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Research Paper

Ghrelin Structure and It's Receptors: A Review

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ABSTRACT: *Ghrelin is a peptide hormone that consists of 28 amino acids. In December 1999, ghrelin was first reported as an endogenous ligand for the former orphan receptor growth hormone secretagogue receptor (GHS-R) 1a. Ghrelin was found to be able to activate this receptor and to stimulate growth hormone release from the pituitary in a dose dependent manner. Ghrelin has been found to be produced primarily in the stomach and then secreted into the blood stream. Within the stomach, ghrelin is produced by enteroendocrine cells in the oxyntic mucosa.*
Subsequently, it has been found that ghrelin exerts pleiotropic activity. It influences not only GH release but also food intake, function of gastrointestinal tract and cardiovascular system, sleep patterns as well as cancer proliferation. This topic review will provide an overview on the ghrelin structure ant it's receptors.

Contents

Keywords*: ghrelin, ghrelin receptor, growth hormone, orexigenic.*

I. INTRODUCTION

Ghrelin is a peptide hormone that consists of 28 amino acids. In December 1999, ghrelin was first reported as an endogenous ligand for the former orphan receptor growth hormone secretagogue receptor (GHS-R) 1a (Kojima et al., 1999). Ghrelin, which was derived from rat stomach extracts, was found to be able to activate this receptor and to stimulate growth hormone release from the pituitary in a dose dependent manner. The name ghrelin comes from this physiological effect: Ghre is the Proto-Indo-European root of the word "grow" (Kojima et al., 1999). Later ghrelin was verified to have other biological activities, such as stimulating appetite and food intake (Vartianen. 2009)**.** Ghrelin has been found to be produced primarily in the stomach and then secreted into the blood stream (Kojima et al., 2000). Within the stomach, ghrelin is produced by enteroendocrine cells in the oxyntic mucosa (figure 1) (Kojima et al., 1999; Gualillo et al., 2001).

Figure 1: Main site of ghrelin secretion.

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(a) An important source of ghrelin is the fundus region of the stomach. The oxyntic mucous contains entero-endocrine cells of different types, of which X/A cells stain most positive for ghrelin.
(b) The molecular composition of n-octanoyl-ghrelin. The octanoic acid tail is vital for receptor binding and thus for biological activity of ghrelin (Kojima *et al*., 1999).
Ghrelin in rats and humans were found to differ in only two amino acid residues, (figure 2) (Kojima *et al.,* 1999, 2005).

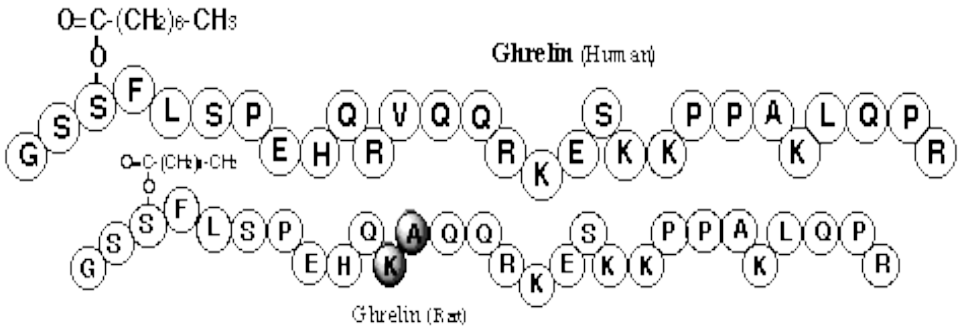

Figure2: Structure of human and rat ghrelin (Kojima *et al*. 1999).

Ghrelin exists in several mammalian, avian and fish species (Kojima *et al.,* 2005). Ghrelin is the first known example of a bioactive peptide modified by acylation (Kojima *et al.,* 2005). Two major forms of ghrelin are present in tissues and blood: des-acylated and octanoylated (Aydin *et al*., 2006a).

II. LOCALIZATION OF GHRELIN EXPRESSION

Stomach is the main source of ghrelin synthesis in all vertebrate species (Ariyasu*et al.,* 2001). Ghrelin is secreted predominantly from the gut and in particular from the X/A cells of the gastric fundus, but lower levels of ghrelin synthesis have been detected in the other areas of gastrointestinal tract, such as in duodenum, jejunum, ileum, and colon (De and Delporte., 2008). In addition, this hormone has been detected in the central nervous system, e.g. in arcuate nucleus and hypothalamus (Lu *et al.,* 2005), and interestingly in pancreas (Date *et al.,* 2002; Volante *et al.,* 2002; Wierup*et al.,* 2002). Also, some cells of the immune system, such as peripheral blood mononuclear cells (lymphocytes and monocytes) produce ghrelin (Mager*et al.,* 2008). Moreover, human ovaries and testes also are capable of secreting this peptide hormone (García*et al.,* 2007). In brief, several cells of the human body are able to produce ghrelin, though the physiological relevance of this is unknown. It could be postulated, that ghrelin might have not only endocrine but also autocrine and paracrine functions.

III. GHRELIN GENE CODES FOR TWO HORMONES

Ghrelin is synthesized as a precursor protein containing 117 amino acids, and is called prepro-ghrelin. Prepro-ghrelin is modified by the action of protein convertase enzymes into mature ghrelin, consisting of 28 amino acids and another peptide hormone, obestatin that consists of 23 amino acids and has opposite effects to ghrelin (Zhang *et al.,* 2005). After post-translational modifications, a fatty acyl group is attached to the third amino acid, serine (Ser3) of the mature ghrelin via an esteric-bond. The most common acyl group found attached on the circulating ghrelin molecules is an octanoyl group (Figure 3). The enzyme that catalyses the reaction in which the octanoateis attached to the hydroxyl group of the Ser3 was identified and is called ghrelin-*O*-acyltransferase (GOAT) (Yang *et al.,* 2008). The other product of ghrelin gene, obestatin, also goes through a post translational modification; the C-terminal glycine residue is amidated. The amidationis believed to be required for the bioactivity of obestatin (Zhang *et al.,* 2005).

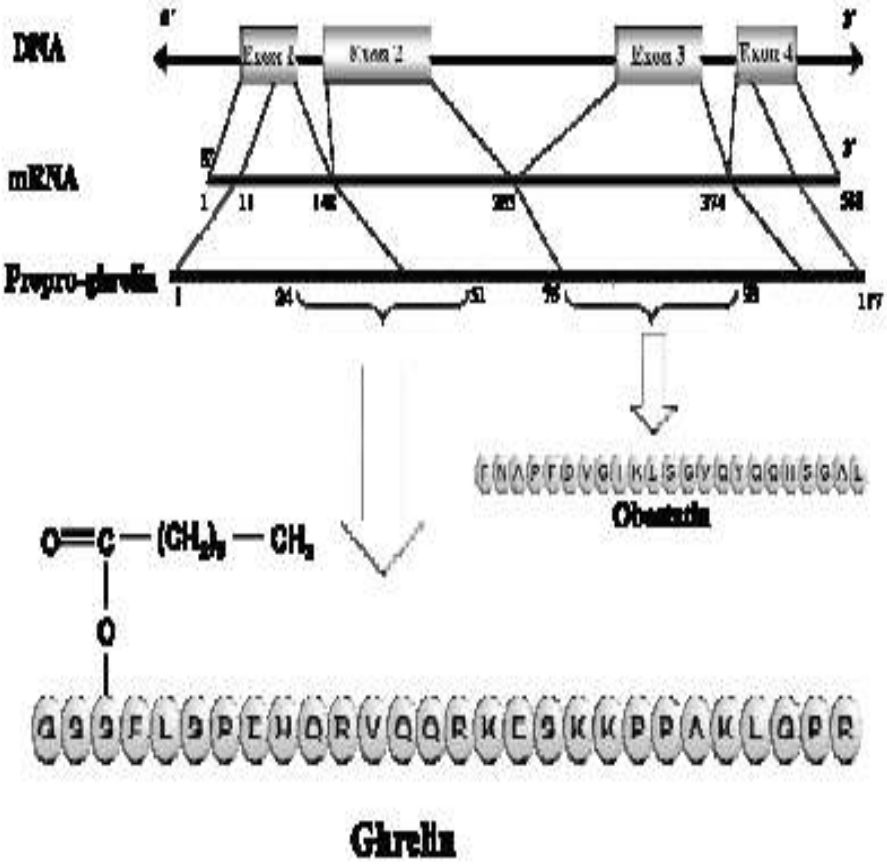

Figure 3: Ghrelin gene consists of four exons and three introns.

The DNA code is transcribed into mRNA, which contains untranslated 5´and 3´regions and coding regions. The coding regions are subsequently translated to form a 117 amino-acid chain, prepro-ghrelin. Mature ghrelin peptide is cleaved from the prepro-ghrelin and an octanoyl group is attached to its third amino acid serine. Another peptide hormone, obestatin, is also derived from the prepro-ghrelin molecule. Amino acids are shown as one letter codes (Vartianen. 2009).

IV. DIFFERENT FORMS OF GHRELIN

Recent studies have revealed that the ghrelin gene can generate a variety of bioactive molecules besides ghrelin. These include acyl forms of ghrelin (i.e., n-decanoyl ghrelin or n-decenoyl ghrelin), des-acyl ghrelin, obestatin and ghrelin-associated peptides originated from the ghrelin gene (Kojima *et al*., 2011).

4.1Desacyl ghrelin (non-acylated ghrelin) and Acylated ghrelin

Ghrelin possess a characteristic fatty acid chain on its N terminal end. Though, the majority of circulating ghrelin actually lacks this acyl group and is known as desacyl or desoctanoyl ghrelin (unacylated ghrelin (UAG) (figure 4). The UAG is the major circulating form and constitutes 80-90% of circulating ghrelin. Although it was originally thought that UAG lacked endocrine and biological actions (Kojima *et al.,* 1999); more recent findings suggest that it has physiological effects and thus it may be considered a metabolically active peptide affecting glucose and lipid metabolism and probably food intake and appears to interact with acylated ghrelin (AG) in the control of metabolism (van der Lely *et al*., 2004; Ghigo*et al*., 2005; Kojima *et at*., 2005). In vitro studies show that AG can lose its acyl side chain in a hydrolysis reaction to form UAG and thus the amount of AG decreased and the level of UAG increased as a function of time in plasma sample *in vitro*. Subsequently, UAG is degraded into small peptide fragments. The half life (T1/2) for hydrolysis is 45 minutes and that for degradation is 204 min, indicating, that UAG is more stable than AG (Rauh*et al.*, 2007).

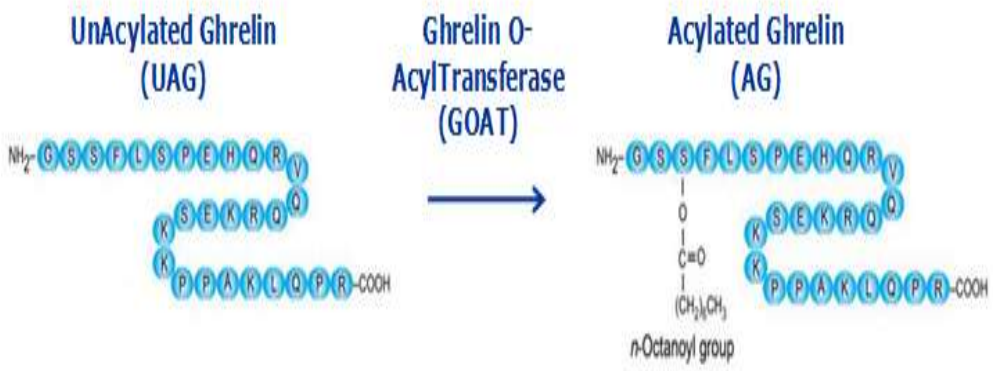

Figure 4: The acylation of UAG into AG
(is catalyzed by Ghrelin O-Acyl Transferase (GOAT) and is essential for binding to GHS-R1a).

4.2Obestatin

It was recently discovered that another peptide hormone derived from the same proprotein as ghrelin, obestatin. The 23 amino acid polypeptide obestatin was assumed to have opposite effects to those of ghrelin (Zhang *et al*., 2005; Soares and Leite-Moreira., 2008).

V. MOLECULAR CHARACTERISTICS OF GHRELIN

After its synthesis, ghrelin is stored in secretory vesicles inside the cell and released subsequently into the bloodstream where it circulates as a free molecule. One study has suggested that ghrelin might be able to interact with high density lipoprotein (HDL) particles in the circulation (Beaumont *et al.,* 2003). Moreover, another study showed that ghrelin might bind to other lipoproteins in plasma and that AG and UAG might be carried by different lipoprotein fractions (De Vriese*et al.,* 2007). Ghrelin is an unstable molecule in a blood or plasma sample. If the sample is not acidified and treated with a proteinase inhibitor, about one third is degraded in one hour at 37°C. Freezing and thawing cycles also affect ghrelin molecules, e.g. after 3 cycles, up to 40 per cent of the ghrelin molecules might be lost, according to (Hosoda*et al.,* 2004).

VI. THE GHRELIN RECEPTOR

6.1 Ghrelin receptor GHS-R1a

Identification of one receptor for ghrelin has been shown. This receptor, growth hormone secretagogue receptor type 1a (GHS-R1a) was known in fact 3 years before ghrelin itself. It was initially identified as a target for growth hormone secretagogues, synthetic molecules that stimulate growth hormone secretion from the pituitary. The discovery of this receptor led immediately to the presumption that a natural ligand for this orphan receptor must exist (Howard *et al.,* 1996). In 1999 ghrelin was shown to bind and activate this receptor and to stimulate growth hormone secretion (Kojima *et al.,* 1999).

6.2 Activation of GHS-R1a

GHS-R1a is activated by ghrelin as well as by synthetic growth hormone secretagogues (Howard *et al.,* 1996; Kojima *et al.,* 1999). This activation leads to the stimulation of the G-protein subunit Gα11, a molecule attached to the receptor in the intra-cellular space (Howard *et al.,* 1996). This causes the activation of intracellular signaling cascades via the phospholipase C (PLC) pathway. Consequently, the production of inositol triphosphate (IP3) is increased, which in turn followed by the release of calcium ions (Ca^{++}) from intracellular stores to the cytosol. PLC can alternatively lead to reactions that phosphorylate and inhibit voltage gated potassium channels on the cell membrane. Depolarization of the membrane together with the elevation in the intracellular level of Ca^{++} lead to the release of growth hormone (Smith *et al.,* 1996). GHS-1a has been shown to mediate also biological functions other than growth hormone release, and to trigger the activation of different intra-cellular cascades depending on the target cell. For example, its effects on cell proliferation are mediated via pathways that involve protein kinase C / mitogen activated protein kinase and tyrosine kinase molecules (Nanzer*et al.,* 2004). Adding to the complexity of the receptor function, GHS-R1a has been shown to have high activity in cells over expressing this receptor. This means that the receptor has basal activity, even when not activated by ghrelin or any other agonist.

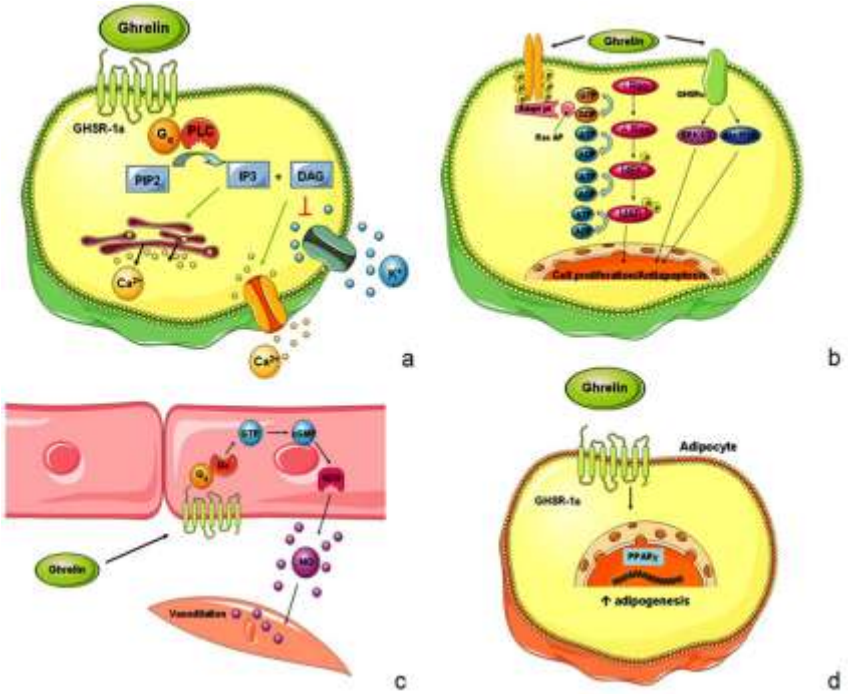

Figure 5: Intracellular pathways involved in ghrelin's effects.

Ghrelin binds to the GHSR-1a, leading to the activation of phospholipase C (PLC). This enzyme converts phosphatidylinositol bisphosphate (PIP2) into inositol triphosphate (IP3) and diacylglyceride (DAG). IP3 induces Ca2+ release from the sarcoplasmatic reticulum while DAG inhibits K+ channels. The increase of intracellular Ca2+ results in membrane depolarization. b. Ghrelin activates a tyrosine kinase receptor, which leads to the activation of the Ras protein. The double phosphorylation of the Ras protein results in the mitogen-activated protein kinase (MAPK), which needs another phosphorylation to enter the nucleus and regulate cell proliferation. c. In the endothelial cells ghrelin stimulates a G-protein-coupled system which activates the guanylate cyclase (GC). This enzyme transforms guanidil triphosphate (GTP) into cyclic guanidil monophosphate (cGMP). CGMP leads to the activation of nitric oxide synthase (NOS) which increases nitric oxide's (NO) levels. NO enters the smooth muscle cell and promotes relaxation. d. In adipocytes ghrelin binds to the GHSR-1a and stimulates the peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a transcription factor regulating genetic transcription and thereby promoting adipogenesis (Hattori 2009).

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