

Ghrelin: Ghrelin as a Regulatory Peptide in Growth Hormone Secretion

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ABSTRACT

Background: Ghrelin is a type of growth hormone (GH) secretagogue that stimulates the release of GH. It is a first hormone linking gastrointestinal-pituitary axis.

Objective: This review highlights the interaction of ghrelin with GHRH and somatostatin to regulate the secretion of GH and intends to explore the possible physiological role of the ghrelin-pituitary-GH axis linkage system.

Observation: Ghrelin is highly conserved among species and is classified into octanoylated (C8:0), decanoylated (C10:0), decenoylated (C10:1) and nonacylated,ghrelin. Acylated ghrelin

is the major active form of human ghrelin. The primary production site of ghrelin is the stomach, and it interacts with stomach ghrelin as well as hypothalamic GHRH and somatostatin in the regulation of pituitary GH secretion. Ghrelin stimulate GH release through the GHS receptor to increase intracellular Ca^{2+} ($[Ca^{2+}]$ levels via IP3 signal transduction pathway. Ghrelin is a specific endogenous ligand for the GHS receptor and provides a definitive proof of the occurrence of a GHS-GHS receptor signalling system in the regulation of GH secretion.

Conclusion: Studies suggests that ghrelin is a powerful pharmacological agent that exerts a potent, time-dependent stimulation of pulsatile secretion of GH.

Keywords: Ghrelin, Growth hormone, Ghrelin-pituitary-Gh axis linkage system

INTRODUCTION

Growth reflects a complex interplay of hormones as well as extrinsic influences and genetic factors. The pulsatility of growth hormone (GH) secretion under physiological conditions is controlled by a complex regulatory system primarily exerted by three hypothalamic neuroendocrine hormones acting as coupled biological oscillators, GH-releasing hormone (GHRH), somatostatin (GHIH) and ghrelin [1]. GHRH, is the principal stimulator of GH synthesis and secretion while somatostatin is a potent noncompetitive inhibitor of the release of GH and modulates the pituitary GH response to GHRH [1]. A third regulator of growth hormone secretion is ghrelin which has a marked growth hormone-stimulating activity.

Ghrelin; a type of Growth Hormone Secretagogue (GHS) was first isolated from the rat stomach in 1999 by Kojima and colleagues [2]. The name "ghrelin" is derived from "ghre" which means grow and "relin" which means release [3]. GHSs act through the GHS-receptor (GHS-R) which is a G protein-coupled receptor (GPCR) and stimulates the release of GH [2]. The isolation of ghrelin from stomach as an endogenous ligand of the growth hormone secretagogue receptor (GHSR) led to a reassessment of hormonal regulation of GH secretion.

Ghrelin cells in the oxyntic mucosa are closed types of cells with no continuity with the lumen of the gastrointestinal tract but lies near the capillary network in the lamina propria. Hence, they can respond to physical stimuli from the lumen, chemical stimuli from the basolateral site, or both and can thus function as endocrine cells delivering ghrelin to peripheral tissues that express GHS-R. Human ghrelin crosses the blood-brain barrier as an intact molecule [3,4]. The ghrelin receptor (GHS-R1a) is conserved across different vertebrate species of mammals, birds and fishes [5-7]. Transcripts for GHSR 1A are expressed at low levels in many tissues, but mostly in the arcuate and ventromedial nuclei of the hypothalamus, pituitary hippocampus and gastrointestinal tract [8,9].

This review highlights the interaction of ghrelin with the hypothalamic hormones; GHRH and somatostatin to regulate the secretion of

GH and intends to explore the interesting aspect of the possible physiological role of the ghrelin-pituitary-GH axis linkage system.

METHODOLOGY

The search was performed in electronic databases (MEDLINE, EMBASE, Cochrane, Google scholar). The electronic database search was conducted using the following MeSH terms and keyword combinations: ("ghrelin"[MeSH Terms] OR "ghrelin"[All Fields]) AND ("growth" [Subheading] OR ("growth"[All Fields] AND "development"[All Fields]) OR "growth"[All Fields] OR "growth and development"[MeSH Terms]). Hand searching was also carried out. Articles were selected on the basis of the abstracts, before examining the full text. The reviewers were not blinded to the journal, authors, or institution of the publications. All applicable references were examined and scrutinized by 2 reviewers.

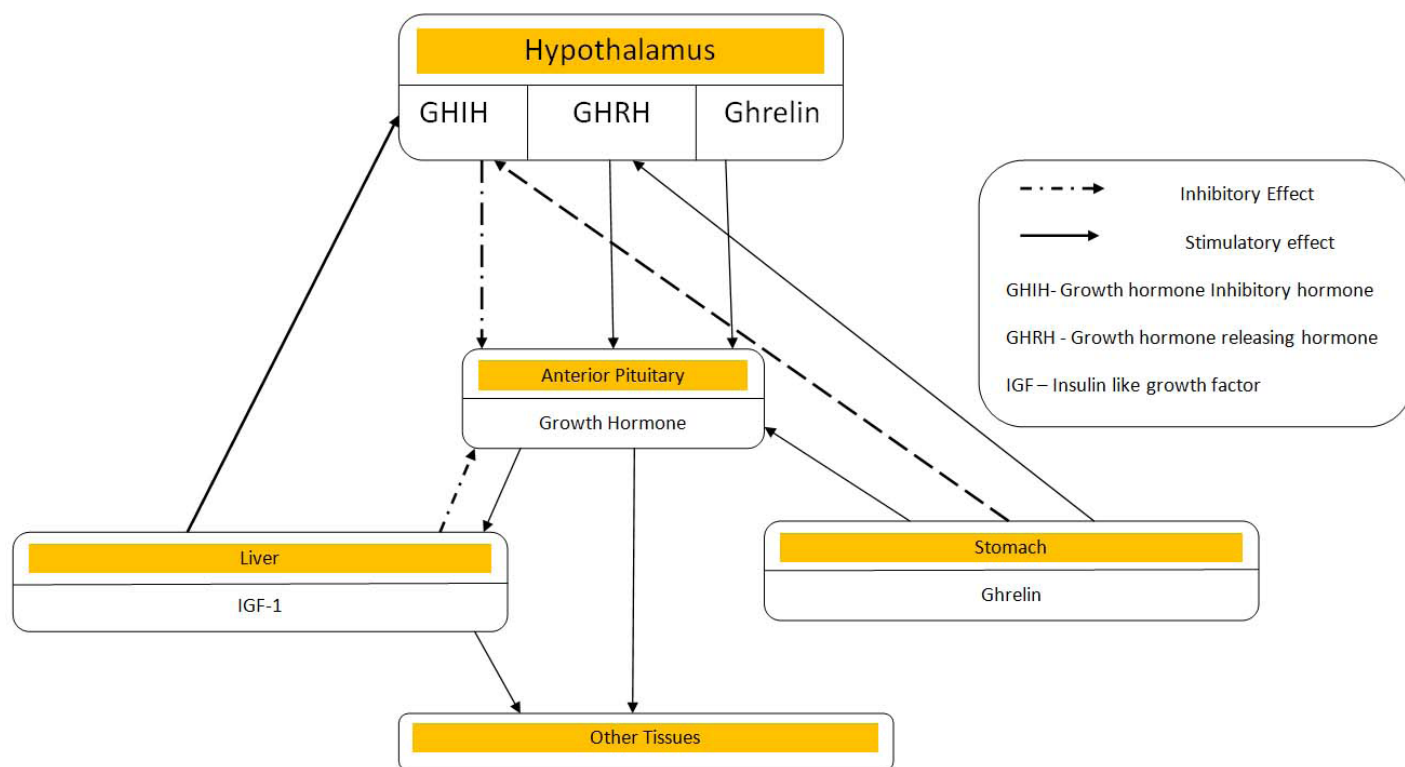
Data extraction: Data extraction was performed by two reviewers on the full texts using a predefined extraction sheet available from the authors. These data included ghrelin/history, ghrelin/physiology, ghrelin/chemistry, ghrelin/ biosynthesis, ghrelin/secretion, ghrelin/ pharmacokinetics, ghrelin/blood, ghrelin/analogs and derivatives, ghrelin/administration and dosage, ghrelin/agonists, ghrelin/ antagonists and inhibitors, ghrelin/diagnostic use, ghrelin/deficiency ghrelin/therapeutic use, ghrelin/drug effects.

Inclusion criteria: Clinical trials (Randomised and non-randomised trials), review articles, systematic reviews, conference proceedings were included in the study. Animal studies were also included.

Exclusion criteria: Studies published in non-indexed journals and in any language other than English were excluded from the study.

Ghrelin gene and synthesis of ghrelin

In humans, the ghrelin gene is located on chromosome 3, at locus 3p25 -2 and is made up of five exons and 3 introns [10]. The first exon is very short (containing only 20 bp) and codes the 5' region [1,11,12]. Ghrelin gene exhibits polymorphism and is not associated with any changes in circulating ghrelin concentration [13].



[Table/Fig-1]: Regulation of growth hormone secretion

mRNA from the *ghrl* gene codes for a 117 amino acid peptide called preproghrelin which is then cleaved to produce proghrelin. Finally, a 28-amino acid acylated ghrelin peptide is produced by protease cleavage and acyl-modification at Ser 3 with an n-octanoyl [11]. Post-transductional mechanisms produce different isoforms of ghrelin, either acylated at Ser with a fatty acid, or have a deletion of the C-terminal Arginine at position 28 [13].

Ghrelin cells: Ghrelin originates in a distinct round to ovoid endocrine cells called "grl cells" [14] which are referred as P/D1 cell in humans and X/A-like cell in rats due to their similarity with the A-cell of the rat pancreas. Ghrelin cells accounts for 23% of chromogranin A-immunoreactive endocrine cells. However, the morphological characteristics and thorough distribution of ghrelin cells in the gastrointestinal tract has not been defined. Ghrelin is also produced in small amounts by other organs like heart, lung, lymphatic tissue, kidney, adrenal glands, thyroid gland, pancreas, gonads, some neoplastic tissues and cancer-cell lines [15-17]. Ghrelin-immunoreactive neurons are present in few regions of the brain like the arcuate and the ventromedial nucleus of the hypothalamus, cerebellum and brainstem [12,18-20]. Ghrelin is also present in placenta along GH, GHRH, somatostatin and insulin-like growth factor 1 (IGF1) which shows that it is also involved in fetal growth [21].

Structure of Ghrelin: A total molecular mass of human ghrelin is 3.370 Kilodalton and the molecular formula is C 149 H 249 N 47 O 42. It is the first and only known peptide hormone in which the hydroxyl group of one of its serine residues is acylated by a fatty acid n-octanoic acid [11,22]. Ghrelin has a hydrophobic moiety: an octanoylated linear chain and the ester bond linking alkyl chain to the serine side chain. The synthetic peptide shows same biological activities as the isolated purified material. Structurally and functionally, ghrelin resembles gut hormone motilin and may have evolved from common ancestral gene [11]. They are therefore now considered to be members of the new motilin-ghrelin peptide superfamily [11]. The human ghrelin and human motilin shows 36% identity.

Ghrelin in different species: Ghrelin are highly conserved among species. The amino acid sequence at positions 11 and 12 is

different in rat and human ghrelin and they are equally potent [2,23] n-octanoylated ghrelin and the shorter form human Ghrelin (hGhr18) is necessary for the GH-releasing effect of ghrelin and the longer forms of Ghrelin are active in vivo [24].

Forms and derivatives of Ghrelin: According to the different types of acylation at Ser3, these peptides could be classified into four groups namely octanoylated (C8:0), decanoylated (C10:0), decenoylated (C10:1) and nonacylated [25]. All peptides are obtained from the same precursor of ghrelin by two alternative pathways and are either 27 or 28 amino acids in length. As in rats, a 28-amino acid peptide octanoylated at Ser3 is the major active form of human Ghrelin. However, the ratio of circulating desacyl ghrelin to acyl ghrelin is 9: 1 while in the human stomach, the ratio is around 1:3 [26,27].

The characteristic feature of AG is the presence of n-octanoyl ester at serine-3 position. The acylation of ghrelin promoted by Ghrelin O-acyltransferase (GOAT) [27] is essential not only for binding with GHS-R1a but also for stimulation of GH secretion [28,29]. Unacylated ghrelin cannot bind and activate the GHS-R 1a and hence is devoid of any effects on endocrine axes. However, it shares other functions of AG through interaction with an unidentified GHS receptor that is different from the classical GHS1a receptor [30,31]. At first UAG was considered an inactive form of ghrelin but now studies have demonstrated that UAG plays a beneficial role in improvement of pancreatic β -cell function and survival [32], and in cardiovascular function [33].

Des-Gln14-ghrelin is an endogenous ligand for the GHSR isolated from the mucosa of the stomach and shares similar hormonal functions as ghrelin [2].

Ghrelin O-acyltransferase (GOAT): GOAT has been regarded as an orphan member of a family of membrane-bound O-acyltransferase enzymes (MBOATs) and is the only known enzyme that has a capacity to acetylate ghrelin. This membrane-bound enzyme can transfer octanoate to serine-3 of ghrelin from octanoyl CoA [34,35]. Since the acyl modification is important for majority of actions of ghrelin, GOAT has emerged as a vital enzyme of interest for research. GOAT expression is tissue specific.

that ghrelin is more potent in releasing GH than the unnatural GHRP/GHSS. Ghrelin and GHRH when administered simultaneously has a synergistic effect on the secretion of GH. Synergistic effect on GH release is also observed by co-administration of GHSs and synthetic ghrelin agonists. This indicates that GHRH is essential for the release of GH. Since GH secretion is maintained in the presence of GHRH and GH-releasing activity of ghrelin *in vivo* is dependent on endogenous GHRH; the primary feed-forward regulator of GH secretion is possibly GHRH. Ghrelin can thus be best regarded as a helper and/or modulator of GHRH in the regulation of secretion of GH [40].

Effect of in-vivo and in-vitro administration of ghrelin on GH secretion: Ghrelin stimulates strong increase in circulating GH levels both *in vitro* and *in vivo* in a dose-dependent manner [41,42]. Intravenous administration of ghrelin stimulates GH release [43] which peaks at 5–15 min and returns to basal levels after 1h. Ghrelin administered intravenously is more potent than GH-releasing hormone (GHRH) in stimulating the release of GH in anaesthetised or freely moving rats, and in humans [44,45]. Intracerebroventricular injection appears to be a more potent route of delivery than intravenous administration. Together, these *in vivo* assays confirms that ghrelin is a potent GH-releasing peptide.

Ghrelin stimulates GH secretion *in vivo* as well as from both pituitary cells in culture [42]. In contrast to the *in-vivo* studies, *in vitro* studies using primary pituitary cells shows that ghrelin stimulates the release of GH, but does not alter the release of other four pituitary hormones [46], thereby suggesting that ghrelin acts directly on the somatotrophs of the pituitary. Also, in contrast to the *in vivo* studies, *in vitro* studies shows that GH-releasing activity of ghrelin is similar with or less than that induced by GHRH [47].

Observations of these *in vivo* and *in vitro* studies suggests that ghrelin is a specific endogenous ligand for the GHS receptor and provides a proof of the occurrence of a GHS–GHS receptor signalling system in the regulation of secretion of GH [48].

Therapeutic efficacy of ghrelin: Though studies have suggested that ghrelin is a powerful pharmacological agent for stimulating the release of GH due to their pharmacokinetic properties and high bioavailability following oral or parenteral administration, the physiological importance of the ghrelin in regulating GH still remains unclear. Several ghrelin analogs have been shown to be useful both as a treatment modality for GH deficiency and as an endocrine diagnostic tool. Co-administration of ghrelin with GHRH and other drugs is currently being investigated to develop novel diagnostic and therapeutic tools [49].

CONCLUSION

Ghrelin, a bioactive peptide is a natural endogenous ligand of the growth hormone secretagogue receptor 1a (GHSR1a). Under physiological conditions, ghrelin administered either centrally or peripherally, exerts a potent, time-dependent stimulation of pulsatile secretion of GH. An intact endogenous GHRH system is required for the GH response to ghrelin.

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