Ghrelin: Biological Characteristics and Regulation of Animal Feed Intake Postprint

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Abstract

Ghrelin, also known as growth hormone-releasing peptide, was first discovered in the stomach of rats and is an endogenous ligand for the growth hormone secretagogue receptor 1A (GHSR-1A). In regions where GHSR-1A is highly expressed, such as the hypothalamic arcuate nucleus (ARC) and paraventricular nucleus (PVN), binding of ghrelin to GHSR-1A produces a series of biological effects. This article reviews the biological characteristics and functions of ghrelin, the mechanisms regulating animal feed intake, factors influencing the regulation of ghrelin gene expression and feedback inhibition, as well as future prospects.

Full Text

Ghrelin: Biological Characteristics and Regulation of Animal Feed Intake

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Abstract

Ghrelin, also known as growth hormone-releasing peptide, was first discovered in the stomach of rats and serves as an endogenous ligand for the growth hormone secretagogue receptor 1A (GHSR-1A). In hypothalamic regions such as the arcuate nucleus (ARC) and paraventricular nucleus (PVN) where GHSR-1A is highly expressed, ghrelin binding to GHSR-1A elicits a series of biological effects. This review summarizes the biological characteristics and functions of ghrelin, its mechanisms for regulating animal feed intake, factors influencing ghrelin gene expression and feedback inhibition, and future research prospects.

Keywords: ghrelin; growth hormone secretagogue receptor 1A; feed intake; astrocytes; agouti-related protein

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor 1A (GHSR-1A). As a novel peptide hormone composed of 28 amino acid residues, it is widely distributed in various tissues that highly express GHSR-1A, acting on brain regions such as the hypothalamic arcuate nucleus (ARC) and paraventricular nucleus (PVN) where GHSR-1A is abundantly expressed, and producing a series of physiological effects upon binding to GHSR-1A [1-2]. Ghrelin exists primarily in two forms in vivo: acylated ghrelin and des-acyl ghrelin, which exhibit competitive interactions with each other [1]. This review will focus on the biological characteristics of ghrelin, its biological functions in regulating animal feed intake, and factors influencing its gene expression and feedback inhibition.

1 Biological Characteristics and Functions of Ghrelin

1.1 Gene Structure

Ghrelin is a mammalian peptide hormone comprising 28 amino acid residues with a molecular weight of 3,314 Da, first discovered by Japanese scientist Kojima et al. [2] in endocrine cells of the gastric mucosa in humans and rats. It serves as an endogenous ligand for GHSR-1A. The complete preproghrelin precursor contains 117 amino acid residues, with an N-terminal 23-peptide segment exhibiting secretory signal peptide characteristics. The signal peptide cleavage site is located between alanine at position 23 (Ala23) and glycine at position 24 (Gly24), with mature ghrelin beginning at residue 24, directly connected to the signal peptide. The final two residues, proline at position 27 (Pro27) and arginine at position 28 (Arg28), function as processing signals. In vivo, ghrelin exists in two primary structural forms: N-octanoylated ghrelin, where the serine residue at position 3 is modified by a fatty acid, and non-acylated ghrelin [1-2]. N-octanoylation is essential for ghrelin' s biological activity. Additionally, the first 10 N-terminal amino acids of mammalian ghrelin are conserved across species. Human preproghrelin is located on chromosome 3p26-p25, spanning 5,199 bp and containing three introns and four exons. The first exon is 554 bp long, comprising a 446 bp 5' untranslated region and the coding sequence for the N-terminal 13 amino acid residues of the signal peptide. The second exon is 117 bp long, with its N-terminal 45 bp encoding the remaining 15 amino acid residues of the signal sequence [3]. Comparative analysis of amino acid sequences across several mammalian species reveals high homology; for instance, human ghrelin differs from rat and mouse ghrelin only at positions 11 and 12, where humans have arginine (Arg) and valine (Val), while rats and mice have lysine (Lys) and alanine (Ala), representing just two amino acid differences (Figure 1 [Figure 1: see original paper]).

1.2 Distribution and Expression

During fasting, ghrelin is secreted by closed-type X/A-like cells in the gastric fundus mucosa, hypothalamic neurons, and various other tissues. Since ghre-

lin receptors are expressed in numerous tissues, ghrelin is widely distributed throughout the body, including the pituitary gland, stomach, intestine, pancreas, thymus, gonads, thyroid, and heart. Furthermore, ghrelin has been detected in hypothalamic nuclei such as the ARC, ventromedial nucleus (VMN), PVN, lateral hypothalamus (LH), midbrain ventral tegmental area (VTA), and other mesolimbic pathway centers, as well as in the nucleus of the solitary tract (NTS), dorsal motor nucleus (DMN), and area postrema (AP) of the dorsal vagal complex [4]. Additionally, ghrelin has been detected in saliva, underscoring its diverse biological functions. Notably, ghrelin, previously thought to exist only in animals, has reportedly been detected in plants as well [5].

1.3 Biological Functions

Due to its widespread distribution across various tissues, ghrelin exhibits diverse biological functions. Current research has identified several major roles: studies have shown that ghrelin can inhibit the secretion of triiodothyronine (T3) and thyroxine (T4) while stimulating adrenocorticotropic hormone secretion and reducing energy expenditure by suppressing hypothalamic-pituitary-thyroid axis activity [6]. As a G protein-coupled receptor highly expressed in the brain, GHSR-1A promotes growth hormone (GH) release upon binding with ghrelin. Ghrelin also enhances the functions of motilin and gastrin, increasing gastric emptying rate and body weight by regulating gastric acid and digestive enzyme secretion and promoting gastrointestinal motility [7]. Most importantly, ghrelin stimulates animal appetite and regulates feed intake by acting on specific neurons in the hypothalamic ARC and PVN, which represents the primary biological function discussed in this review (Figure 2 [Figure 2: see original paper]).

2 Mechanisms of Ghrelin in Regulating Animal Feed Intake

Feed intake is a critical determinant of whether animals can fully express their production performance and genetic potential. Therefore, investigating nutritional strategies to modulate feed intake represents a highly significant research area. Studies have demonstrated that the central nervous system (CNS) plays a pivotal role in regulating feed intake, with the hypothalamus serving as the key site where CNS control is exerted. Complex food-related signals are integrated and processed within the CNS, activating feeding-related centers such as the hypothalamus to influence appetite and modulate feed intake [8]. Additionally, certain brain-gut peptides can regulate feed intake by affecting gastric emptying rate. Recently discovered peptides with such functions include ghrelin, cholecystokinin (CCK), pancreatic polypeptide (PP), and peptide YY (PYY) [9]. The following two sections will elaborate on the mechanisms underlying feed intake regulation.

2.1 Pathways Regulating Animal Feed Intake

A central melanocortin circuit formed by the ARC exists in the mediobasal hypothalamus, and ghrelin primarily regulates feed intake through its actions in the hypothalamic ARC. Two major neuronal populations within the ARC sense peripheral metabolic signals to control feed intake: one population secretes agouti-related protein (AgRP) and neuropeptide Y (NPY), and ghrelin utilizes this pathway to stimulate appetite and increase feed intake [10-11]. The other population secretes pro-opiomelanocortin (POMC), which is processed to produce melanocortin-4 receptor (MC4R) or α-melanocyte-stimulating hormone (αMSH), thereby reducing feed intake and exerting antagonistic effects against ghrelin (Figure 3 [Figure 3: see original paper]) [12-14].

The appetite-stimulating effect of ghrelin can be suppressed by leptin produced from adipose tissue. Since leptin receptors are also expressed on NPY/AgRP neurons, ghrelin and leptin likely co-regulate feed intake through the NPY/AgRP neuronal system, demonstrating their antagonistic relationship [15-16]. Under normal fasting conditions, plasma ghrelin levels are sufficient to stimulate appetite, and increasing ghrelin levels either centrally or peripherally can trigger feeding behavior [17]. Des-acyl ghrelin, another form of ghrelin present in vivo with significantly lower activity, has been shown by Asakawa et al. [18] to reduce feed intake by acting on the hypothalamic ARC and PVN, slowing gastric emptying rate and decreasing appetite, thus antagonizing the effects of ghrelin.

2.2 Mechanism of AgRP Neurons in Regulating Feed Intake

One of the primary pathways through which ghrelin regulates feed intake is via AgRP neurons. Recent studies by Nathan et al. [19] and Yang et al. [20] have identified a crucial factor in AgRP neuronal regulation of appetite: astrocytes. Astrocytes are non-neuronal glial cells traditionally believed to primarily maintain a healthy microenvironment for neuronal function. However, current research has revealed that astrocytes represent a specialized glutamatergic cell type that actively participates in energy homeostasis regulation.

Glial sensors regulate AgRP neurons through reactive gliosis activated by astrocytes. Adenosine monophosphate (AMP)-dependent gliotransmission stimulates synaptic activity of AgRP neurons, altering blood-brain barrier (BBB) regulation and increasing BBB permeability to ghrelin, thereby activating appetite-stimulating hormone responses that constitute the primary mechanism of feed intake regulation [21]. During gliotransmission, astrocytes release adenosine triphosphate (ATP), which is rapidly converted to AMP by ectonucleotidases and subsequently modulates synaptic transmission through G protein-coupled adenosine A1 receptors (Figure 4 [Figure 4: see original paper]). Using chemogenetic Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology, Nathan et al. [19] and Yang et al. [20] administered clozapine-N-oxide (CNO) to mice to activate astrocytes in the ARC, resulting in decreased ac-

tion potential firing rates of AgRP neurons expressing the excitatory DREADD receptor hM3Dq. This significantly reduced feed intake, blunted responses to ghrelin, and enhanced anorexigenic leptin responses. Conversely, when mice expressed the inhibitory DREADD receptor hM4Di, opposite effects were observed. Subsequent injection of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective adenosine A1 receptor antagonist, into the mouse ARC markedly potentiated and prolonged ghrelin-induced feeding behavior, enhanced rapid re-feeding, and transiently increased feed intake. Importantly, DPCPX in the ARC completely abolished the feeding inhibition observed in hM3Dq DREADD mice previously treated with CNO, reversing the action potential firing rates in AgRP neurons. These findings demonstrate that ghrelin' s regulation of feed intake is a transient, rapid, and temporary modulation [22].

3 Regulation of Ghrelin Gene Expression

The regulation of animal feed intake is an extremely complex process influenced by numerous factors including neural, humoral, and hormonal signals. Recent findings have revealed that nutritional status during feeding plays a particularly important role among these factors [22]. While peptide hormones generally cannot freely enter the brain, the BBB possesses specialized transport mechanisms that enable certain peptides to cross this otherwise impermeable barrier and access the central nervous system [23]. Various nutrients can regulate feed intake not only through synthesis and secretion of brain-gut peptides but also by traveling via the bloodstream to the CNS, crossing the BBB to influence central feeding regulation. Glucose, amino acids, and free fatty acids can all modulate feed intake by affecting intracellular AMP-activated protein kinase (PK) pathways [24].

3.1 Gamma-Aminobutyric Acid (GABA)

GABA is a major inhibitory neurotransmitter in the animal brain and has long been widely used as a safe, low-toxicity feed additive to improve feed intake, alleviate heat stress, modulate immune function, and enhance animal production performance [25]. GABAergic synapses are critical for coordinated neuronal activity, and GABA' s stimulatory effects on neurons take precedence over most normal physiological processes in the brain [26]. Gastón et al. [27] found that dietary supplementation with GABA stimulates the activity of GABAergic presynaptic neurons in the hypothalamic ARC, leading to a significant increase in ghrelin gene mRNA expression through a series of physiological processes.

3.2 Glutamate

Glutamate is the most abundant excitatory amino acid neurotransmitter in the CNS and is widely used as a flavoring agent in both human foods and animal diets. Evidence indicates that AgRP neuron regulation is clearly dependent on glutamatergic input signals [27]. Lee et al. [28] demonstrated that ingestion of

glutamate salts induces morphological changes in astrocytes, triggering a series of biological effects that increase ghrelin gene mRNA expression and enhance feed intake.

3.3 Energy Level

Chowdhury et al. [29] found that carbohydrates can increase levels of anorexigenic hormones such as leptin that produce satiety signals. When carbohydrate levels become excessive, AgRP neuronal activity is suppressed, synaptic transmission is weakened, and ghrelin gene mRNA expression is downregulated, ultimately inhibiting feed intake.

3.4 Minerals and Vitamins

Amini et al. [30] demonstrated through experiments that calcium ion intake exhibits a linear positive correlation with ghrelin secretion levels. Increased calcium content enhances action potential firing rates at AgRP neuronal synapses, leading to elevated ghrelin gene mRNA expression. Additionally, Wang et al. [31] found that vitamin D3 intake increases systemic calcification and calcium ion levels, thereby promoting upregulation of ghrelin gene mRNA expression and stimulating feed intake.

4 Feedback Inhibition of Ghrelin

Koutkia et al. [32] conducted extensive experimental studies and found through correlation analysis that endogenous ghrelin secretion exhibits an inverse, negative relationship with GH levels in the body. High energy status suppresses ghrelin gene mRNA expression, whereas decreased energy levels upregulate ghrelin gene mRNA expression. However, when energy levels drop abruptly, ghrelin gene mRNA expression does not increase significantly but instead shows feedback inhibition, which physiologically mitigates hunger sensation while simultaneously increasing GH levels.

Animal growth and production fundamentally depend on feed intake, a basic life-sustaining activity closely linked to endogenous ghrelin secretion. Ghrelin promotes feed intake through a series of physiological actions mediated by AgRP neurons and astrocytes in the hypothalamic ARC. Enhancing feed intake has long been a priority in animal production, and in-depth research into ghrelin' s structure, function, mechanisms, and expression regulation prompts us to reconsider strategies for improving feed intake and more effectively increasing weight gain and production performance. The discovery of ghrelin offers broader applications for enhancing animal feed intake, but since research on ghrelin is still in its early stages, many aspects require further investigation. For example, developing safer and more effective ghrelin-based biological products to improve feed intake and promote animal growth, as well as the development and utilization of ghrelin feed additives, remain important research directions. Although some specific mechanisms of ghrelin action are not yet fully elucidated, it is evident

that ghrelin holds broad application prospects in the regulation of animal feed intake.

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