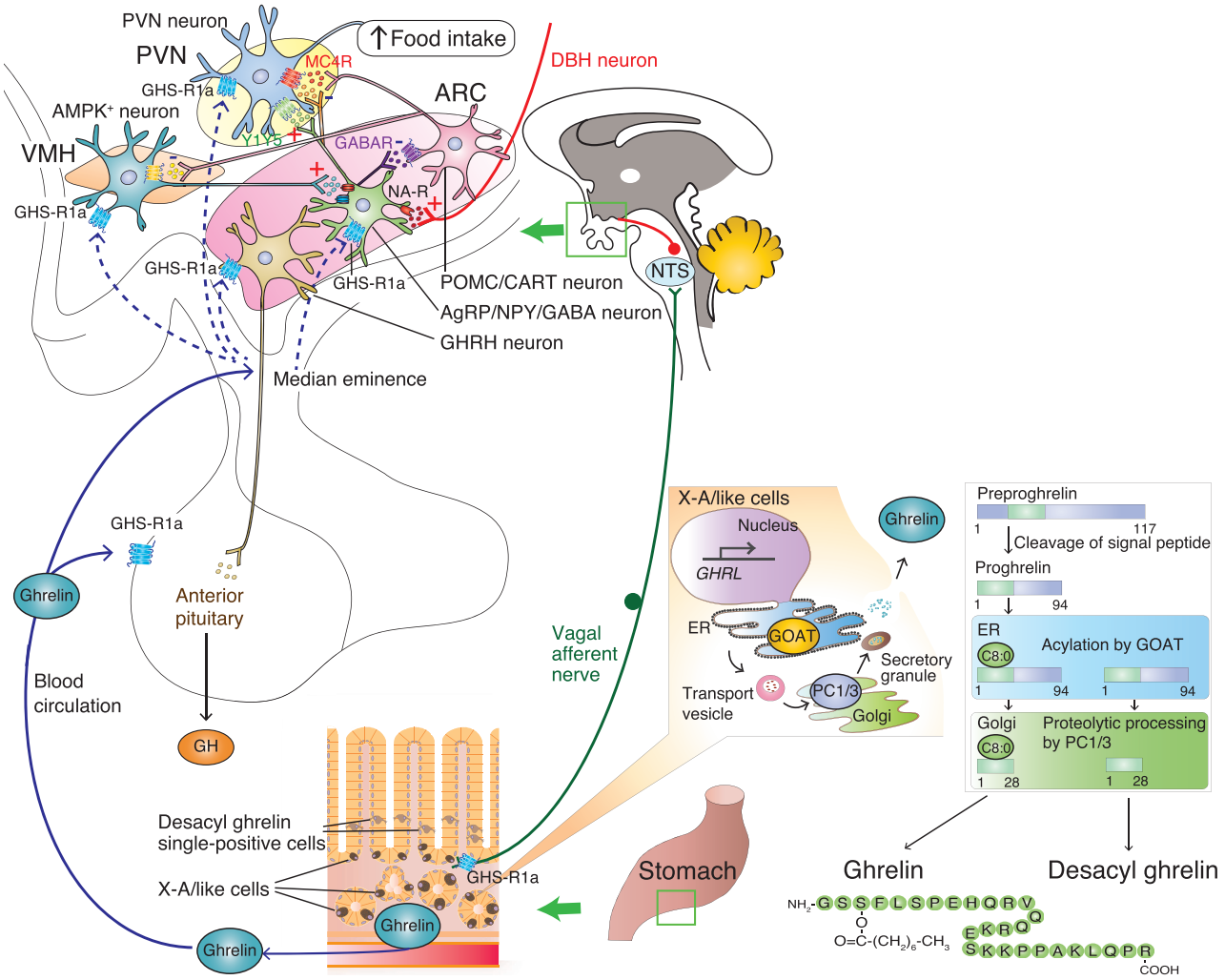

Figure 1. Synthesis of Ghrelin and the Routes Conveying Ghrelin Signals to the Hypothalamus

In X/A-like cells of the stomach, GOAT is localized to the ER, where proghrelin is acylated. Acylated proghrelin is transported to the Golgi body, and then cleaved by PC1/3 to form ghrelin. PC1/3 might also cleave non-acylated proghrelin to produce desacyl ghrelin. Ghrelin is a 28-amino acid peptide in which Ser3 is modified by *n*-octanoic acid. Two routes have been proposed to convey gastric-derived ghrelin signals to the hypothalamus: the vagal afferent nerve and the blood circulation. The vagus nerve is the 10th cranial nerve, contains both efferent and afferent fibers, and conveys information to and from the viscera and brain. Afferent endings within the gastrointestinal mucosa are more optimally positioned to monitor bioactive substances released from enteroendocrine cells. GHS-R1a is synthesized in the nodose ganglion of the vagal afferent nerve, and then transported to the stomach. Ghrelin binds to GHS-R1a and suppresses the electric activity of the vagal afferent nerve. This electrical signal reaches the NTS, where the signal is relayed to DBH neurons. DBH axon terminals make synapses with NPY neurons in the ARC. Circulating ghrelin is transported across the blood-brain barrier and binds to neurons in the vicinity of fenestrated capillaries of the median eminence. GHS-R1a in the hypothalamus is predominantly expressed in ARC, VMH, and PVN. The ARC contains orexigenic neurons expressing NPY and AgRP, anorexigenic neurons expressing POMC and CART, and neurons expressing GABA. Both the NPY/AgRP neurons and the POMC/CART neurons project to the PVN. Elevated activity of POMC neurons increases α-melanocyte-stimulating hormone (α-MSH) release in the PVN, which in turn acts on melanocortin 4 receptor (MC4R)-expressing neurons to suppress food intake. NPY acts on Y1 and Y5 receptors in the PVN to stimulate food intake, whereas AgRP antagonizes MC4R. Ghrelin induces food intake by potently activating NPY/AgRP neurons. Ghrelin also increases food intake by stimulating inhibitory GABAergic input to POMC neurons. Ghrelin stimulates GH release by acting on GHRH neurons or by directly activating somatotrophs in the anterior pituitary. GHRH, growth hormone releasing hormone; NA-R, noradrenergic receptor.

GHS-R1a's ability to respond to ghrelin. The active conformation of GHS-R1a is defined by several structural elements, including extracellular loop II (ECL2) (Holst et al., 2004; Mokrosinski et al., 2012; Valentin-Hansen et al., 2012).

Several lines of evidence suggest that GHS-R1a constitutive activity is closely related to continuous GH secretion. A genetic screen identifies a GHS-R1a mutation (Ala204Glu) located in GHS-R1a ECL2 in patients with idiopathic short stature (Pantel et al., 2006). This mutation decreases the constitutive activity by restricting its conformational dynamics (i.e., the protein is frozen in the inactive conformation) (Damian et al., 2015). Conversely, human somatotroph adenomas overexpress GHS-R1a, and inverse agonist inhibits GH secretion from these tumors (Korbonits et al., 2001; Mear et al., 2014). Central administration of GHS-R1a inverse agonist suppresses food intake and body weight gain while increasing energy expenditure (Abegg et al., 2017; Petersen et al., 2009). Thus, constitutive GHS-R1a activity might provide a high set point for signal transduction activity and

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help organisms to maintain feeding and energy storage at levels sufficient for survival.

In summary, GHS-R1a is expressed in the hypothalamic and extra-hypothalamic regions, as well as in the metabolic organs. The diversity of ghrelin's roles in behavioral control may arise from heterodimerization of GHS-R1a with other GPCRs.

GOAT

GOAT is the only enzyme known to catalyze acyl modification of ghrelin. GOAT belongs to the membrane-bound *O*-acyltransferase (MBOAT) family, which consists of 16 enzymes. GOAT is a polytopic integral membrane protein and contains 11 TM helices and one re-entrant loop (Taylor et al., 2013). GOAT mRNA is predominantly restricted to stomach and pancreas in human tissue, and is highly expressed in ghrelin-producing cells of the stomach (Gutierrez et al., 2008; Sakata et al., 2009). GOAT in the ER binds *n*-octanoyl-CoA to proghrelin before proghrelin is transported to the Golgi (Ohgusu et al., 2009; Taylor et al., 2013; Yang et al., 2008) (Figure 1). The minimal length of the GOAT substrate requires four amino acids of the ghrelin N terminus, particularly Gly1, Ser3, and Phe4. Dietary medium-chain triglyceride (MCT) can be directly utilized for ghrelin acylation (Nishi et al., 2005).

In addition to the stomach and pancreas, it is reported that GOAT mRNA is broadly expressed in human tissues (Lim et al., 2011), as well as in plasma (Goebel-Stengel et al., 2013). These observations raise the possibility that circulating desacyl ghrelin is acylated by GOAT, and then bound by GHS-R1a to exert local effects. However, these findings still require rigorous proof, taking into account methodological issues such as the accuracy of qPCR and the specificity of the GOAT antibody. Further investigations are required to validate the presence of GOAT in many human tissues and the bloodstream.

Long-term fasting inhibits ghrelin acylation, even though total ghrelin levels are elevated under these conditions. Gastric *Mboat4* expression is decreased upon fasting, suggesting that the ghrelin-GOAT system informs the CNS about the availability, rather than the absence, of nutrients (Kirchner et al., 2009). GOAT expression represses ghrelin translation. The core clock gene *Bmal1* also regulates mRNA expression of GOAT and ghrelin, as well as the circadian rhythmicity of ghrelin secretion (Laermans et al., 2015).

GOAT is required for prevention of hypoglycemia under famine conditions via GH-mediated maintenance of blood glucose levels (Zhao et al., 2010a). GOAT-knockout mice have normal body weight and fat mass when they are fed either chow diet or high-fat diet (HFD). On an MCT-enriched diet, GOAT-knockout mice have lower body weight and fat mass than wild-type mice, whereas transgenic mice overexpressing both human ghrelin and GOAT on an MCT-enriched diet exhibit higher body weight and fat mass and reduced energy expenditure in comparison with littermates (Kirchner et al., 2009). Endogenous ghrelin's effect on adiposity is highly dependent upon MCT in the diet.

GOAT also localizes in both plasma membranes and trafficking vesicles of tibial marrow adipocytes (Hopkins et al., 2017). In the plasma membrane, GOAT utilizes octanoic acid within marrow adipocytes to acylate desacyl ghrelin. GOAT-mediated local acylation of exogenous desacyl ghrelin permits activation of GHS-R1a and subsequent adipogenesis in the bone marrow. These observations regarding desacyl ghrelin and GOAT in the plasma membrane suggest the existence of a novel endocrine mechanism for target-cell-mediated transacylation.

GOAT has been proposed as a promising target for therapeutic intervention aimed at modulating obesity and glucose metabolism. GO-CoA-Tat is a synthetic peptide-based bisubstrate analog in which ghrelin peptide and octanoyl-CoA are linked by a non-cleavable bridge (Barnett et al., 2010); consequently, it potently antagonizes GOAT and inhibits production of acyl ghrelin, but not desacyl ghrelin. GO-CoA-Tat administration decreases adiposity and improves glucose tolerance in mice fed an MCT-enriched diet. Pretreatment of human islet cells with GO-CoA-Tat increases insulin response to glucose challenge. These findings suggest that GO-CoA-Tat could facilitate development of drugs targeting GOAT. Collectively, GOAT seems to act to sense and communicate to the brain about peripheral nutrient availability for ensuring efficient metabolism and energy storage. GOAT is essential for prevention of hypoglycemia under famine.

Ghrelin's Effect on Feeding Regulation in the Hypothalamus

GHRPs and GHSs stimulate food intake by activating hypothalamic neurons involved in regulation of homeostatic feeding (Bailey et al., 1998; Vaccarino et al., 1985). The hypothalamus, which comprises a dozen nuclei, receives and emits a variety of signals related to both hunger and satiety. Visceral cues from the stomach and intestines, as well as reward and motivational triggers from various brain regions, converge in the hypothalamus. Some key molecules regulating feeding behavior are produced in the arcuate nucleus (ARC); these include orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP), as well as anorectic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (Figure 1). GHS-Rs are expressed in 94% of NPY/AgRP neurons and 8% of POMC neurons (Willesen et al., 1999). NPY/AgRP-neuron-selective re-expression of GHS-R1a in a tamoxifen-inducible AgRP-CreER^{T2} transgenic mouse model restores ghrelin's orexigenic activity (Wang et al., 2013). NPY/AgRP-neuron-specific GHS-R1a-knockout mice are resistant to diet-induced obesity (Wu et al., 2017). Furthermore, a designer receptor exclusively activated by a designer drug-based chemogenetic approach in a *Ghsr-IRES-Cre* knockin mouse reveals that (1) activation of GHS-R1a-expressing neurons in the mediobasal hypothalamus (MBH) nuclei, including ARC, ventromedial nucleus of the hypothalamus (VMH), and paraventricular nucleus (PVN), is required for normal feeding by both peripheral ghrelin administration and fasting and (2) activation of MBH GHS-R1a-expressing neurons is sufficient to induce feeding (Mani et al., 2017). Ghrelin also amplifies gamma-aminobutyric acid (GABA)-inhibitory postsynaptic currents in the axonal terminal of NPY/AgRP neurons, thereby suppressing POMC neurons (Tong et al., 2008).

The lateral hypothalamic area (LHA), a region implicated in the control of energy homeostasis and motivated behaviors, produces two orexigenic neuropeptides, orexin and melanin-concentrating hormone (Sakurai et al., 1998; Shimada et al., 1998). The LHA has direct connections with the ARC, PVN, and VTA, and receives direct inputs from neurons of the ventral temporal subregion of the hippocampus (vHP). Direct injection

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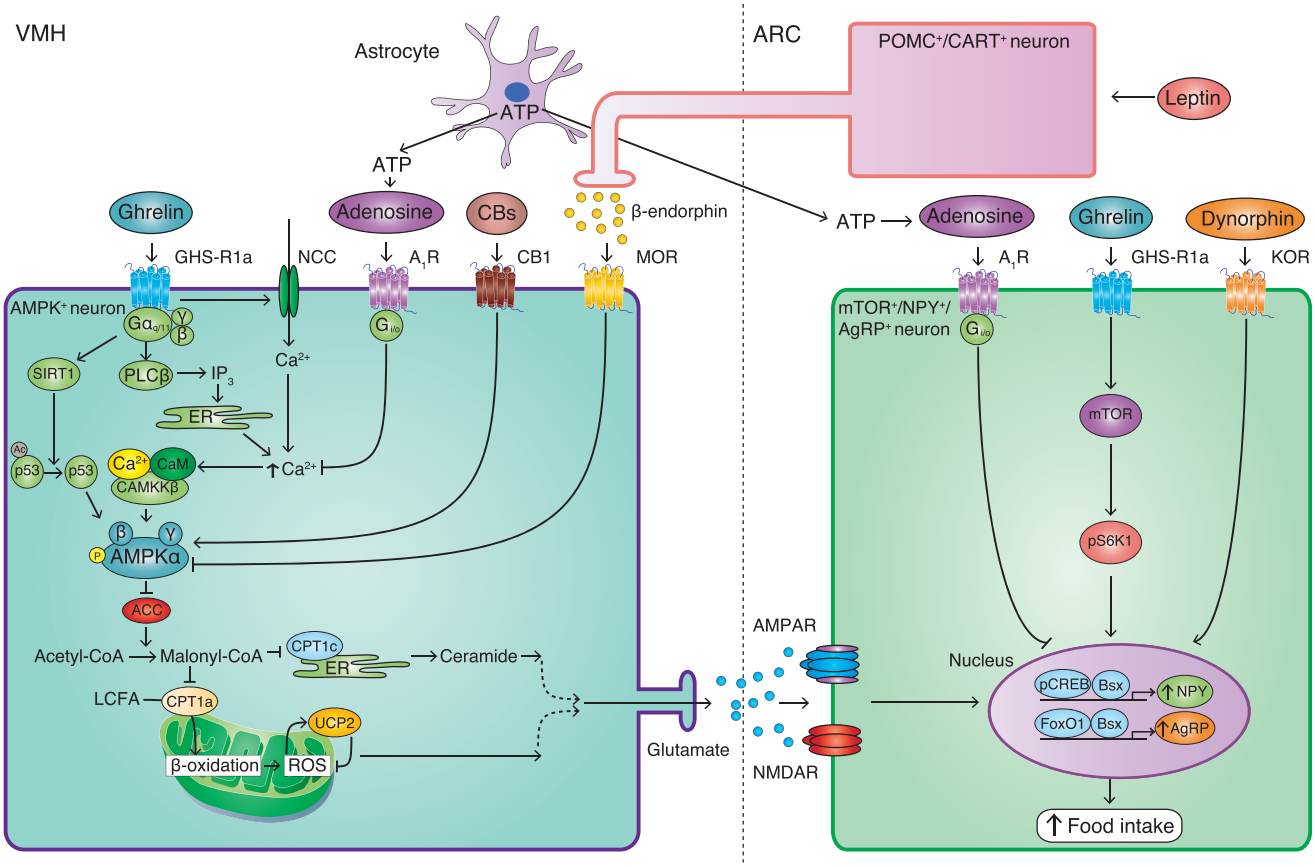

Figure 2. Ghrelin-Mediated Feeding Signaling in the Hypothalamic ARC and VMH

The ghrelin-GHS-R1a axis stimulates AMPK activity in the VMH by activating the SIRT1-p53 pathway and CaMKKβ. Cannabinoids are necessary for the stimulatory effects of ghrelin on AMPK activity. AMPK activation and subsequent ACC inhibition lead to a reduction in malonyl-CoA and release of CPT1a and CPT1c. CPT1a activation promotes long-chain fatty acid entry into mitochondria and increases β-oxidation. Fatty acid oxidation stimulates generation of ROS, as well as UCP2 expression to neutralize ROS. In addition to the AMPK-CPT1a-UCP2 axis, CPT1c-mediated ceramide metabolism is an essential mediator of the effect of ghrelin on feeding. These ghrelin-induced metabolic changes might potentiate glutamate release from the presynaptic terminals of NPY/AgRP neurons that express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and *N*-methyl-D-aspartate receptor (NMDAR). In the ARC, ghrelin binds to GHS-R1a, upregulates mTORC1-pS6K1 signaling, and increases expression of AgRP and NPY. The κ-opioid receptor pathway also mediates the actions of ghrelin in the ARC (Romero-Picó et al., 2013b). As a result, ghrelin increases the expression of transcription factors such as pCREB, FoxO1, and BSX in the ARC, leading to the activation of NPY/AgRP neurons, resulting in food intake. After feeding, persistent orexigenic activity is switched off by leptin-mediated opioid release from POMC/CART neurons. μ-opioid receptor signaling is involved in leptin-mediated inactivation of presynaptic AMPK. Astrocytes suppress ghrelin-induced food intake through adenosine A_1 receptors (A_1R) localized on pre- and post-synaptic neurons. CBs, cannabinoids; KOR, κ-opioid receptor; MOR, μ-opioid receptor; mTOR, mammalian target of rapamycin; NCC, N-type calcium channel.

of ghrelin into the vHP stimulates feeding along with activation of LHA orexin neurons, and this effect is abolished by central pretreatment with an orexin antagonist (Hsu et al., 2015). In support of this idea, both intra-VTA ghrelin and orexin potentiate chow intake, and intra-VTA blockade of orexin receptors attenuates ghrelin-induced intake of rewarding food (Cone et al., 2014). Taken together, ghrelin-GHS-R1a signaling induces feeding directly through the activation of NPY/AgRP neurons and indirectly through the vHP-LHA-VTA pathway.

Ghrelin-GHS-R1a Signaling for Activation of NPY/AgRP Neurons

Several lines of research have elucidated the intracellular and intercellular events activated by ghrelin in NPY/AgRP neurons. Ghrelin changes mitochondrial respiration and increases the expression or activity of the transcription factors cAMP-responsive element-binding protein (CREB), Forkhead box protein O1 (FoxO1), and brain-specific homeobox protein homolog (BSX) (Lage et al., 2010; Nogueiras et al., 2008). These changes in transcription factor activity are essential for the functions of NPY/AgRP neurons in the control of energy balance (Sakkou et al., 2007). The ghrelin-GHS-R1a axis increases intracellular Ca^{2+} levels and activates CaM-dependent protein kinase kinase β (CaMKKβ), leading to activation of AMP-activated protein kinase (AMPK) (Anderson et al., 2008; Hawley et al., 2005) (Figure 2). Ghrelin also triggers the sirtuin-1 (SIRT1)/p53 pathway, which is essential for upregulation of hypothalamic AMPK (Velásquez et al., 2011). The endogenous cannabinoid system is equally essential for the stimulatory effects of ghrelin on AMPK activity and food intake (Kola et al., 2008). Ghrelin stimulates the biosynthesis of 2-arachidonoyl glycerol (an endogenous cannabinoid) in parvocellular neurons of the PVN to inhibit the release of excitatory neurotransmitter glutamate from presynaptic axons innervating the PVN neurons, thus promoting food intake.

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Ghrelin-induced AMPK activation suppresses acetyl-CoA carboxylase (ACC) activity, decreases malonyl-CoA level, and activates carnitine palmitoyl transferase 1a (CPT1a) (Andrews et al., 2008). CPT1a promotes transport of long-chain fatty acids into mitochondria, where they undergo β-oxidation (Lam et al., 2005). Uncoupling protein 2 (UCP2) is an inner mitochondrial anion carrier protein that uncouples the oxidative phosphorylation from ATP production, with the resultant energy dissipating as heat. Fatty acid oxidation increases the generation of ROS, which in turn induces UCP2 expression in order to scavenge ROS. Like the AMPK-CPT1a-UCP2 pathway, CPT1c, a brain-specific isoform of CPT1 localized in the ER of neurons, is an essential mediator of ghrelin action (Ramírez et al., 2013). Ghrelin induces synthesis of C18:0 ceramide via CPT1c in the hypothalamus, and central administration of ceramide induces food intake and NPY and AgRP expression in *Cpt1c*-knockout mice. CPT1c is allosterically inhibited by malonyl-CoA (Wolfgang and Lane, 2011), suggesting that GHS-R1a-AMPK-induced malonyl-CoA reduction activates both CPT1a and CPT1c to activate NPY/AgRP neurons.

Hypothalamic mammalian target of rapamycin complex 1 (mTORC1) is an alternative metabolic pathway involved in ghrelin-induced feeding. Central ghrelin administration upregulates mTORC1/p70-S6 kinase 1 (S6K1) signaling in NPY/AgRP neurons, and inhibition of mTORC1 abolishes ghrelin-induced upregulation of NPY and AgRP (Martins et al., 2012; Stevanovic et al., 2013). Importantly, mTORC1/NPY/AgRP-expressing neurons are located in the ARC, whereas AMPK-expressing neurons, which send synaptic projections to ARC neurons, are located in the VMH (Figure 2). Local administration of an adenovirus encoding a dominant-negative isoform of AMPK into the VMH blocks ghrelin-induced food intake (López et al., 2008). Ghrelin decreases malonyl-CoA levels and increases CPT1 activity in the VMH, but not in the ARC (Gao et al., 2013). Ghrelin triggers potentiation of glutamate release from the presynaptic terminal onto NPY/AgRP neurons (Liu et al., 2012). Ghrelin administration or food deprivation results in persistent upregulation of excitatory synaptic input to AgRP neurons, which is mediated by a presynaptic positive-feedback loop dependent on AMPK (Yang et al., 2011).

Astrocytes are also key players in the regulation of ghrelin-induced activation of NPY/AgRP neurons. Cell-type-specific astrocyte activation within the MBH decreases both basal and ghrelin-induced food intake and promotes leptin-induced inhibition of food intake (Yang et al., 2015). Genetic inactivation of astrocytes increases and prolongs ghrelin-induced food intake.

Overall, ghrelin promotes homeostatic feeding by operating the ARC-VMH-PVN hypothalamic circuit. Future research is required to clarify the mechanisms that coordinate and orchestrate ghrelin's orexigenic actions in the VMH and ARC.

Routes Conveying Peripheral Ghrelin's Signals to the Hypothalamus

Two different routes have been proposed to convey the gastric-derived ghrelin signals to the brain: the vagal afferent nerve and the blood circulation. Afferent information from the alimentary tract is conveyed to the nucleus tractus solitarius (NTS) in the medulla oblongata. GHS-R1a is synthesized in the nodose ganglion of the vagal afferent nerve, and then transported to the stomach. Ghrelin binds to the receptor and suppresses the electric activity of the vagal afferent nerve (Asakawa et al., 2001; Date et al., 2002). This electrical signal reaches the NTS where the signal is relayed to the dopamine β-hydroxylase (DBH, a noradrenaline synthetic enzyme)-containing neurons (Date et al., 2006). DBH-positive neurons reside in the commissural NTS, directly project to the hypothalamus, and make synapses with NPY/AgRP neurons in the ARC. Peripheral administration of ghrelin increases noradrenaline release in the ARC, which activates NPY/AgRP neurons.

Controversy persists regarding the indispensability of the vagal afferent system and central noradrenergic system for conveying the peripheral ghrelin signal to the hypothalamus. Blockade of the gastric vagal afferent pathway by application of capsaicin, a specific afferent neurotoxin, or total subdiaphragmatic vagotomy abolishes peripheral ghrelin-induced feeding and activation of NPY-producing neurons (Asakawa et al., 2001; Date et al., 2002). Conversely, a study using subdiaphragmatic vagal deafferentiation reported that the vagal afferent is not responsible for feeding induced by intraperitoneal ghrelin administration (Arnold et al., 2006). In regard to the role of the central noradrenergic system, bilateral midbrain transection rostral to the NTS or toxin-induced loss of DBH-expressing neurons in the hindbrain abolishes ghrelin-induced feeding (Date et al., 2006). However, another study reported that GHS-induced activation of the ARC occurs independent of noradrenergic tone (Bailey et al., 2000). Vagotomized human patients do not increase their food intake in response to peripheral ghrelin injections (le Roux et al., 2005). Therefore, the vagal afferent nerve-NTS-noradrenergic neuron-ARC route may be at least partially required for transmission of the peripheral ghrelin feeding signal to the hypothalamus.

The other route for conveying the gastric-derived ghrelin signal to the brain is the blood circulation. Intravenously administered human ghrelin, but not mouse ghrelin, can be transported to the hypothalamus across the blood-brain barrier in mice (Banks et al., 2002, 2008). Circulating ghrelin binds to neurons in the vicinity of fenestrated capillaries of the median eminence, very close to the ARC (Schaeffer et al., 2013).

GHS-R1a is also expressed in various extra-hypothalamic regions where the blood-brain barrier is well preserved. Ghrelin has multifaceted neuronal functions, but it remains unclear whether its roles in the brain are attributed to circulating ghrelin, brain-derived ghrelin, or ligand-independent GHS-R1a signaling. GHS-R1a in the hippocampus controls dopaminergic regulation of memory by heterodimerizing with D1R in the absence of ghrelin (Kern et al., 2015). However, other studies demonstrate that circulating ghrelin enters the hippocampus and binds to GHS-R1a-expressing neurons, where it promotes dendritic spine synapse formation and enhances memory performance (Diano et al., 2006). Subcutaneous ghrelin administration induces c-*fos* in the hindbrain where GHS-R1a is expressed, suggesting that circulating ghrelin may reach and bind to the neurons of the hindbrain, beyond the blood-brain barrier (Scott et al., 2012). Collectively, these observations indicate that the vagal afferent nerve and the blood circulation convey gastric-derived ghrelin signals to the brain.

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Ghrelin's Orexigenic Effects Mediated through Extra-hypothalamic Regions

In addition to the hypothalamus, ghrelin exerts orexigenic effects through extra-hypothalamic areas including the hippocampus, amygdala, and VTA. These regions are important for the mesolimbic dopaminergic system, which controls motivational aspects of multiple behaviors, including hedonic feeding (Alvarez-Crespo et al., 2012; Carlini et al., 2004; Kanoski et al., 2013). Ghrelin's orexigenic effect on NPY/AgRP neurons is restricted to mice fed a non-palatable diet, whereas these neurons are dispensable when highly palatable food is available (Denis et al., 2015). Ghrelin bound to VTA neurons increases dopamine levels in the shell of the nucleus accumbens, a key area in the reward system that receives dopamine neuron projections from the VTA, thereby stimulating food intake and food motivation (Abizaid et al., 2006; Jerlhag et al., 2007; Skibicka et al., 2011). In concert, restricted expression of GHS-R1a in catecholaminergic neurons (most of which are dopaminergic neurons in the VTA) in GHS-R1a-null mice is sufficient to restore ghrelin-induced acute food intake and stress-induced food-reward behaviors (Chuang et al., 2011). Interestingly, GHS-R1a-expressing MBH neurons send axonal projections to the amygdala, suggesting the presence of second-order neurons located downstream of MBH GHS-R1a neurons and a functional link between homeostatic and hedonic feeding (Mani et al., 2017). The ability of ghrelin to elicit food-reinforced behavior is dependent on intact dopaminergic and opioid signaling in the VTA (Romero-Picó et al., 2013a; Skibicka et al., 2012; Weinberg et al., 2011).

Peripheral ghrelin administration to healthy volunteers during fMRI enhances the activity in amygdala, orbitofrontal cortex (OFC), and hippocampus (Goldstone et al., 2014; Malik et al., 2008). The effects of ghrelin on amygdala and OFC response are correlated with self-rated hunger ratings. Circulating ghrelin levels co-vary with fMRI activity in the amygdala in response to palatable food stimuli upon fasting (Kroemer et al., 2013). Intriguingly, healthy-weight women exhibit positive associations between fasting ghrelin and fMRI activity in the right amygdala, hippocampus, insula, and OFC in response to high-calorie foods, whereas these associations are absent in patients with anorexia nervosa (Holsen et al., 2014). Furthermore, circulating ghrelin levels under satiety are positively associated with increased activity of the ventral striatum during the expectation of food-related reward in healthy individuals (Simon et al., 2017). Carriers of obesity-risk alleles have an attenuated postprandial reduction of circulating ghrelin levels, along with modulation of the neural responses to food images in homeostatic and brain reward regions, further suggesting the close links between ghrelin, obesity, and altered hedonic food susceptibility (Karra et al., 2013). Taken together, ghrelin interacts with the dopamine-reward system and favors food consumption during both fasting and satiety by enhancing the hedonic and incentive responses to food-related cues.

The role of the hindbrain in regulation of ghrelin-induced food intake remains controversial. Direct microinjection of ghrelin into the dorsal vagal complex stimulates food intake at a dose lower than the lowest effective dose that induces food intake upon microinjection into the ARC (Faulconbridge et al., 2003). Ghrelin administration into the fourth ventricle induces c-*fos* expression in the NTS, but not in the hypothalamus, suggesting the presence of independent hindbrain circuits that respond to ghrelin (Faulconbridge et al., 2008). On the other hand, hindbrain-specific GHS-R1a expression in GHS-R1a-deficient mice is not sufficient for ghrelin-induced feeding, indicating that direct sensing of ghrelin in GHS-R1a-expressing hindbrain neurons is not involved in ghrelin's orexigenic effect (Scott et al., 2012).

GHS-R1a is expressed in neurons of the hippocampus and ARC, and ghrelin plays crucial roles in mnemonic processes, which commit to conditioned meal responses including meal anticipation, food preferences, and food-seeking behaviors (Betley et al., 2015; Russo et al., 2017). The ghrelin-GHS-R1a axis promotes the synaptic accumulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor and increases long-term potentiation in the hippocampus (Ribeiro et al., 2014). The molecular mechanisms underlying ghrelin's role in learning and memory have been extensively reviewed (Andrews, 2011; Ferrario et al., 2016; Volkow et al., 2011).

The Roles of Endogenous Ghrelin on Feeding and Coordination of Energy Homeostasis

Although it has been clearly demonstrated that exogenous ghrelin potently influences food intake and adiposity, the requirement of endogenous ghrelin for feeding remains undetermined. Deletion of ghrelin does not influence food intake (McFarlane et al., 2014; Sun et al., 2003, 2006; Wortley et al., 2004). Similar negative findings were obtained in mice lacking GHS-R1a or GOAT (Sun et al., 2004; Zhao et al., 2010a). These lines of evidence suggest that the ghrelin-GOAT system may not be an essential endogenous regulator of food intake.

Circulating ghrelin levels rise before a meal and drop afterward (Cummings et al., 2001; Shiiya et al., 2002). Gastric GOAT expression levels rise under *ad libitum* conditions and decrease with fasting. Acylation of ghrelin is directly influenced by ingested dietary MCT, and ghrelin signaling in the hypothalamus requires neuronal fatty acid metabolism. Furthermore, as noted above, the endogenous ghrelin-GOAT system is crucial for whole-body energy storage on an MCT-enriched diet. Thus, the endogenous ghrelin surge before a meal and elevation of GOAT expression upon feeding might prepare the organism for incoming food, ensuring efficient metabolism and storage of energy, rather than serving as a meal initiation cue or hunger signal in response to persistent starvation (Kirchner et al., 2009; Müller et al., 2015). The fact that ghrelin stimulates expression of genes involved in lipogenesis further supports this idea (Perez-Tilve et al., 2011; Sangiao-Alvarellos et al., 2009; Theander-Carrillo et al., 2006).

Obesity impairs ghrelin's functions in homeostatic feeding and reward processing, leading to a condition called ghrelin resistance. The molecular mechanisms underlying ghrelin resistance have been extensively reviewed in the recent literature (Cui et al., 2017; Zigman et al., 2016).

Ghrelin's Effects on Glucose Metabolism

The majority of human and animal studies have demonstrated that ghrelin suppresses insulin secretion (Broglio et al., 2001; Dezaki et al., 2004; Reimer et al., 2003). In healthy humans, ghrelin administration reduces glucose-stimulated insulin secretion (GSIS) and deteriorates glucose tolerance (Tong et al., 2010). Ablation of the ghrelin gene increases GSIS and improves insulin

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sensitivity in HFD-fed mice and leptin-deficient *ob/ob* mice (Dezaki et al., 2006; Sun et al., 2006). GHS-R1a antagonists enhance insulin secretion and improve glucose tolerance in diet-induced obese rodents (Esler et al., 2007) and in hepatocyte nuclear factor-1α-knockout mice (Brial et al., 2015).

Suppression of insulin secretion by ghrelin has been attributed to GHS-R1a expression in β cells (Doi et al., 2006; Kageyama et al., 2005; Wang et al., 2010). GHS-R1a blockade in isolated islets increases insulin secretion and cytosolic Ca^{2+} in single β cells (Dezaki et al., 2004). However, the roles of the aforementioned GHS-R1a signaling mechanisms in the regulation of insulin secretion remain controversial; because GHS-R1a is a $G\alpha_q$-coupled receptor, its activation would be predicted to enhance rather than inhibit insulin secretion. Several potential explanations have been offered. Ghrelin inhibits insulin secretion by non-canonical GHS-R1a signaling, in which $G\alpha_i$ rather than $G\alpha_q$ is coupled to GHS-R1a (Dezaki et al., 2007), and GHS-R1a:SST5 heteromerization is required for coupling of GHS-R1a to $G\alpha_i$ (Park et al., 2012). By performing comprehensive transcriptome analysis of α, β, or δ cells from individual islets of triple-transgenic reporter mice, which enable fluorescence-activated cell sorting purification of these three types of endocrine cells, two independent research groups showed that GHS-R1a is expressed exclusively in δ cells (Adriaenssens et al., 2016; DiGruccio et al., 2016). SST5 was not detected in any murine islet endocrine cells in either study. Ghrelin increases intracellular [Ca^{2+}] in δ cells and robustly potentiates glucose-stimulated somatostatin secretion. Somatostatin receptor antagonism prevents suppression of insulin secretion by ghrelin. Intriguingly, several single-cell RNA sequencing studies of human islets have shown that expression of *GHSR* is restricted to δ cells (Lawlor et al., 2017; Muraro et al., 2016; Segerstolpe et al., 2016).

Ghrelin administration impairs insulin sensitivity in healthy humans (Vestergaard et al., 2007) and adult-onset GH-deficient patients (Gauna et al., 2004). Ghrelin's effects on insulin resistance are independent of GH, cortisol, and free fatty acids (FFAs) (Vestergaard et al., 2017). Ghrelin directly stimulates glucose output from hepatocytes (Gauna et al., 2005). In addition, ghrelin reduces glycogen synthase kinase and enhances expression of proliferator-activated receptor-γ co-activator-1α (PGC-1α), an activator of gluconeogenesis, in the liver (Barazzoni et al., 2007).

The ghrelin-GHS-R1a axis also regulates glucose homeostasis via central mechanisms. Global neuronal deletion of GHS-R1a improves insulin sensitivity (Lee et al., 2016). More specifically, hindbrain-specific GHS-R1a expression in systemic GHS-R1a-null mice protects against exacerbated fasting-induced blood glucose reduction (Scott et al., 2012). AgRP-neuron-selective re-expression of GHS-R1a in GHS-R1a-null mice fully restores the lowered blood glucose upon caloric restriction (Wang et al., 2013). Appropriate ghrelin levels during post-natal life are essential for lifelong glucose homeostasis and body weight control through normal development of ARC axonal projections; chronic exposure or blockade of ghrelin during the post-natal period causes abnormal maturation of neuronal projections from the ARC to the PVN, with a predominance of projections from AgRP/NPY neurons, resulting in metabolic disturbances in adulthood such as hyperglycemia and elevated body weight (Steculorum et al., 2015). Future studies are needed to clarify how GHS-R1a-expressing neurons cooperate with other central and peripheral tissues to control blood glucose.

In summary, GHS-R1a is expressed exclusively in δ cells in both humans and rodents, and ghrelin seems to exert an insulinostatic effect within pancreatic islets in a δ-cell-mediated paracrine manner. The ghrelin-GHS-R1a axis in hindbrain or AgRP neurons prevents hypoglycemia under starvation.

Regulation of Ghrelin Secretion

Biosynthesis and secretion of ghrelin rise upon fasting and drop after feeding (Cummings et al., 2001; Shiiya et al., 2002; Toshinai et al., 2001). Ghrelin secretion is entrained to habitual eating patterns in humans (Frecka and Mattes, 2008). Circulating ghrelin level is negatively correlated with body mass index: plasma ghrelin concentrations are lower in obesity and higher in emaciation (Cummings et al., 2002; Nagaya et al., 2001; Tschöp et al., 2001). Post-prandial ghrelin suppression is proportional to ingested caloric load (Callahan et al., 2004) and dependent on the macronutrient composition of the meal (Monteleone et al., 2003). Exposure to high D-glucose or long-chain fatty acids solution suppresses ghrelin release from isolated gastric mucosal cells (Lu et al., 2012; Sakata et al., 2012). Insulin suppresses ghrelin secretion via the PI3K-Akt pathway (Gagnon and Anini, 2012).

Activation of sympathetic nerves stimulates ghrelin secretion, whereas adrenergic antagonists suppress ghrelin secretion (Mundinger et al., 2006; Zhao et al., 2010b). Concordantly, ghrelin-producing cell-specific deletion of β_1-adrenergic receptor blunts ghrelin secretion and causes profound hypoglycemia upon caloric restriction (Mani et al., 2016). Another key regulator of ghrelin secretion is the chemosensory signaling pathway. Gavage of bitter-taste receptor agonists stimulates ghrelin secretion, whereas this effect is blunted in α-gustducin-knockout mice (Janssen et al., 2011). In addition, ghrelin-secreting MGN3-1 cells sense L-Phe, L-Ala, and monosodium glutamate via their respective taste receptors and secrete ghrelin following exposure to these amino acids (Vancleef et al., 2015).

The circulating ghrelin level is altered by cephalic phase, as demonstrated by the rise in ghrelin level when food is anticipated in a fixed meal-feeding model (Drazen et al., 2006). Ghrelin secretion is also regulated by the cholinergic system: plasma ghrelin levels are increased and decreased by cholinergic agonists and antagonists, respectively (Broglio et al., 2004; Hosoda and Kangawa, 2008).

Ghrelin-producing cells in the stomachs of ghrelin-hrGFP reporter mice express several kinds of GPCRs for neurotransmitters, hormones, paracrine lipid messengers, and metabolites (Engelstoft et al., 2013). Agonists of $G\alpha_s$-coupled receptors for epinephrine and norepinephrine stimulate ghrelin secretion, whereas agonists of $G\alpha_{i/o}$- or $G\alpha_{q/11}$-coupled receptors for somatostatin and lactate suppress secretion. Future studies should seek to determine whether and how the candidate factors mentioned in this section coordinately (or solely) regulate ghrelin secretion.

The Role of Ghrelin in Cellular Homeostasis

Ghrelin and Autophagy

Autophagy, an evolutionarily conserved pathway for bulk digestion of cytoplasmic organelles, plays a crucial role in

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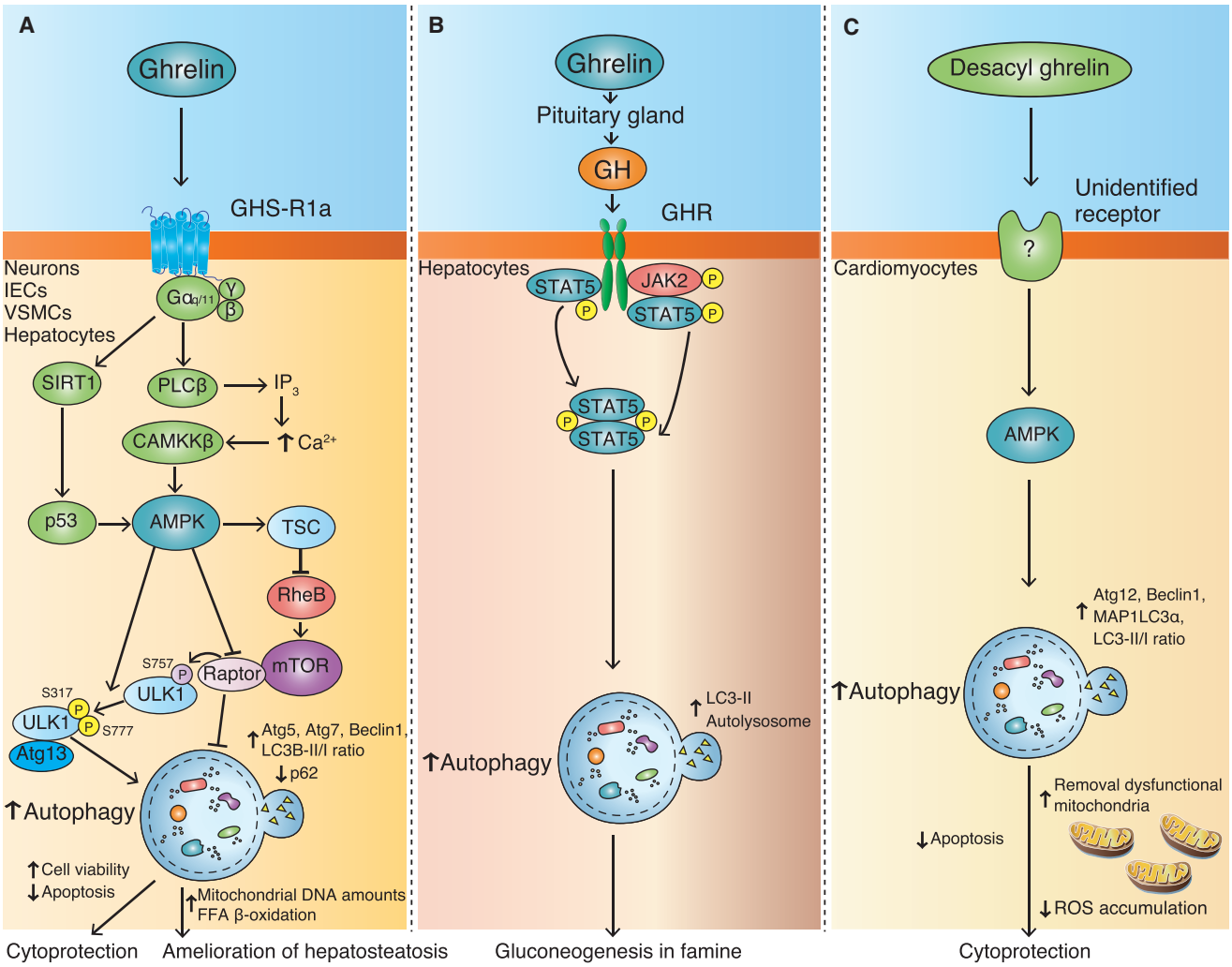

Figure 3. The Role of Ghrelin's Proautophagic Properties in Cellular Homeostasis

(A) Ghrelin enhances autophagy in a GHS-R1a-dependent manner. Activated AMPK inhibits mTOR via activation of TSC and inactivation of Raptor. Raptor-induced inhibitory phosphorylation of ULK1 is reduced, leading to activation of ULK1 kinase activity, and activated ULK1 triggers autophagy (Kim et al., 2011). Ghrelin exerts a cytoprotective effect by inducing autophagy in neurons, intestinal epithelial cells (IECs), and vascular smooth muscle cells (VSMCs). Ghrelin's proautophagic property improves hepatosteatosis by increasing the abundance of mtDNA and inducing mitochondrial FFA β-oxidation. CaMKKβ and the SIRT1-p53 axis also mediate signaling to AMPK in the setting of autophagy, as in the case of hypothalamic ghrelin signaling.

(B) Under fasting, fat-depleted conditions, GH maintains blood sugar levels by stimulating hepatic autophagy and subsequent gluconeogenesis. Ghrelin is essential for the maintenance of GH levels under starved, fat-depleted conditions. GH signaling induces autophagy via pSTAT. The molecule that connects the GH-pSTAT axis and autophagy is currently unknown.

(C) Desacyl ghrelin stimulates AMPK activity, induces autophagy, and decreases ROS accumulation and apoptosis, thereby protecting cardiomyocytes from ischemic injury.

Atg, autophagy-related; LC3, microtubule-associated protein 1A/1B-light chain 3; MAP1LC3α, microtubule associated protein 1 light chain 3α; RheB, Ras homolog enriched in brain; TSC, tuberous sclerosis complex; ULK1, Unc-51 like kinase-1.

the maintenance of cellular homeostasis and survival (Mizushima et al., 2008). Ghrelin exerts a cytoprotective activity in some tissues by inducing autophagy (Figure 3A). For example, ghrelin enhances cell viability and suppresses apoptosis by stimulating autophagy in a cell culture model of Alzheimer disease (Cecarini et al., 2016). Ghrelin stimulates autophagy by decreasing phospho-mTOR levels in cerebral cortical neurons (Ferreira-Marques et al., 2016). Ghrelin also enhances autophagy and decreases cell damage in intestinal epithelia and vascular smooth muscle cells (Wan et al., 2016; Xu et al., 2017).

Ghrelin activates AMPK in hepatocytes, promotes autophagy, stimulates mitochondrial biogenesis, and induces mitochondrial FFA β-oxidation, and thus ameliorates hepatic triglyceride overaccumulation (Ezquerro et al., 2016) (Figure 3A). In concert, ghrelin attenuates hepatic lipotoxicity by enhancing autophagy via restoration of the AMPK/mTOR signaling pathway (Mao et al., 2015a). The ghrelin-GH-autophagy axis is essential for survival in famine. Under fasting, fat-depleted conditions, organisms activate hepatic autophagy to perform gluconeogenesis and maintain blood glucose levels. This process is mainly orchestrated by the action of GH (Ezaki et al., 2011). Under starved,

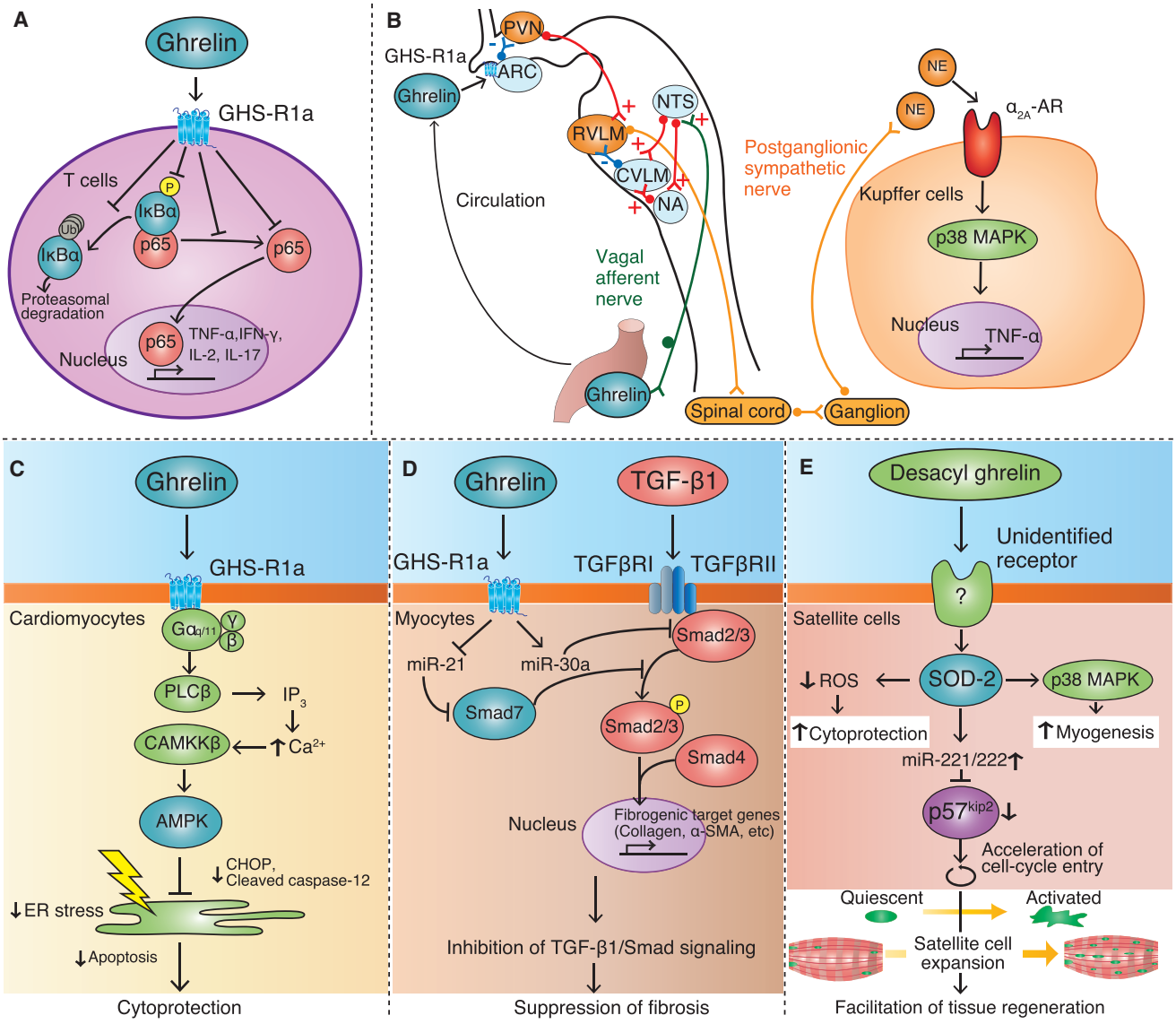

Figure 4. The Roles of Ghrelin in Inflammation, Cytoprotection, Fibrosis, and Maintenance of Resident Stem Cells

(A) Ghrelin suppresses proinflammatory cytokine production in T cells by inhibiting the NF-κB pathway. Ghrelin prevents phosphorylation and proteasomal degradation of IκBα, thereby suppressing nuclear translocation of p65.

(B) Ghrelin suppresses inflammation via its sympathoinhibitory action. The rostral ventrolateral medulla (RVLM) is the dominant source of sympathetic excitatory signals to the periphery. The RVLM receives inhibitory inputs from the caudal ventrolateral medulla (CVLM), nucleus ambiguus (NA), and nucleus tractus solitarius (NTS), as well as excitatory inputs from the paraventricular nucleus (PVN). Enhanced norepinephrine (NE) release from postganglionic sympathetic nerve leads to α_{2A}-adrenergic receptor (α_{2A}-AR)-mediated mitogen-activated protein kinase (MAPK) p38 activation and TNF-α production in Kupffer cells. Ghrelin suppresses sympathetic nerve activity through the vagus nerve-NTS-CVLM-RVLM inhibitory pathway. Ghrelin also inhibits the PVN via the arcuate nucleus (ARC).

(C) Ghrelin exhibits cytoprotective effects on cardiomyocytes by suppressing ER stress in a GHS-R1a/CaMKKβ/AMPK-dependent manner.

(D) Ghrelin upregulates antifibrotic (miR-30a) microRNA and downregulates profibrotic (miR-21) microRNA and the TGF-β1-Smad pathway, thereby ameliorating skeletal muscle fibrosis after injury.

(E) Desacyl ghrelin stimulates SOD-2 activity and increases expression of miR-221 and miR-222, which in turn suppress $p57^{kip2}$ expression in satellite cells of skeletal muscle. This accelerates cell-cycle entry and expansion of satellite cells, thereby facilitating muscle regeneration. Desacyl ghrelin-mediated SOD-2 upregulation also increases myogenesis and decreases ROS generation, further promoting tissue regeneration after injury.

CHOP, CCAAT-enhancer-binding protein homologous protein; IFN-γ, interferon-γ; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α; α-SMA, α-smooth muscle actin.

fat-depleted conditions, GOAT-knockout mice exhibit insufficient GH upregulation, a decline in hepatic autophagy, and lethal hypoglycemia (Zhang et al., 2015) (Figure 3B).

A comprehensive screen based on *in vivo* delivery of arrayed cDNA libraries aimed at identifying tissue-protective factors revealed robust, specific expression of the ghrelin gene in heart and skeletal muscle after acute ischemia (Ruozi et al., 2015). Transduction of the ghrelin gene into the heart rescues cardiomyocytes from ROS accumulation and apoptosis, and restores cardiac functions after myocardial infarction in an

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autophagy-dependent manner (Figure 3C). Desacyl ghrelin also decreases ROS production, lowers tissue inflammation, and enhances insulin-stimulated glucose uptake in skeletal muscle in an autophagy-dependent manner (Gortan Cappellari et al., 2016).

The Roles of Ghrelin in Inflammation, Fibrosis, Stem Cells, and Regeneration

Inflammation and fibrosis are increasingly appreciated as major hallmarks of dysfunction of metabolic organs (Sun et al., 2011, 2013). Ghrelin decreases inflammation by inhibiting the nuclear factor κB (NF-κB) pathway and mitigates fibrosis (Imazu et al., 2011; Moreno et al., 2010; Tsubouchi et al., 2014; Wu et al., 2007b). Ghrelin directly suppresses production of proinflammatory cytokines in monocytes and T cells (Dixit et al., 2004) (Figure 4A). Ghrelin inhibits differentiation of Th17 cells, an important subset of T cells that exert proinflammatory functions (Xu et al., 2015). Ghrelin knockdown increases interleukin-17 (IL-17) production in primary human T cells, suggesting a role of endogenous ghrelin in regulation of Th17 cell function (Dixit et al., 2009).

Ghrelin also suppresses systemic inflammation through its sympathoinhibitory functions (Cheyuo et al., 2012). The rostral ventrolateral medulla (RVLM) is the dominant source of sympathetic excitatory signals to the periphery. Ghrelin inhibits the excitatory output of the PVN to the RVLM, resulting in suppression of sympathetic output. Sepsis-induced sympathoexcitation promotes norepinephrine release from postganglionic sympathetic nerves, stimulates the α_{2A}-adrenergic receptor on Kupffer cells, and augments tumor necrosis factor alpha (TNF-α) production (Yang et al., 2001) (Figure 4B). Both central and peripheral administration of ghrelin decreases circulating levels of TNF-α and norepinephrine in sepsis (Wu et al., 2007c). Ghrelin also decreases release of sepsis-induced proinflammatory cytokines via activation of the vagus nerve (Wu et al., 2007a). Furthermore, ghrelin exerts cytoprotective effects by suppressing ER stress. Ghrelin administration decreases myocardial apoptosis and ameliorates cardiac dysfunction after chemical or ischemic myocardial injury (Zhang et al., 2009, 2013). Ghrelin prevents ER stress and reduces apoptosis in primary rat cardiomyocytes (Zhang et al., 2013) (Figure 4C).

Ghrelin prevents organ fibrosis by reducing fibroblast activity. Ghrelin inhibits the expression of transforming growth factor β1 (TGF-β1) and phospho-Smad3 in association with attenuated extracellular matrix deposition and reduced myofibroblast accumulation (Mao et al., 2015b; Sun et al., 2015). Ghrelin also decreases collagen production (Ota et al., 2013). Furthermore, ghrelin upregulates antifibrotic (miR-30a) microRNA and downregulates profibrotic (miR-21) microRNAs in skeletal muscle after injury, resulting in inactivation of the TGF-β1/Smad pathway and mitigation of fibrosis (Katare et al., 2016) (Figure 4D).

Desacyl ghrelin plays a crucial role in tissue renewal by stimulating resident stem cell activity, and also protects skeletal muscle against ischemic injury (Togliatto et al., 2013). In particular, desacyl ghrelin increases the number of satellite cells, a resident stem cell of skeletal muscle. Desacyl ghrelin promotes cell-cycle entry in satellite cells via modulation of the superoxide dismutase-2 (SOD-2)-miR-221/222-$p57^{kip2}$ pathway (Figure 4E). In concert, desacyl ghrelin enhances self-renewal of satellite cells, facilitates myoblast differentiation, and ameliorates the dystrophic phenotype in mdx mice, a model of Duchenne muscular dystrophy (Reano et al., 2017).

Ghrelin, SIRT1, and Mitochondria

SIRT1, a nicotinamide adenine dinucleotide-dependent protein deacetylase, is crucial in cell metabolism, longevity, and stress response (Brooks and Gu, 2009). Ghrelin signaling prolongs lifespan in murine models of aging, concomitant with elevation of SIRT1 expression (Fujitsuka et al., 2016; Yang et al., 2016). Desacyl ghrelin administration to *ob/ob* mice protects against ischemia-induced functional impairment, and also decreases ROS generation in intramuscle vessels (Togliatto et al., 2015). Desacyl ghrelin upregulates both SIRT1 and SOD-2 by restoring endothelial cell miR-126 expression, which in turn leads to deacetylation of p53 and histone 3 Lys56 and protects endothelial cells from cellular senescence. In addition, desacyl ghrelin protects microvascular endothelial cells from oxidative stress-induced apoptosis while increasing SIRT1 activity (Shimada et al., 2014).

Numerous studies have confirmed the close link between ghrelin and mitochondrial functions. In a murine model of Parkinson disease, continuous administration of ghrelin improves mitochondrial respiration, increases mitochondrial biogenesis in a UCP2-dependent manner, and decreases dopaminergic neuron loss (Andrews et al., 2009). Ghrelin administration ameliorates renal damage and increases PGC-1α expression and mitochondrial number in the kidney (Fujimura et al., 2014). In a murine model of chronic kidney disease, ghrelin also attenuates the decline in exercise endurance while increasing muscle mass and mitochondrial abundance (Tamaki et al., 2015).

In summary, ghrelin maintains cellular homeostasis by driving autophagy, potentiating stem cell function, mitigating inflammation and fibrosis, decreasing ROS production, and improving mitochondrial and ER functions.

Clinical Pharmacology Studies of Ghrelin

The clinical pharmacology of ghrelin has been studied under a variety of pathological conditions (Takiguchi et al., 2015). Based on a previous review (Garin et al., 2013) and our own literature search, as of November 30, 2017, the results of clinical trials in which ghrelin was administered to healthy participants or patients with various disorders have been published in 167 articles (Table S1). Among these papers, 108 articles measured circulating GH level, 41 examined appetite, 17 evaluated food intake, 9 measured body weight, 47 measured blood glucose level, 38 measured blood insulin, and 9 examined insulin sensitivity. Overall, the results of these trials indicate that ghrelin increases circulating GH (94%, 102 of 108 articles), stimulates appetite (88%, 36 of 41 articles), promotes food intake (88%, 15 of 17 articles), increases body weight (78%, 7 of 9 articles), elevates blood glucose (68%, 32 of 47 articles), and decreases insulin sensitivity (78%, 7 of 9 articles) across diverse subject populations. By contrast, the outcome of ghrelin administration on circulating insulin remains controversial: 14 articles (37%) reported that ghrelin decreases plasma insulin, whereas 24 (63%) reported no such effect. Ghrelin infusion increases respiratory quotient in healthy and obese subjects, implying that it promotes adiposity in humans (Huda et al., 2009). Ghrelin infusion also increases palatability of food in obese subjects, suggesting the involvement of ghrelin in enhancement of food-reward behavior (Druce et al., 2005). The adverse effects in these studies were tolerable, with a predominance of flushing and borborygmus with mild severity.

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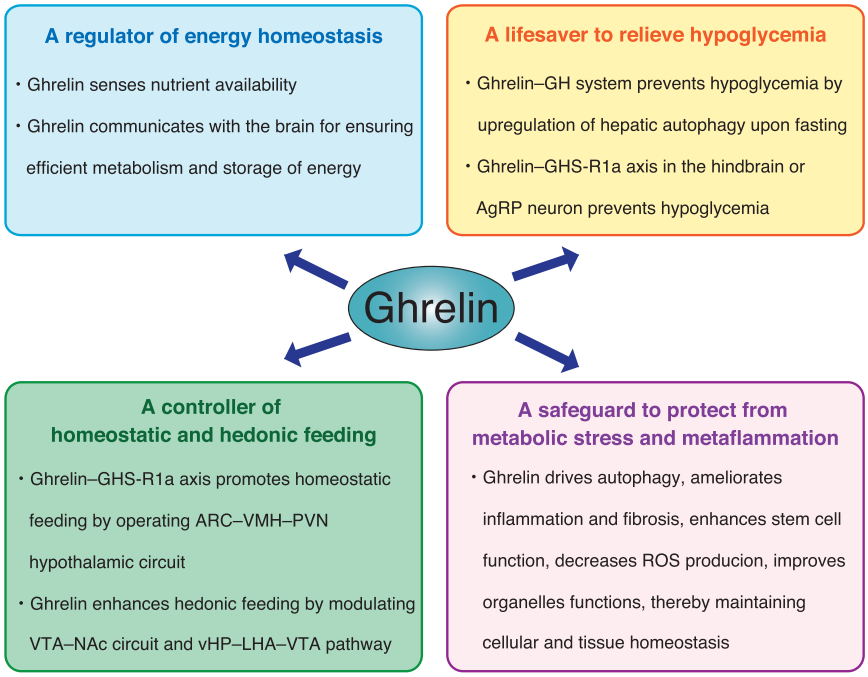

Figure 5. The Role of Ghrelin in Energy Homeostasis

Ghrelin serves as a crucial regulator of energy homeostasis by sensing peripheral nutrient availability and transmitting this information to the brain for ensuring efficient metabolism and storage of energy, a lifesaver that relieves the organism from hypoglycemia under famine, a controller of homeostatic and hedonic feeding, and a candidate safeguard that protects metabolically critical organs (e.g., adipose tissue, liver, and skeletal muscle) from metabolic stress (e.g., hypoxia, production of reactive oxygen species, disruption of mitochondrial function, and endoplasmic reticulum stress) and metaflammation. NAc, nucleus accumbens.

Serious adverse events such as pneumonia, enteritis, and lung cancer were extremely rare (Garin et al., 2013).

Cachexia is a multifactorial syndrome characterized by ongoing appetite loss, skeletal muscle wasting, and body weight reduction. Ghrelin administration to patients with chronic heart failure increases food intake and body weight, ameliorates muscle wasting, and improves exercise capacity and left-ventricular function (Nagaya et al., 2004). Ghrelin confers these clinical benefits and also improves respiratory functions in patients with chronic respiratory failure (Miki et al., 2012).

Ghrelin's effects on surgical stress and chemotherapy-associated adverse reactions have also been explored. After total gastrectomy, the plasma concentration of ghrelin is reduced to 10%–20% of its preoperative level due to the removal of ghrelin-producing cells (Doki et al., 2006). Esophageal reconstruction leads to an approximately 50% reduction in ghrelin due to truncal vagotomy, devascularization, and mobilization of the stomach. Ghrelin administration to patients undergoing total gastrectomy increases their oral food intake and minimizes post-operative body weight loss (Adachi et al., 2010).

The potential of ghrelin as a therapeutic agent for treatment of various pathophysiological conditions can be attributed to its ability to promote GH secretion, stimulate anabolic activity, suppress the effects of inflammation, and regulate the autonomic nervous system. Ghrelin's roles in cytoprotection have been explored in the context of treating diabetic polyneuropathy and the post-operative state following hip joint surgery (Akamizu et al., 2008; Ueno et al., 2017). Ghrelin can ameliorate the complications induced by the chemotherapeutic agent cisplatin, including hyperalgesia, cachexia, and male gonadal toxicity (Garcia et al., 2008, 2015; Whirledge et al., 2015).

Ghrelin appears to function in learning and memory, reward-seeking behavior, prevention of anxiety, sleep, and higher neurological functions. Moreover, animal studies demonstrate that ghrelin has potential as a novel palliative and neuroprotective agent in some neurodegenerative diseases (Dickson et al., 2011; Lee et al., 2010; Shi et al., 2017). Future research will be required to translate these proof-of-concept studies into clinical trials.

Conclusion

The discovery of ghrelin represents a major turning point in the study of stomach-brain interactions, and has made enormous contributions to our understanding of systemic homeostasis. In this review, we present clear evidence that ghrelin exerts a multifactorial homeostatic force (Figure 5; Table S2). Because functional failure of organelles is a critical event in loss of metabolic homeostasis, as well as the initiation or propagation of metaflammation (Arruda and Hotamisligil, 2015; Fu et al., 2012; Hotamisligil, 2010), ghrelin's protective effects on organelles and proautophagic property raise the possibility of new therapeutic approaches against metabolic diseases. Clinical applications of ghrelin, focused on its prohomeostatic traits, have been attempted in patients with various disorders, and the safety and promising effects of these interventions have been demonstrated.

Many aspects of ghrelin biology remain unresolved, including the regulatory signaling pathways responsible for ghrelin and GOAT expression in the stomach, the contribution of GHS-R1a constitutive activity to neuronal functions, and the identity of the desacyl ghrelin receptor. Future research on ghrelin's homeostatic force will provide clues in therapeutic interventions for patients with metabolic diseases.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at https://doi.org/10.1016/j.cmet.2018.02.008.

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