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## Ghrelin, a Gastric Hormone with Diverse Functions

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### 1. Introduction

Ghrelin is a 28 amino acid peptide hormone derived from the X/A like endocrine cells located in the gastric mucosa. Since its discovery more than a decade ago, about 5000 papers have been reported. The scope of these reports is broad and ranges from the gene structure, transcriptional regulation, posttranslation modification, ligand-receptor binding activities, intracellular signaling, to various biological functions such as food intake, energy balance, glucose and lipid metabolism, cardiovascular disease and immunomodulation. In this chapter, we will summarize the advance of our knowledge on this fascinating hormone, ghrelin.

### 2. The history of ghrelin discovery

#### 2.1 Development of GHS (growth hormone secretagogues)

Growth hormone (GH) was first identified for its effect on longitudinal growth. Recombinant growth hormone is therefore used as the treatment for individuals with short stature in various conditions (Chipman, 1993; Jorgensen & Christiansen, 1993). However, GH replacement therapy encounters several drawbacks such as low bioavailability and side effects upon its administration. Moreover, most growth hormone-deficient individuals exhibit a secretory defect rather than a primary deficiency in the production of growth hormone.

A synthetic hexapeptide, His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> (GHRP-6), was identified as a potent stimulator on GH release *in vitro* and *in vivo* by an unknown mechanism (Bowers et al., 1984). Because of its poor oral bioavailability and short half-life in human serum, GHRP-6 was selected only as a structural model to design non-peptide mimetics (Bowers et al., 1992). However, as a peptide, GHRP-6 did not readily lend itself to optimization of pharmacokinetic properties by medicinal chemistry. The benzodiazepine like template containing aromatic substitution was therefore discovered as potential non-peptide structure to model the GHRP-6 structure (Bowers et al., 1980). Based on the structure-activity relation, a non-peptidyl GH secretagogue L-692, 429, was identified. This non-peptidyl GH secretagogue synergizes with GHRP-6 to stimulate GH release and cAMP production (Smith et al., 1993).

In parallel with developing structure activity relationships for the benzolactams, alternative approach was proposed. It has been suggested that the derivatives of frequently occurring units was used as a useful approach to design receptor agonists and antagonists (Evans et al., 1988). These recurring structural units were termed “privileged structures” and had been recognized (Patchett et al., 1995) as hydrophobic double ring systems contributing to receptor binding of many antagonists of biogenic amines. By replacing d-Trp by *O*-benzyl-d-serine and incorporating a methanesulfonyl amido group, a new drug with excellent oral bioavailability and specificity in its release of GH without significant effect on plasma levels of other hormones was discovered and named L-163,191 (MK-0677) (Patchett et al., 1995).

## 2.2 Discovery of GHS-R

Extensive researches had been focused on the regulation of GH secretion in the 90s, due to its potential in the therapeutics of GH deficient related diseases. GH secretion is mainly regulated by GH-releasing hormone (GHRH) (Frohman & Jansson, 1986) and somatostatin (Katakami et al., 1986, which are stimulatory and inhibitory hormones respectively. Studies on GHS synthetic peptides and non-peptides which stimulate GH release indicated a distinct third pathway in addition to GHRH and somatostatin and the presence of a unique receptor (Bowers et al., 1984). Their activity was not blocked by the opiate receptor antagonist naloxone. Furthermore, these molecules were neither GHRH receptor agonists nor somatostatin receptor antagonists (Cheng et al., 1989; Blake & Smith, 1991; Cheng et al., 1991). This novel receptor was identified in porcine and rat anterior pituitary membranes using MK-0677 as a ligand. The binding of this receptor is  $Mg^{2+}$ -dependent and could be inhibited by GTP- $\gamma$ -S, indicating that it is a G-protein-coupled receptor (GPCR) (Pong et al., 1996). Activation of this GPCR by GHS results in the stimulation and amplified pulsatile release of growth hormone, and it was therefore named as GHS-R. This GHS-R defines a novel neuroendocrine pathway for the control of pulsatile GH release. The full-length sequence of this receptor was identified by using the expression-cloning strategy in *Xenopus* oocytes. Activation of GHS-R induces a transient increase in the concentration of intracellular calcium in somatotrophs (Bresson-Bepoldin & Dufy-Barbe, 1994), the increase in the level of inositol trisphosphate (IP3) (Mau et al., 1995) and activity of protein kinase-C (PKC) (Cheng et al., 1991).

## 2.3 Identification of ghrelin

Cloning of the GHS-R cDNA allows the engineering of cell lines stably expressing the GHS-R, which are essential for identification of endogenous GHS-R ligands. Through a process of reverse pharmacology, the endogenous nature ligand for GHS-R1a was identified from the gastric extracts and named as ghrelin. By detecting the intracellular calcium concentration in CHO cells expressing GHS-R1a as a functional readout, Kojima *et al.* screened several tissue extracts from brain, lung, heart, kidney, stomach and intestine, and unexpectedly discovered the active agonist for the GHS-R1a in the stomach rather than in the brain (Kojima et al., 1999). The active component was purified by successive chromatography including gel filtration, ion-exchange high-performance liquid chromatography (HPLC) and reverse-phase HPLC (RP-HPLC) (Kojima et al., 1999). Its amino acid sequence in rat was finally determined as GSSFLSPEHQKAQQRKESKKPPAKLQPR in which the serine-3 (Ser<sup>3</sup>) is n-octanoylated. This octanoylation is necessary for its binding and activation of the GHS-

R. This 28 amino acid peptide was named ghrelin based on the word root “ghre” in proto-indo-european language for “growth” to depict its ability of stimulating the release of growth hormone (Kojima et al., 1999). Ghrelin is the only known peptide modified by a fatty acid, and it shares no structure homology with growth hormone secretagogues. Human ghrelin was cloned using the primers based on rat ghrelin cDNA under low stringency condition. Only two amino acids are different between human and rat preproghrelin (Kojima et al., 1999), indicating that ghrelin is highly conserved between species.

### 3. The gene structure of ghrelin

#### 3.1 Ghrelin gene

Localized on the chromosome 3p25–26, the human ghrelin gene comprises five exons, same as the mouse gene (Tanaka et al., 2001a; Kanamoto et al., 2004). The short first exon contains only 20 base pairs, which encode part of the 5'-untranslated region. There are two different transcriptional initiation sites in the ghrelin gene; one occurs at -80 and the other at -555 relative to the ATG initiation codon, resulting in two distinct mRNA transcripts (transcript-A and transcript-B) (Kanamoto et al., 2004).

The 5'-flanking region of the human ghrelin gene contains a TATA box-like sequence (TATATAA; -585 to -579), as well as putative binding sites for several transcription factors such as AP2, basic helix-loop-helix (bHLH), hepatocyte nuclear factor-5, NF- $\kappa$ B, and half-sites for estrogen and glucocorticoid response elements (Tanaka et al., 2001a; Kishimoto et al., 2003; Kanamoto et al., 2004). Neither a typical GC nor a CAAT box was found.

#### 3.2 Ghrelin gene-derived transcripts

The 28 amino acids of the functional ghrelin peptide are encoded by ghrelin gene which consists of 4 introns and 5 exons, including a non-coded exon 1. In rat, mouse and pig, the codon for Gln<sup>14</sup> (CAG) is used as an alternative splicing signal to create two different ghrelin mRNAs (Hosoda et al., 2000b). One encodes the ghrelin precursor, and another encodes des-Gln<sup>14</sup>-ghrelin precursor: a spliced variant without the codon for glutamine in position 14 (pre-pro-des-Gln<sup>14</sup>- ghrelin mRNA) (Hosoda et al., 2003). Des-Gln<sup>14</sup>-ghrelin is identical to ghrelin, except for the deletion of Gln<sup>14</sup>. Nearly all of the cDNA clones isolated from human stomach encodes the pre-pro-ghrelin mRNA, only a few cDNA clones encodes the pre-pro-des-Gln<sup>14</sup>-ghrelin mRNA (Hosoda et al., 2003).

Another splicing variant was detected in the mouse testis (Tanaka et al., 2001b). This variant, a ghrelin gene-derived transcript (GGDT), comprises the 68-bp 5'-unique sequence located between exons 3 and 4 of the ghrelin gene, and the remaining 252 bp sequence identical to the exons 4 and 5 of mouse ghrelin gene. GGDT encodes a 54 amino acid peptide consisting of 12 amino acid residues which is unrelated to the ghrelin gene and the COOH-terminal 42-amino acid sequence of mouse ghrelin precursor. The function of this variant is not clear.

In addition, a human exon 3-deleted mRNA transcript has been described in breast and prostate cancer (Yeh et al., 2005). The murine homologue (exon 4-deleted mRNA transcript) has also been identified in comprehensive murine tissues (Jeffery et al., 2005). These variants are likely products of the ghrelin gene attributed to alternative splicing mechanisms.

#### 4. Posttranslational modification of ghrelin

*In vitro* and *in vivo* studies have demonstrated that the prohormone convertase 1/3 (PC1/3) is the only identified enzyme responsible for processing of proghrelin into ghrelin (Zhu et al., 2006). This endoprotease co-localizes with ghrelin in gastric endocrine cells and processes the 94 amino acids human ghrelin precursor into the 28 amino acids mature ghrelin through limited proteolytic cleavage (Ozawa et al., 2007).

Octanoylation of the third amino acid Serine is the most unique posttranslational modification of ghrelin. Ghrelin is the only protein currently known to be octanoylated. This octanoylation is essential for binding of ghrelin with its receptor, GHS-R1a. The enzyme that catalyzes the octanoylation of ghrelin was identified by two individual study groups and designated as ghrelin O-acyltransferase (GOAT) in 2008 (Gutierrez et al., 2008; Yang et al., 2008a). GOAT is a member of the family membrane-bound O-acyltransferases, with its structure conserved among different species. Transcripts for both GOAT and ghrelin are present predominantly in stomach and pancreas. Genetic disruption of the GOAT gene in mice leads to complete absence of octanoylated ghrelin in circulation. The design of GOAT inhibitors may therefore provide a new approach for the treatment of obesity and diabetes. *In vitro* study has demonstrated that GOAT activity could be significantly inhibited by an octanoylated ghrelin pentapeptide and other end-products (Yang et al., 2008b), suggesting the existence of a negative feedback regulation. In addition to the octanoylation, different acylation by other fatty acid has been reported. Studies have revealed some naturally occurring variants of ghrelin such as decanoyl ghrelin based on the different acylation on the serine-3 residue, which exhibit physiological function similar to octanoylated ghrelin (Ghigo et al., 2005).

In addition, ghrelin has been reported to be phosphorylated by protein kinase C  $\alpha$ ,  $\beta$ , and  $\delta$  at serine-18 residue which affects the secondary structure and membrane binding property of ghrelin (Dehlin et al., 2008). The intracellular function of phosphorylated ghrelin remains unknown, but the phosphorylation may associate with subcellular localization of ghrelin, especially des-acyl ghrelin.

#### 5. Ghrelin family members

The preproghrelin, encoded by ghrelin mRNA transcript A, contains a 23 amino-acid signal peptide and a 94 amino-acid proghrelin (1-94). The latter includes the 28 amino acid mature ghrelin (1-28) and a 66 amino acid tail (29-94) (Kojima et al., 1999; Jeffery et al., 2005). This proghrelin can be cleaved by prohormone convertase 1/3 (PC1/3) in mouse gastric mucosa to generate bona fide ghrelin *in vivo* (Zhu et al., 2006). Different products of ghrelin gene have been generated by alternative splicing and post-translational modification.

##### 5.1 N-octanoyl ghrelin

Ghrelin was purified from the rat stomach through a series of steps of chromatography: gel filtration, two ion-exchange HPLC steps, and a final reverse-phase HPLC (RP-HPLC) procedure. The second ion-exchange HPLC yielded two active peaks, from which ghrelin and des-Gln<sup>14</sup>-ghrelin were purified, respectively (Hosoda et al., 2000b). Analysis of ghrelin

revealed the unique structure of a 28-amino acid peptide with its third amino acid Serine *n*-octanoylated. This modification is essential for ghrelin's activity for binding with GHSR1a. It is the first known case of a peptide hormone modified by a fatty acid. The sequence of ghrelin is highly conserved between species. Only two amino acid residues are different between rat and human (Kojima et al., 1999). There is no structural homology between ghrelin and peptide GHSs such as GHRP-6 or hexarelin.

### 5.2 Des-Gln<sup>14</sup>-ghrelin

A second type of ghrelin peptide has been purified from rat stomach and identified as des-Gln<sup>14</sup>-ghrelin (Hosoda et al., 2000b). Except for the deficiency of Gln<sup>14</sup>, des-Gln<sup>14</sup>-ghrelin is identical to ghrelin, with the same *n*-octanoic acid modification and potency of activities. The deletion of Gln<sup>14</sup> attributes to the CAG codon encoding Gln, which is a splicing signal. Analysis of ghrelin gene structure reveals that an intron exists between Gln<sup>13</sup> and Gln<sup>14</sup> of the ghrelin sequence. The 3'-end of the intron has two tandem CAG sequences. The AGs of these sequences may serve as the splicing signals (McKeown, 1992). When the first AG is used as the splicing signal, prepro-ghrelin mRNA is produced and the second CAG is translated into Gln<sup>14</sup>. On the other hand, when the second AG is used, prepro-des-Gln<sup>14</sup>-ghrelin mRNA is generated to produce des-Gln<sup>14</sup>-ghrelin missing Gln<sup>14</sup> (Hosoda et al., 2000b). These findings confirm that des-Gln<sup>14</sup>-ghrelin is processed from the ghrelin gene by alternative splicing rather than a distinct genomic product from ghrelin. Des-Gln<sup>14</sup>-ghrelin is present in low amounts in the stomach, indicating that ghrelin is the major functional form.

### 5.3 Des-acyl ghrelin

Des-acyl ghrelin, the nonacylated form of ghrelin, also exists at significant levels in both stomach and blood (Hosoda et al., 2000a). In blood, des-acyl ghrelin circulates in amount far greater than acylated ghrelin. The clearance rates of inactive forms of peptide hormones are often reduced compared with their respective active forms. Ghrelin in the plasma binds to high-density lipoproteins (HDLs) that contain a plasma esterase, paraoxonase, and clusterin (Beaumont et al., 2003). Because a fatty acid is attached to the third amino acid Serine of ghrelin via an ester bond, paraoxonase may be involved in de-acylation of acyl-modified ghrelin. Thus parts of des-acyl ghrelin may originate from the de-acylation of acyl ghrelin.

Des-acyl ghrelin does not compete the binding sites of GHS-R1a with acyl ghrelin in hypothalamus and pituitary. Consistent with this finding, des-acyl ghrelin shows no GH-releasing and other endocrine activities associated with activation of GHS-R1a. However, des-acyl ghrelin shares with active acyl-ghrelin some non-endocrine actions, including the modulation of cell proliferation and adipogenesis (Cassoni et al., 2004). Emerging evidences suggest the existence of a distinct receptor other than GHS-R1a, which des-acyl ghrelin may bind with. Des-acyl ghrelin has been reported to either stimulate (Toshinai et al., 2006) or inhibit (Asakawa et al., 2005; Matsuda et al., 2006) food intake in a GHS-R1a independent pathway. It has been showed that ghrelin and des-acyl ghrelin both recognize common high-affinity binding sites on H9c2 cardiomyocytes (Baldanzi et al., 2002), which do not express the classical ghrelin receptor GHS-R1a, suggesting the existence of a novel ghrelin receptor subtype in the cardiovascular system.

## 5.4 Obestatin

Recent evidence suggests that the 66 amino acid tail of proghrelin (29–94) can either circulate as a full-length peptide (C-ghrelin) or be processed to smaller peptides, mainly obestatin (11–23). Obestatin was proposed as a novel peptide derived from C terminal of proghrelin (Zhang et al., 2005). This new peptide was named ‘obestatin’ based on its appetite-suppressing potential. The structure of obestatin was deduced as a 23 amino acid sequence of proghrelin 53–75 according to mass spectrometric analysis. Because of the C-terminal Gly–Lys motif, amidation of obestatin was assumed and confirmed as a prerequisite for its biological activity (Zhang et al., 2005). Detail mechanism on how this peptide is processed to a 23 amino acid peptide remains to be explored. Obestatin was originally extracted from rat stomach and has subsequently been shown to be an active circulating peptide (Zizzari et al., 2007; Harada et al., 2008). However, it had been found that no evidence for obestatin peptide circulating as distinct entity in the human and rat blood (Bang et al., 2007). Specific immunoassays directed to the N-terminus, C-terminus, and mid-region of proghrelin (29–94) all detect the same molecule of Mr ~7kDa, suggesting that the only form present in circulation is close to the full length 66- amino acid C-ghrelin (Bang et al., 2007). Further studies are necessary to clarify this issue.

## 5.5 Other ghrelin forms

Several other minor forms of the ghrelin peptides were isolated in the purification of human ghrelin from the stomach (Hosoda et al., 2003). These peptides could be classified into four groups by the type of acylation at the third amino acid Serine: nonacylated, octanoylated (C8:0), decanoylated (C10:0), and decenoylated (C10:1). Two kinds of different length peptides, either 27 or 28 amino acids, were found. The 27 amino acid peptide lacks the C terminal Arg28 and derives from the same ghrelin precursor. Both synthetic octanoylated and decanoylated ghrelins increase the concentration of intracellular Ca<sup>2+</sup> in GHS-R-expressing cells and stimulate GH release in rats to a similar degree.

The expression of a novel human mRNA transcript designated as exon 3-deleted mRNA transcript has been reported in breast and prostate cancer (Yeh et al., 2005). A murine homologue, exon 4-deleted mRNA transcript, has also been identified in a widely range of tissues (Jeffery et al., 2005). Deletion of exon 3 from the preproghrelin transcript results in a truncated C-peptide with a unique 16-amino-acid peptide tail, COOH-terminal D3 peptide, which begins with a potential peptide cleavage site [Arg–Arg]. Previous studies indicate that this unique COOH-terminal D3 peptide (RPQPTSDRPQALLTSL) does not affect cell proliferation or cell apoptosis in prostate cancer cell lines. However, the comprehensive expression of this C-ghrelin suggests that it may possess some unidentified functions.

## 6. Tissue distribution of ghrelin

### 6.1 Stomach and other gastrointestinal organs

In all vertebrate species, stomach is the primary organ producing ghrelin (Ariyasu et al., 2001). Ghrelin immune-reactive cells are more abundant in the fundus than in the pylorus (Date et al., 2000; Tomasetto et al., 2001; Yabuki et al., 2004). In situ hybridization and immunohistochemical analysis indicate that ghrelin-containing cells are a distinct endocrine cell type found in the gastric mucosa (Date et al., 2000; Rindi et al., 2002). Four types of

endocrine cells have been identified in the oxyntic mucosa: ECL cells, D cells, enterochromaffin (EC) cells, and X/A-like cells (Capella et al., 1969; Solcia et al., 1975; Grube & Forssmann, 1979). The granule contents of X/A-like cells had not been identified until the discovery of ghrelin. The X/A-like cells contain round, compact, electron dense granules that are filled with ghrelin (Date et al., 2000; Dornonville de la Cour et al., 2001; Yabuki et al., 2004). The X/A-like cells account for about 20% of the endocrine cell population in adult oxyntic glands and can be stained by an antibody specific to the NH<sub>2</sub>-terminal, acylated portion of ghrelin, indicating that ghrelin in the secretory granules of X/A-like cells has already been acylated. However, the number of X/A-like cells and ghrelin concentration in the fetal stomach is very low and gradually increases after birth (Hayashida et al., 2002).

Ghrelin-immunoreactive cells are also found in the duodenum, jejunum, ileum, and colon (Date et al., 2000; Hosoda et al., 2000a; Sakata et al., 2002), among which ghrelin concentration gradually decreases from the duodenum to the colon. The pancreas is another ghrelin-producing organ especially during the embryo development and the production of ghrelin decreases rapidly after birth. The cell type that produces ghrelin in the pancreatic islets remains controversial, whether it might be the  $\alpha$  cells,  $\beta$  cells, newly identified islet epsilon ( $\epsilon$ ) cells, or an unknown novel type of islet cells (Date et al., 2002; Wierup et al., 2002; Prado et al., 2004; Wierup et al., 2004).

## 6.2 Central nervous system – Hypothalamus

Ghrelin is the endogenous ligand for GHS-R which is mainly expressed in the hypothalamus and pituitary (Howard et al., 1996; Guan et al., 1997). Despite that the content of ghrelin is very low (Kojima et al., 1999; Hosoda et al., 2000a), ghrelin immunoreactivity has been detected in the hypothalamic arcuate nucleus, an important region for controlling appetite (Kojima et al., 1999; Lu et al., 2002). In addition, ghrelin immunoreactive cell bodies in the hypothalamus are distributed in a continuum filling the internuclear space between the lateral hypothalamus (LH), arcuate (ARC), ventromedial (VMH), dorsomedial (DMH), and paraventricular hypothalamic nuclei (PVH) and the ependymal layer of the third ventricle (Cowley et al., 2003). These ghrelin-containing axons innervate neurons that contain neuropeptide Y (NPY) and agouti-related protein (AgRP) and may stimulate the release of these orexigenic peptides. These histological findings are consistent with the functional studies in which injection of ghrelin into the cerebral ventricles of rats potently stimulates food intake.

Ghrelin has also been found in the pituitary gland (Korbonits et al., 2001a; Korbonits et al., 2001b), where it may influence the release of GH in an autocrine or paracrine manner. Stimulation of primary pituitary cells with ghrelin increases their intracellular Ca<sup>2+</sup> concentration (Bennett et al., 1997; Guan et al., 1997; McKee et al., 1997a). The expression level of ghrelin in the pituitary is high in the neonates and declines with puberty. Pituitary tumors, such as adenomas, corticotroph tumors, and gonadotroph tumors contain ghrelin peptides as well.

## 6.3 Other tissues

Two major forms of ghrelin: *n*-octanoyl and des-acyl ghrelin, are found in circulation (Hosoda et al., 2000a). The concentrations of *n*-octanoyl ghrelin and total ghrelin in human



plasma are 10–20 fmol/ml and 100–150 fmol/ml respectively. Plasma ghrelin concentration increases in fasting condition and reduces after habitual feeding (Cummings et al., 2001; Tschop et al., 2001a). In addition, plasma ghrelin level is lower in obese subjects than the age matched lean controls (Tschop et al., 2001b; Hansen et al., 2002; Shiiya et al., 2002).

Ghrelin mRNA is also detected in the kidney, especially in the glomeruli (Mori et al., 2000; Gnanapavan et al., 2002). Moreover, peptide extracts from mouse kidney contain both *n*-octanoyl and des-acyl ghrelin. Significant relationship between plasma ghrelin concentration and the serum creatinine level has been demonstrated. In patients with end-stage renal disease, plasma ghrelin level increases 2.8-fold compared with those with normal renal function (Yoshimoto et al., 2002). This finding suggests that the kidney is an important organ for clearance and/or degradation of ghrelin.

In the reproductive system, ghrelin immune-reactive cells have been identified in interstitial Leydig cells and at a low level in Sertoli cells (Barreiro et al., 2002; Tena-Sempere et al., 2002). The ghrelin receptor has also been detected in germ cells, mainly in pachytene spermatocytes, as well as in somatic Sertoli and Leydig cells (Gaytan et al., 2004). During embryo development, ghrelin-immunoreactive cells are detectable in cytotrophoblast cells in first-trimester human placenta but disappear in third-trimester placenta (Gualillo et al., 2001). Ghrelin-containing cells are also detected in syncytiotrophoblast cells of the human placenta and in the cytoplasm of labyrinth trophoblasts of the rat placenta.

#### 6.4 Ghrelin producing cell lines

Ghrelin can be detected in several cultured cell lines. One of which is TT cells, a human thyroid medullary carcinoma cell line (Kanamoto et al., 2001). TT cells express ghrelin mRNA. Conditioned medium and cellular extracts of TT cells contain both *n*-octanoyl ghrelin and des-acyl ghrelin. Other cultured cells such as the kidney-derived cell line NRK-49F (Mori et al., 2000), gastric carcinoid ECC10 cells (Kishimoto et al., 2003), and the cardiomyocyte cell line HL-1 (Iglesias et al., 2004) express ghrelin as well.

Some patients with neuroendocrine tumors also accompany with high level of ghrelin in plasma. It had been reported that a patient with a malignant neuroendocrine pancreatic tumor with ghrelin immunoreactivity and a high circulating ghrelin level (Corbetta et al., 2003). A patient with a metastasizing gastric neuroendocrine tumor has also been reported to have extremely high circulating level of ghrelin (Tsolakis et al., 2004). In the latter case, the patient developed diabetes mellitus and hypothyroidism. In both cases, GH and IGF-I levels were within the normal range, and the patients had no clinical features of acromegaly.

### 7. Regulation of ghrelin biosynthesis

In the 5'-flanking region of the ghrelin gene, several E-box consensus sequences exist (Kanamoto et al., 2004). Destruction or site-directed mutagenesis of these sites decreases the promoter activity in TT cells. Upstream stimulatory factors (USF), members of the bHLH-LZ family of transcription factors, bind to these E-box elements and may thus modulate the expression of ghrelin gene. Ghrelin promoter activity in ECC10 cells is up-regulated by glucagon and its second messenger cAMP (Kishimoto et al., 2003), suggesting that a high level of ghrelin production may be related to increased glucagon in fasting condition.

The biosynthesis of ghrelin is tightly linked with the energy status and regulated by different nutritional ingredients. Fasting increases ghrelin, des-acyl ghrelin and C-ghrelin, while demonstrates no effect on obestatin (Zhang et al., 2005; Bang et al., 2007). Conversely, feeding decreases ghrelin, des-acyl ghrelin and C-ghrelin (Zhang et al., 2005; Bang et al., 2007). Postprandial acylated ghrelin level falls more quickly than total ghrelin. The postprandial decrease of ghrelin can be attributed mainly to the increase of the serum glucose concentration, because the total ghrelin, acyl ghrelin, and des-acyl ghrelin all decrease significantly after a high-carbohydrate meal (Sedlackova et al., 2008). Other nutrients, such as high-fat meal, induces minor (Monteleone et al., 2003) but more persistent (Erdmann et al., 2003; Foster-Schubert et al., 2008) post-prandial suppression in circulating total ghrelin than high-carbohydrate meal in humans. Ingestion of either medium-chain fatty acids (MCFAs, such as n-octanoic acid) or medium-chain triglycerides (MCTs, such as glyceryl trioctanoate) increases the stomach contents of acyl ghrelin without changing the total ghrelin in mouse (Nishi et al., 2005). This finding suggests that medium-chain fatty acids can be utilized directly for the acyl modification of ghrelin. Oral ingestion of a physiological dose of essential amino acids leads to a continuous rise in serum ghrelin level in humans (Groschl et al., 2003; Knerr et al., 2003). Insoluble dietary fiber ingestion may influence ghrelin level as well (Gruendel et al., 2007).

Chronic energy imbalance such as obesity or anorexia also alters ghrelin production. Body mass index in human is negatively correlated with the production of ghrelin. Plasma ghrelin concentration is low and post-prandial decrease in ghrelin is attenuated in the obese individuals (Erdmann et al., 2005). Patients with anorexia nervosa have significant increase in serum total and acyl ghrelin levels (Harada et al., 2008), which return to normal when the disease is cured and the body weight restored (Otto et al., 2001). Furthermore, total ghrelin level is inversely associated with fat cell volume (Purnell et al., 2003) and specifically in women with total fat mass and fat mass/lean mass ratio, whereas in men with abdominal fat mass and fat distribution index (Makovey et al., 2007).

There may also be a developmental regulation of ghrelin gene-derived peptides expression. It has been showed that plasma total ghrelin concentration decreases abruptly after birth, contrasting with a 3-fold increase in the concentration of acylated ghrelin (Chanoine & Wong, 2004).

Little has been known on the intracellular mechanism by which ghrelin expression is regulated. Our studies suggest that gastric mammalian target of rapamycin (mTOR) activity is critical for the modulation of ghrelin production (Xu et al., 2009; Xu et al., 2010).

## 8. Ghrelin receptors and their distribution

The GHS receptor gene is located on chromosome 3q26.2 and encodes for two transcripts, 1a and 1b which encode a full-length receptor GHS-R1a and a shortened version GHS-R1b respectively (McKee et al., 1997a). Type 1a encodes a 366-amino acid polypeptide containing all seven TM (transmembrane) domains, while type 1b encodes a truncated 289-amino acid polypeptide with only five TM domains. Both sequences are identical from the Met translation site to Leu at the 265th amino acid position (Howard et al., 1996).

The GHS-R1a is comprehensively distributed in a variety of tissues and has high affinity with ghrelin and GHSs. GHS-R1b also has a widespread expression but does not compete

with GHS-R1a to bind ghrelin or synthetic GHS (Gnanapavan et al., 2002) and its function remains controversial. GHS-R1a is highly expressed in the hypothalamus and pituitary, consistent with ghrelin's functional role in the control of appetite and growth hormone release.

### 8.1 Type 1a GHS receptor

The GHS-R1a gene contains a single, ~2kb intron separating two exons and encodes a GPCR with seven transmembrane (TM) domains (McKee et al., 1997b). Its ligand activation domains are in the second TM domain (TM2) and the third TM domain (TM3). These key amino acid residues are essential for binding and activation by different ligands, and have been evolutionarily conserved for 400 million years, highlighting its importance in fundamental physiological processes (Palyha et al., 2000). GHS-R1a mRNA is detected at 18 and 31 weeks of gestation, indicating that ghrelin might be active early in fetal development (Hayashida et al., 2002; Nakahara et al., 2006). Comparison of the predicted human rat, pig and sheep GHS-R1a amino acid sequences reveals 91.8–95.6% sequence homology (Palyha et al., 2000).

#### 8.1.1 GHS-R1a structure

The human GHS-R1a consists of 366 amino acids with a molecular mass of approximately 41 kDa (Howard et al., 1996; Schwartz et al., 2006) and belongs to family A of G-protein-coupled receptors (GPCRs) (Bockaert & Pin, 1999). It spans the membrane with seven  $\alpha$ -helix hydrophobic domains which joint each other by three intra- and extracellular domains, beginning with an extracellular N-terminal domain and ending with an intracellular C-terminal domain (Bockaert & Pin, 1999). The N-terminal domain forms a  $\beta$ -hairpin structure and the TM domains a round calyx-like structure which is attributed to Pro residues in the center of the TM helices. TM3 occupies the central position among the TM segments and TM5 is the most peripheral (Pedretti et al., 2006).

GHS-R1a possesses three conserved residues Glu-Arg-Tyr at the intracellular end of transmembrane 3 (TM3) domain in position 140–142 (ERY/DRY motif), which are important for the isomerization between the active and inactive conformation, and two cysteine (Cys116 and Cys198) residues on the extracellular loop 1 and 2 respectively forming a disulfide bond (Bockaert & Pin, 1999; Petersenn, 2002). Mutagenesis of TM sites reveals much of the functional domains within the GHS-R1a. Amino acids like E124, D99, and R102 of TM2 and TM3 contribute to the ligand activation, while E124, F119, and Q120 of TM3 mediate binding activity (Palyha et al., 2000). Consistent with this finding, investigation using antibody-binding studies directed at different regions of the ghrelin receptor, has also identified the amino acid residue E124 as an important binding site for GHSs, mediating a salt bridge interaction with basic moieties that appear to act independent of receptor activation (Feighner et al., 1998). Meanwhile, the importance of the hydrophobic octanoyl moiety attached to ghrelin's serine-3 residue to GHS-R1a binding has been demonstrated (Bednarek et al., 2000), and an 'active core' sequence of Gly-Ser-Ser (octanoyl)-Phe essential for receptor activation has been identified.

#### 8.1.2 Ligand binding domains

GHS-R1a transduces information provided by both ghrelin and GHSs. This concept has been explained based on the existence of a common binding domain as demonstrated by

molecular modeling and site-directed mutagenesis studies (Feighner et al., 1998). The binding domain should allow a conserved structure of agonists to recognize a complementary binding pocket that accommodates the variable part of the ligand overlapping in the agonist binding site (Bondensgaard et al., 2004). This domain is imposed by the orientation of transmembrane segments that determine the stereo- and geometric specificity of the ligand's entry and binding to the transmembrane core.

The binding domain of GHS-R1a involves six amino acids located in TM3, TM6 or TM7 (Holst et al., 2006). In addition, binding of the natural ligand ghrelin with GHS-R1a requires the ligand to interact with one pocket formed by polar amino acids in TM2/TM3 and one formed by non-polar amino acids in TM5/TM6, respectively (Pedretti et al., 2006). The synthetic peptidyl and non-peptidyl GHSs share a common binding pocket in the TM3 region of the GHS-R1a, although there are other distinct binding sites in the receptor that seem to be selective for particular classes of agonists (Feighner et al., 1998). While the main binding pocket for small amines is deep in the pocket created by the TM domains, small peptides bind to extracellular epitopes as well. The basic amine common to peptidyl (GHRP-6) and non-peptidyl (MK-0677) GHS establishes an electrostatic interaction with Glu<sup>124</sup> in the TM3 domain (Feighner et al., 1998). Substitution of glutamine for glutamic acid [Glu<sup>124</sup>Gln mutant] in human GHS-R1a inactivates its function. Furthermore, mutation of Arg<sup>283</sup> in TM6, which interacts with Glu<sup>124</sup>, abolishes both agonist-induced activation and constitutive signaling (Feighner et al., 1998; Holst et al., 2003). In addition, disruption of the disulfide bond between Cys<sup>116</sup> and Cys<sup>198</sup> in the extracellular portion of the receptor by mutating Cys<sup>116</sup> into alanine [Cys<sup>116</sup>Ala mutant] completely abolishes the activity of all agonists (Feighner et al., 1998; Palyha et al., 2000).

### 8.1.3 Constitutive activity

Evidences on the physiological relevance of GHS-R1a constitutive activity are steadily emerging. The molecular mechanism of such constitutive activity relates to three aromatic residues located in TM6 and TM7, namely PheVI:16, PheVII:06 and PheVII:09. These residues promote the formation of a hydrophobic core between helices 6 and 7 to ensure proper docking of the extracellular end of TM7 into TM6 (Holst et al., 2004). Phe<sup>279</sup>Leu and Ala<sup>204</sup>Glu polymorphisms have been reported to be associated with short stature and obesity respectively (Wang et al., 2004), indicating that constitutive activity of GHS-R1a *in vivo* might be imperative for normal growth and development of the human body (Holst & Schwartz, 2006). The Phe<sup>279</sup> mutation is a conservative amino acid exchange in the sixth transmembrane domain of GHS-R1a, and the Ala<sup>204</sup> is located in the second extracellular loop. Both amino acid residues are highly conserved (Wang et al., 2004).

When transfected to HEK-293 cells, the derived GHS-R1a receptor displays reduced basal activity and lower expression at the plasma membrane (Pantel et al., 2006), although responsiveness to ghrelin is intact (Holst & Schwartz, 2006; Pantel et al., 2006). In addition, GHS-R1a transfection leads to constitutively stimulated CRE (cAMP-responsive element) activity in HEK-293 cells (Holst et al., 2003). When overexpressed in COS-7 cells, GHS-R1a possesses a constitutive activity in the turnover of IP<sub>3</sub>, which is approximately 50% of the maximal agonist induced activity (Holst et al., 2003; Holst et al., 2006). However, GHS-R1a does not show any constitutive activity in the pituitary cell line RC-4B/ C40, indicating that GHS-R1a constitutive activity may depend on the cellular context (Falls et al., 2006). The

only known ligand blocking GHS-R1a constitutive activity is D-Arg<sup>1</sup>-D-Phe<sup>5</sup>-D-Trp<sup>7,9</sup>-Leu<sup>11</sup>-substance P (Holst et al., 2003; Holst et al., 2006).

#### 8.1.4 Endocytosis of GHS-R1a

Kinetic studies of GHS-R1a internalization based on radioligand binding experiments and confocal microscopy have demonstrated that GHSR-1a is internalized in a time dependent manner, which peaks at approximately 20 min after ghrelin stimulation. The ghrelin- GHSR-1a complex is internalized principally by a clathrin-mediated mechanism and through the endosomal trafficking pathway (Camina et al., 2004). Once the ligand-receptor complex is internalized into vesicles, GHSR-1a is sorted into endosomes to be recycled back to membrane. About 360 min after agonist removal, the level of GHS-R1a receptors on the cell surface rises close to 100% of its original level. The process is not affected by cycloheximide, suggesting that GHS-R1a originates from endosomes rather than de novo receptor synthesis. This notion is further demonstrated by the observation that fluorescence associated with GHS-R1a-EGFP in CHO cells reappears on the membrane in the similar kinetics after a ghrelin pulse. Moreover, fluorescence emitted by the EGFP-labeled GHS-R1a in cells co-localizes with the early endosomal protein 1 but not with cathepsin (lysosomal marker), indicating that GHS-R1a is not targeted to lysosomal compartments. Thus, most of GHS-R1a appearing at the cell surface originates from endocytosed receptors, leading to the complete restoration of binding capacity and functionality.

Function experiments also demonstrate the theory of GHS-R1a recycling. GH response to twice consecutive pulses of ghrelin is blunted when pulses are separated by short interval (60 min), but it restores the initial amplitude when the second pulse is administered after 180, 240 or 360 min, which fits well with the kinetics of receptor recycling. The slower recycling (3–6 h) of this receptor compared to other GPCRs is determined by the stability of the complex GHSR-1a/ $\beta$ -arrestin during clathrin-mediated endocytosis because this complex appears to dictate the profile of the receptor re-sensitization (Luttrell & Lefkowitz, 2002).

In the normal healthy organism, ghrelin receptor desensitization and endocytosis govern the ability of cells to respond to ghrelin and thereby regulate intracellular signaling to avoid a permanent stimulation of target cells. Moreover, receptor re-sensitization determines the frequency of the response to ghrelin. Deficiencies in this attenuation system may lead to an uncontrolled or defective stimulation of target cells, which causes alteration in its intracellular signaling and subsequent pathological changes.

#### 8.2 Non-type 1a GHS receptors

Remarkable differences in the binding profile among ghrelin, synthetic peptidyl (hexarelin) and non-peptidyl (MK-0677) GHSs have been reported (Ong et al., 1998a; Ong et al., 1998b; Papotti et al., 2000; Holst et al., 2004) , suggesting the presence of a novel unidentified receptor subtype in tissues that do not express GHS-R1a or express the receptor at a low level. The existence of various GHS-R1a homologues, the lack of a definite phenotype in GHS-R1a knockout mice as well as the presence of multiple endogenous ghrelin-like ligands, indicate the existence of multiple receptors for ghrelin and GHSs.

### 8.2.1 Other GPCR homologues of GHS-R1a

Based on its peptide sequence and intracellular signaling, GHS-R1a is often included in a small family of receptors for small polypeptides such as the receptor for motilin (52% homology), neurotensin receptor-1 (NTS-R1) and NTS-R2 subtype (33–35% homology), neuromedin U receptor-1 (NMU-R1) and NMU-R2 subtype (~ 30% homology), and the orphan receptor GPR39 (27–32% homology) (McKee et al., 1997b; Tan et al., 1998).

GHS-R1a shares the highest homology with GPR38, the receptor for motilin (McKee et al., 1997b; Petersenn, 2002). GPR38 is mostly distributed in the thyroid gland, bone marrow, stomach and gastrointestinal smooth muscles, and less widely expressed in the neuroendocrine tissues than GHS-R1a. Both GPR38 and GHS-R1a receptors mediate the pulsatile biological effects upon continuous stimulation and increase gastrointestinal motility. In response to motilin, GPR38 couples with Gαq/Gα13, increases cytosolic free Ca<sup>2+</sup> by an IP<sub>3</sub>-dependent Ca<sup>2+</sup> release mechanism (Huang et al., 2005).

Another group of GPCR homologues of GHS-R1a are NTS receptor, namely NTS-R1 and NTS-R2. NTS is a 13-amino-acid polypeptide, which has high sequence homology with neuromedin N. In the CNS, NTS-Rs have been identified in the hypothalamus, amygdala and nucleus accumbens. NTS-Rs involve in modulation of the dopaminergic system in the CNS, while act on the small intestine endocrine cells to increase acid secretion and regulate smooth muscle contraction in the peripheral tissues (Vincent et al., 1999). NTS-R1 mainly functions through Gq/11-PLC/IP<sub>3</sub>, but it also activates cGMP/cAMP signaling and ERK1/2 phosphorylation (Amar et al., 1985; Bozou et al., 1989; Poinot-Chazel et al., 1996; Vincent et al., 1999). NTS-R2 induces intracellular Ca<sup>2+</sup> signaling and ERK phosphorylation (Botto et al., 1997; Sarret et al., 2002).

Neuromedin U is a 23~25 amino acids polypeptide which is the ligand for other members of this receptor family, the NMU-R1/R2 receptors. NMU is highly conserved among species and widely distributed in the body especially at high levels in the brain where it mediates effects on food intake opposite to ghrelin (Jethwa et al., 2006), likely by cross-talking with the anorectic leptin system (Jethwa et al., 2006; Nogueiras et al., 2006). The NMU-R1 subtype is mainly expressed in the periphery, while NMU-R2 is mostly in the brain (Brighton et al., 2004). Both receptors are involved in the regulation of smooth muscle contraction, gastric acid secretion, insulin secretion, ion transport in the gut, feeding behavior and stress (Brighton et al., 2004). Both NMU-R1 and NMU-R2 signal through Gq/11, PLC and Ca<sup>2+</sup> (Brighton et al., 2004).

The other GHS-R1a homologue is GPR39, which remains controversial about whether or not obestatin binds it (Zhang et al., 2005; Dun et al., 2006; Lauwers et al., 2006; Tremblay et al., 2007). GPR39 is distributed in a variety of tissues, but mostly in brain regions (McKee et al., 1997b). Compared with GHS-R1a and GPR38, GPR39 has a very long C-terminal and two potential palmitoylation sites, which creates a 4th intracellular loop (Morello & Bouvier, 1996; McKee et al., 1997b). Activation of GPR39 by Zn<sup>2+</sup> induces PLC signaling, CRE- and SRE-dependent transcriptional activity, and cAMP production (Holst et al., 2007). Except for the sequence homology with GHS-R1a, the relationship between GPR39 and ghrelin system remains indefinable.

### 8.2.2 Type 1b GHS receptor

Type 1b GHS receptor (GHS-R1b) contains 298 amino acids corresponding to the first five TM domains encoded by exon 1, plus a unique 24 amino acid tail encoded by an alternatively spliced intron sequence. Neither ghrelin nor GHS binds or causes any response to GHS-R1b receptor (Smith et al., 1997) in cells transfected with GHS-R1b. Since GHS-R1b is comprehensively expressed in various tissues (Gnanapavan et al., 2002), it is reasonable to assume that this receptor possesses some unidentified biological functions. A report in 2007 has revealed that GHS-R1b decreases the cell surface expression of GHS-R1a and acts as a repressor of the constitutive activity of GHS-R1a when overexpressed in HEK-293 cells (Chu et al., 2007). This finding suggests that GHS-R1b may act as an endogenous modulator for GHS-R1a constitutive activity. This fascinating action may attribute to its capability of forming heterodimers with full length GPCR receptors, causing altered biological properties compared to the original receptor.

### 8.2.3 CD36 receptor

CD36, a membrane glycoprotein of Mr ~84kDa belonging to the scavenger receptor type-B family (Bodart et al., 1999), distributes in a wide range of tissues including adipose tissue, platelets, monocytes/macrophages, dendritic cells, and microvascular endothelium (Febbraio et al., 2001). CD36 is implicated in multiple physiological functions such as fatty acid/lipid transportation, insulin resistance, antigen presentation and regulation of angiogenesis, as well as pathophysiological processes related to the formation of macrophage foam cell and atherosclerotic lesions (Febbraio et al., 2001). In a perfused heart preparation, hexarelin elicits vasoconstriction. This effect is likely mediated via CD36 because no similar effect occurs in CD36 null mice and rats (Bodart et al., 2002). The signal transduction pathways mediating the vasoconstrictive effect of hexarelin involve both L-type calcium channels and protein kinase C (Bodart et al., 1999).

The effect of hexarelin on the inhibition of cholesterol accumulation might be also mediated by CD36. Hexarelin reduces internalization of oxLDL by interfering with CD36 on the same interaction site as that of oxLDL (Avallone et al., 2006). In addition, hexarelin increases cholesterol efflux in macrophages through activation of CD36 and GHS-R1a which enhances expression of the ABCA1 and ABCG1 transporters and therefore improves cholesterol efflux from macrophages (Avallone et al., 2006).

## 8.3 Tissue distribution of ghrelin receptors

The ghrelin receptor is ubiquitously distributed in both the CNS and peripheral tissues. In the rat brain, GHS-R1a signals are detected in hypothalamic nuclei including anteroventral preoptic nucleus, anterior hypothalamic area, suprachiasmatic nucleus, lateroanterior hypothalamic nucleus, supraoptic nucleus, ventromedial hypothalamic nucleus, arcuate nucleus, paraventricular nucleus and tuberomammillary nucleus as well as in the pituitary gland (Guan et al., 1997). This distribution is consistent with its physiological function associated with energy metabolism and GH release. In addition to the hypothalamus, GHS-R1a mRNA is also expressed in several other discrete regions of the rat brain such as dentate gyrus, CA2 and CA3 regions of the hippocampal formation, thalamic regions, and several

nuclei within the brain stem including pars compacta of the substantia nigra, ventral tegmental area, median and dorsal raphe nuclei and laterodorsal tegmental nucleus (Guan et al., 1997). GHS-R1a may therefore play a role in the control of other functions such as memory, anxiety and some endocrine physiology.

In the peripheral tissues, GHS-R1a mRNA is detected in the stomach and intestine (Date et al., 2000), pancreas (Guan et al., 1997), kidney (Mori et al., 2000), heart and aorta (Nagaya et al., 2001c), adipose tissues (Gnanapavan et al., 2002), as well as in various endocrine neoplasms (de Keyzer et al., 1997; Korbonits et al., 2001a; Papotti et al., 2001). Such a wide distribution of GHS-R1a is consistent with the reported broad functions beyond the control of GH release and food intake. Existence of a novel ghrelin receptor subtype with a binding profile different from the GHS-R1a in the peripheral tissues, in human thyroid and breast tumors, as well as in related cancer cell lines has been reported but requires further confirmation (Cassoni et al., 2001; Volante et al., 2002).

## 9. Intracellular signaling pathways of ghrelin receptors

Binding of ghrelin to GHS-R1a induces a profound change in the transmembrane  $\alpha$  helices, which alters the conformation of intracellular loops and uncovers masked G protein binding sites interacting with G proteins. This interaction promotes the release of guanosine diphosphate (GDP) bound to the G protein  $\alpha$  subunit and exchange for guanosine triphosphate (GTP), followed by activation of G protein subunits which initiates intracellular signaling responses via a series of intracellular molecules.

Intracellular calcium is the well-characterized signaling mode used by GHS-1a receptor related studies, as its endogenous ligand ghrelin was discovered by monitoring intracellular calcium flux in recombinant cells expressing the GHSR-1a (Howard et al., 1996; Kojima et al., 1999). Ghrelin activates at least two signal transduction pathways associated with calcium regulation. In neuropeptide Y (NPY)-containing neurons of the hypothalamus, ghrelin induced increase in intracellular calcium concentration is dependent on the calcium influx through N-type calcium channels activated by the AC-cAMP-PKA signaling pathway following the binding of the Gs protein to GHS-R1a (Kohno et al., 2003). In addition to this cAMP/ PKA pathway, ghrelin also evokes the intracellular calcium signaling by an alternative pathway through Gq/PLC/IP3 (Howard et al., 1996). Coupling of ghrelin to the GHS-R1a activates phospholipase C to generate IP3 and diacylglycerol (DAG). IP3 then triggers the release of calcium from IP3 sensitive intracellular calcium stores (Deghenghi et al., 2001), whereas DAG is responsible for the activation of PKC. The initial calcium current is followed by sustained calcium entry through L- and T-type calcium channels via membrane depolarization (Chen et al., 1990; Smith et al., 1993). Different binding sites on the GHSR-1a have been proposed to potentially account for these two distinct components of ghrelin receptor signaling (Cassoni et al., 2001).

In addition to the well-characterized activation of calcium currents, ghrelin also activates the MAP kinase (MAPK) signaling pathway. In 3T3-L1 preadipocytes, ghrelin induces a rapid activation of ERK1/2, while inhibition of ERK signaling by PD98059 significantly attenuates the proliferative activities of ghrelin (Kim et al., 2004). In human and rat adrenal zona glomerulosa cells, the proliferative effect of ghrelin involves the activation of tyrosine kinase-dependent MAPK p42/p43 mechanism and appears to be independent of PKA and



PKC (Carreira et al., 2004; Mazzocchi et al., 2004). Pretreatment of cells with *Gi/o* inhibitor (pertussis toxin), PKC inhibitors (staurosporin and GF109203X), or PI3K inhibitor (wortmannin) significantly attenuates ghrelin-induced ERK1/2 phosphorylation, suggesting that multiple signaling pathways are involved in activation of MAPK induced by ghrelin (Mousseaux et al., 2006).

AMP-activated protein kinase (AMPK) plays a pivotal role in the regulation of energy metabolism. Ghrelin increases food intake through the activation of AMPK and acetyl-CoA carboxylase (ACC), and the inhibition of fatty acid synthase (FAS) in the ventral medial hypothalamus (Lopez et al., 2008). The molecular mechanism by which ghrelin regulates AMPK is still unknown, although tumor suppressor LKB1 may be an upstream regulator of AMPK kinase (Hardie, 2004). It is worth noting that AMPK's metabolic activity induced by ghrelin receptor is tissue-specific. In the rat liver, ghrelin inhibits AMPK activity to evoke a series of lipogenic and glucogenic gene expression and an increase in triglyceride content without changing the mitochondrial oxidative enzyme activities (Barazzoni et al., 2005). Ghrelin can also inhibit vascular inflammation through the activation of the calmodulin-dependent kinase kinase (CaMKK), AMPK and endothelial nitric oxide synthase (eNOS) (Xu et al., 2008).

Insulin signaling pathway is also regulated by ghrelin through the insulin receptor substrate (IRS-1) associated PI3K activity and Akt phosphorylation. In hepatoma cells, ghrelin increases IRS-1 associated PI3K activity (Murata et al., 2002) and inhibits Akt kinase activity. In addition, ghrelin partially reverses the down-regulation of insulin on phosphoenol pyruvate carboxykinase (PEPCK) mRNA expression, a rate-limiting enzyme of gluconeogenesis.

## 10. Ghrelin's functions

Few gastrointestinal peptide hormones attract more attention than ghrelin, not only because it is the only identified orexigenic hormone circulating in the blood, but also it possesses the unique structure of octanoyl acid modification. In addition to its classical effect on GH release, ghrelin exercises a wide range of physiological functions ranging from regulation of food intake and energy metabolism, control of glucose and lipid homeostasis, modulation of cardiovascular function, to immunomodulation.

### 10.1 Ghrelin and energy balance

#### 10.1.1 Ghrelin and obesity

Hyperphagia, weight gain, and increased adiposity occurs after chronic administration of ghrelin either systemically or intracerebroventricularly (Tschop et al., 2000; Nakazato et al., 2001). Chronic central administration of ghrelin increases adipose deposition independently of food intake (Nakazato et al., 2001) in pair-fed animals. Further studies demonstrate that central ghrelin stimulates triglyceride (TG) uptake and lipogenesis, while inhibits lipid oxidation in white adipocytes (Theander-Carrillo et al., 2006). Peripheral daily administration of ghrelin for two weeks causes a significant increase in fat mass as measured by dual energy X-ray absorptiometry (DXA) (Tschop et al., 2000). Consistent with these observations, the ghrelin receptor null mice are protected against the full development of diet-induced obesity (Zigman et al., 2005).

### 10.1.2 Ghrelin and cachexia/anorexia

Cachexia is characterized by involuntary weight loss due to persistently negative nitrogen balance resulting from a diverse series of pathological conditions such as malnutrition, chronic infectious diseases, immunodeficiency and malignancy. Cachexia is always accompanied by anorexia, while the reduction in food intake alone cannot explain the metabolic changes and wasting associated with cachexia. Ghrelin and ghrelin receptor agonists are fascinating candidates for the treatment of cachexia, due to their unique anabolic effects such as stimulating GH secretion, food intake, gastric motility and adiposity. High levels of circulating acylated ghrelin are detected in cachexia patients associated with lung cancer (Shimizu et al., 2003), chronic heart failure (Nagaya et al., 2001b), renal failure (Yoshimoto et al., 2002), chronic liver disease (Tacke et al., 2003) and anorexia nervosa (Otto et al., 2001), which may represent a compensatory response to an organism's wasting. Both ghrelin and acylated to total ghrelin ratio are elevated in cancer induced cachexia and inversely associated with body mass index (BMI). Whether increased circulating ghrelin levels indicate a ghrelin resistance in cachexia remains to be determined.

### 10.2 Ghrelin and food intake

Both animal experiments and clinical studies demonstrate that ghrelin induces a rapid increase in food intake in rodents and humans (Muccioli et al., 2002). Originating from the stomach, ghrelin activates arcuate NPY/AgRP neurons to increase the secretion of NPY, AgRP and GABA. NPY subsequently modulates the activity of postsynaptic secondary order neurons in the paraventricular nucleus, dorsomedial nucleus and lateral hypothalamic area to stimulate food intake, while GABA inactivates the proopiomelanocortin neurons and inhibits the anorectic melanocortin signaling pathway.

The intracellular mechanisms that mediate NPY/AgRP neuronal activation in response to ghrelin in appetite regulation have been demonstrated to be associated with the lipid metabolism in the hypothalamus (Lopez et al., 2008). After binding of ghrelin, the ghrelin receptor activates hypothalamic AMPK. AMPK then phosphorylates the acetyl-CoA carboxylase, decreases malonyl-CoA level, and suppresses lipid synthesis (Kola et al., 2005), leading to the activation of carnitine palmitoyltransferase 1 (CPT1). Activated CPT1 then accelerates lipid transport into mitochondria to catabolize lipid. Ghrelin regulates mitochondrial oxidation in the hypothalamic cells mainly through uncoupling protein 2 (UCP2) (Andrews et al., 2008), ninety percent of which co-expresses the ghrelin receptor. Experiments on isolated hypothalamic synaptosomes demonstrate that ghrelin increases oxygen consumption and reactive oxygen species (ROS) (Yamagishi et al., 2001), which are always associated with increased transcription and activity of UCP2 (Echtay et al., 2002). The produced ROS are de-gradated by UCP2 (Brand et al., 2004) in order to allow continuous CPT1-promoted fatty acid  $\beta$  oxidation that supports the bioenergetic needs to maintain firing of NPY/AgRP neurons and stimulate food intake. Compound C, an AMPK inhibitor, suppresses appetite stimulated by ghrelin. In UCP2-deficient mice, compound C fails to affect appetite. Taken together, these results suggest that lipid metabolism through AMPK/CPT1/UCP2 in the hypothalamic neurons is a key modulator for the regulation of appetite by ghrelin.

### 10.3 Ghrelin and glucose homeostasis

It has been demonstrated that ghrelin contributes to the modulation of glucose homeostasis. Blocking the effect of endogenous ghrelin by D-Lys3-GHRP-6 significantly improves the intraperitoneal glucose tolerance test (IGTT), indicating that endogenous ghrelin involves in the regulation of insulin release and blood glucose homeostasis (Dezaki et al., 2004). Although ghrelin has been demonstrated to inhibit the activity of the glucosensing neurons in the dorsal vagal complex of rats (Penicaud et al., 2006; Wang et al., 2008), most available data validate that ghrelin modulates insulin secretion and sensitivity, and hepatic glucose production.

The inhibitory effect of ghrelin on insulin secretion has been profoundly demonstrated. In the dissected and perfused rat pancreas, ghrelin significantly inhibits the insulin release in response to increasing glucose concentrations, while demonstrates no significant effect on basal insulin release (Egido et al., 2002). Moreover, the level of insulin released from the perfused pancreas is markedly increased by either blocking the GHS-R1a or immunoneutralizing the endogenous ghrelin. Glucose stimulated insulin release is also enhanced in the pancreas islets isolated from ghrelin-null mice (Dezaki et al., 2006).

*In vitro* experiments show that ghrelin attenuates the inhibitory effects of insulin on expression of PEPCK and up-regulates the gluconeogenesis in cultured rat hepatoma cells (Murata et al., 2002). It also decreases the phosphorylation levels of protein kinase B (PKB) and glycogen synthase kinase (pGSK). All of these results provide evidences that ghrelin has a direct effect on hepatic glucose metabolism (Murata et al., 2002). However, GHS-R1a expression in the liver has not yet been demonstrated. Whether the effect of ghrelin on hepatic glucose metabolism is exerted via a novel ghrelin receptor subtype remains to be explored.

### 10.4 Ghrelin and lipid metabolism

Chronic central administration of ghrelin increases adipose deposition independently of food intake (Nakazato et al., 2001; Theander-Carrillo et al., 2006). Furthermore, various fat storage promoting enzymes such as lipoprotein lipase, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD1) are markedly increased, while the fat oxidation rate limiting enzymes such as carnitine palmitoyl transferase-1 $\alpha$  (CPT1) and uncoupling proteins (UCPs) are significantly decreased (Theander-Carrillo et al., 2006).

Peripheral administration of ghrelin has also been implicated on the regulation of lipid metabolism, with effects on liver, skeletal muscle and adipose tissue. In the liver, ghrelin increases lipogenic genes expression and triglyceride content, while reduces AMPK activity leading to lower fatty acid oxidation (Barazzoni et al., 2005). In the gastrocnemius muscle, ghrelin increases mitochondrial oxidative enzyme activities and reduces triglyceride content (Barazzoni et al., 2005). Ghrelin selectively increases peroxisome proliferator activated receptor  $\gamma$  to reduce muscle fat content in skeletal muscle (Barazzoni et al., 2005). In adipocytes, ghrelin stimulates lipogenesis partly by the insulin-induced glucose uptake (Patel et al., 2006), antagonizes lipolysis induced by isoproterenol (Larhammar, 1996), and stimulates the proliferation and differentiation of preadipocytes (Kim et al., 2004).

### 10.5 Ghrelin and gastrointestinal motility

Ghrelin is a strong gastrokinetic agent, having the motilin-like ability to stimulate motor activity in the gastrointestinal tract (Trudel et al., 2002). Ghrelin triggers the migrating motor complex in the fasted state (Fujino et al., 2003; De Winter et al., 2004; Tack et al., 2006) and accelerates gastric emptying in the postprandial state in animals and humans (Asakawa et al., 2001b; De Winter et al., 2004; Binn et al., 2006; Levin et al., 2006). Ghrelin also accelerates the transit of the small intestine but has no effect on the colon (Trudel et al., 2002). The contractile response of the stomach to the intravenous administration of ghrelin has also been reported (Masuda et al., 2000). The prokinetic effect of ghrelin on motility is mediated through activation of vagal afferents because atropine or vagotomy blocks the contractions induced by ghrelin in these urethane-anesthetized rats (Fujino et al., 2003; Fukuda et al., 2004).

Post-operative ileus is a major cause of complications and prolonged hospitalization. Ghrelin reverses the delay in gastric emptying in post-operative patients (Trudel et al., 2002). Treatment with TZP-101, a synthetic ghrelin receptor agonist or RC-1139, a ghrelin analogue, shows promising results of acceleration of gastric emptying in rats with ileus induced by morphine or surgery (Poitras et al., 2005; Venkova et al., 2007). In addition, intravenous administration of ghrelin has been shown to accelerate gastric emptying in patients with diabetic, idiopathic and post-vagotomy gastroparesis (Murray et al., 2005; Tack et al., 2005; Binn et al., 2006). However, controversy still exists on the use of ghrelin for treatment of gastroparesis because that the study number of patients was small and the methods employed to measure gastric emptying varied from study to study (Tack et al., 2005; Peeters, 2006). In addition, the unwanted effects of ghrelin on glucose and lipid metabolism should be considered. The future of ghrelin application in gastrointestinal motility disorder is largely dependent on our understanding of the mechanism by which ghrelin stimulates gastrointestinal motility (Peeters, 2006).

### 10.6 Ghrelin and memory, depression and anxiety

It was firstly demonstrated that ghrelin can increase memory retention when injected i.c.v. in rats (Carlini et al., 2002). Latter studies showed similar results when injected into the hippocampus, amygdala and dorsal raphe nucleus in rats (Carlini et al., 2004), as well as injected i.c.v. in chronically food-restricted mice (Carlini et al., 2008). Results from step-down tests suggest that ghrelin might modulate specific molecular intermediates involved in the memory acquisition/consolidation processes (Carlini et al., 2010a). The precise mechanism by which ghrelin affects memory is currently unknown. It has been revealed that circulating ghrelin can reach the hippocampus, increase spine synapse density in CA1, and induce long-term potentiation (LTP), which are paralleled by enhanced spatial learning and memory (Diano et al., 2006). Further studies suggest that ghrelin enhances spatial memory by activating the PI3K signaling pathway. The NOS/NO pathway might also involve in the effects of ghrelin on memory consolidation. Intra-hippocampal administration of ghrelin increases the NOS activity dose-dependently and reduces the threshold for LTP generation in dentate gyrus of rats (Carlini et al., 2010b). Additional studies have showed that ghrelin's effects on memory may depend on the availability of 5-HT (Carlini et al., 2007). In contrast to the enhanced effects of ghrelin on memory in rodents, ghrelin could decrease memory retention in neonatal chicks (Carvajal et al., 2009).

The connection between ghrelin and depression was firstly demonstrated by administrating ghrelin antisense DNA which induces an anti-depressant response (Kanehisa et al., 2006). This observation is in agreement with an increase in depression-like behavior in rats with central administration of ghrelin (Schanze et al., 2008). In contrast to these findings, Lutter and colleagues have found that increasing ghrelin levels produces antidepressant-like responses which may be induced by the activation of orexin neurons in the lateral hypothalamus (Lutter et al., 2008). It has also been revealed that chronic social defeat stress, a rodent model of depression, persistently increases ghrelin levels, whereas growth hormone secretagogue receptor (GHSR) null mice show increased deleterious effects of chronic defeat (Kluge et al., 2009). Clinical studies also reveal conflicting results. One study has reported that the levels of acylated and des-acylated ghrelin are lower in depressive patients before and after citalopram treatment than in the control group (Olszanecka-Glinianowicz et al., 2010). Other studies have shown no significant difference in patients with major depression (Asakawa et al., 2001a; Nakashima et al., 2008; Ogaya et al., 2011). Despite of these controversial findings, emerging evidences suggest that ghrelin plays an important role in depression. Ghrelin gene polymorphism has been found to be associated with depression (Carlini et al., 2002). In the brain regions critical for the regulation of cognitive behavior, ghrelin has been reported to increase expression of glutamate receptor metabotropic 5 (Grm5) mRNA, GABAA-3 $\alpha$ (Gabra3) and GABAA-5 $\alpha$  receptor (Gabra5) subunit mRNA in the amygdala, to influence the central serotonin system (Schanze et al., 2008), and to inhibit 5-HT release (Carlini et al., 2004). Since it has been reported that decreased 5-HT activity can elicit depressive like behavior (Temel et al., 2007), it is reasonable to believe that ghrelin might be associated with depression.

Animal and clinical studies indicate that ghrelin is also associated with anxiety. Both central and peripheral administration of ghrelin induces anxiogenesis (Asakawa et al., 2001a; Carlini et al., 2002; Carlini et al., 2004; Carvajal et al., 2009). Ghrelin gene expression is increased by stresses in mice (Asakawa et al., 2001a; Hansson et al., 2011) and psychological stress may increase plasma ghrelin levels in humans (Rouach et al., 2007). Administration of anti-sense DNA for ghrelin in rats has been reported to induce an antidepressant response, while blockade of ghrelin decrease anxiety-like behavior (Kanehisa et al., 2006). The enhanced effects of ghrelin on anxiety may be mediated by corticotropin-releasing hormone (CRH) (Asakawa et al., 2001a) and inhibition of serotonin release (Carlini et al., 2004). In contrast to these observations, increasing circulating ghrelin has been reported to produce anxiolytic-like responses, while no longer were these anxiolytic-like behavioral responses observed when GHSR-null mice were calorie restricted (Lutter et al., 2008). These findings are consistent with a report showing low ghrelin cell activity in high-anxiety Wistar Kyoto rats (Kristensson et al., 2007). The reasons for the conflicting results are currently unknown (Andrews, 2011).

The finding of ghrelin's effects on brain cognitive functions will provide new therapies for mental disorders.

### 10.7 Ghrelin and cardiovascular disease

Numerous studies suggest that ghrelin exercises a wide array of cardiovascular activities including the vasodilation, improvement of myocardial function (Chang et al., 2004; Li et al., 2006) and endothelium protection (Li et al., 2004; Tesauro et al., 2005; Rossi et al., 2007).

These effects may involve both peripheral and central mechanisms (Lin et al., 2004). Microinjection of ghrelin into the nucleus of the solitary tract significantly decreases the mean arterial pressure and heart rate through its action on this nucleus (Matsumura et al., 2002; Lin et al., 2004). The direct effects of ghrelin on cardiovascular function are supported by the mRNA expression of both ghrelin and its receptor in the heart and aortas (Nagaya et al., 2001a; Gnanapavan et al., 2002). In addition, [<sup>125</sup>I-His9] ghrelin, a radio-labeled ghrelin, has been shown to bind to the heart and to peripheral vascular tissue (Katugampola et al., 2001). *In vitro* studies demonstrated that ghrelin inhibits apoptosis of cardiomyocytes (Baldanzi et al., 2002), improves myocardial function during ischemia/reperfusion (Chang et al., 2004) and reduces infarct size (Frascarelli et al., 2003). Moreover, the radiolabeled ghrelin signal increases in atherosclerotic regions (Katugampola et al., 2001), suggesting that ghrelin receptor expression is up-regulated and ghrelin may participate in the development of atherosclerosis.

Congestive heart failure (CHF) is an often-fatal condition in which the heart muscles become weakened and lack the strength to adequately pump blood throughout the body. In patients with CHF, ghrelin decreases mean arterial pressure without increasing heart rate. Chronic treatment with ghrelin improves left-ventricular (LV) function and exercise capacity, as well as attenuates the development of LV remodeling and cachexia in a rat model of chronic heart failure (Nagaya et al., 2001c; Nagaya et al., 2004). Treatment with synthetic GHS such as GHRP-6 or hexarelin improves LV function, prevents cardiac damage after ischemia, and attenuates fibroblast proliferation (Locatelli et al., 1999; Iwase et al., 2004; Xu et al., 2007). In summary, emerging data supports the role of ghrelin and its analogs as novel therapeutic candidates for CHF.

### 10.8 Ghrelin and immunomodulation

The expression of ghrelin and GHS-R in cells of the immune system has been detected in several lymphoid organs (Gnanapavan et al., 2002) and leukocyte subsets including T and B cells, monocytes (Dixit et al., 2004). Such widespread expression of ghrelin receptor in the immune system supports a role for ghrelin in the regulation of immune-related functions. This notion is further supported by previous studies demonstrating that ghrelin regulates immune cell proliferation, activation and secretion of proinflammatory cytokines (Dixit et al., 2004; Taub, 2008). Chronic administration of a ghrelin analogue to old mice for 3 weeks has been demonstrated to stimulate growth, cellularity and differentiation of the thymus, and to increase T-cell production (Koo et al., 2001) which enhances resistance to the initiation of neoplasms and subsequent metastasis in animals inoculated with a transplantable lymphoma cell line, EL4. In addition, GHSs promotes thymic engraftment in bone marrow transplant of SCID mice (Koo et al., 2001).

Upon binding with ghrelin, GHS-R elicits a potent intracellular calcium release in primary and cultured human T cells. In addition, GHS-R is preferentially associated with GM1<sup>+</sup> lipid rafts upon cellular activation (Dixit et al., 2004). These findings suggest that GHS-R is actually expressed on the surface of T cells and functionally active. Activation of T cells by ghrelin forms the lamellipodia and remodeling of actin cytoskeleton, leading to polarization and directional migration (Dixit et al., 2004).

Monocytes are important sources of proinflammatory cytokines. Initial studies show that ghrelin acts to inhibit the production of IL-1 $\beta$  and IL-6 via the GHS-R because this inhibition

of cytokines is blunted by GHS-R antagonists (Dixit et al., 2004). All these data suggest a novel role for ghrelin in immune cell function as a negative regulator of inflammatory cytokine.

### 10.9 Ghrelin and cell differentiation

Dependent on the type of cells, ghrelin can either stimulate or inhibit the cell differentiation. In 3T3-L1 cell lines, over-expression of ghrelin significantly inhibits differentiation of adipocytes (Zhang et al., 2004) and markedly decreases mRNA and protein levels of PPAR $\gamma$ , a marker of adipocyte differentiation. Over-expression of ghrelin in adipose tissue under the control of FABP4 promoter (Zhang et al., 2008) significantly decreases the amount of adipose tissue and renders the mice resistant to diet induced obesity, which indicate that ghrelin may impair the development of adipose tissue (Zhang et al., 2008).

In C2C12 cells lines, ghrelin significantly increases the differentiation of premyocytes into myocytes. Expression of both Myo D and myosin heavy chain protein are elevated in cells overexpressing ghrelin (Zhang et al., 2007), indicating the differentiation of myocyte. Exogenous ghrelin stimulates the proliferation of C2C12 myoblasts and promotes these cells to differentiate and fuse into multinucleated myotubes by activating p38 (Filigheddu et al., 2007).

In both osteoblast cell lines and primary cultured osteoblasts, ghrelin stimulates an increase in cell proliferation and differentiation (Fukushima et al., 2005; Delhanty et al., 2006). The expression of GHS-R1a mRNA and immunoreactivity in osteoblast cells has been demonstrated (Fukushima et al., 2005). The proliferative effect of ghrelin is suppressed by inhibitors of extracellular-signal-regulated kinase (ERK) and phosphoinositide-3 kinase (PI3K), indicating that ghrelin stimulates human osteoblast growth via MAPK/PI3K pathways.

## 11. Cross talk between ghrelin and other hormones

Normal functions of the organism rely on the precise coordination of various hormones. As expected, ghrelin also interacts with other hormones to exercise its biological functions.

### 11.1 Growth hormone/insulin-like growth factor-1 (IGF-1) axis

Ghrelin is known to evoke a specific, dose-dependent release of GH either *in vitro* or *in vivo* (Kojima et al., 1999). This effect is mediated by GHS-R because GHS-R-null mice fail to show the ghrelin-induced GH secretion (Sun et al., 2004). Although ablation of the ghrelin receptor does not reduce food intake or fat mass, GHS-R $^{-/-}$  mice do demonstrate modest reductions in body weight and exhibit biochemical alterations in IGF-1 levels (Sun et al., 2004). IGF-1 accounts for most of the perceptible, anabolic effects of GH such as the linear growth and increase in skeletal muscle mass (Root, A.W. & Root, M.J., 2002). These results indicate that ghrelin may play a more modulatory role in GH release (Sun et al., 2004).

Administration of growth hormone significantly decreases the serum concentration of acyl ghrelin (Eden Engstrom et al., 2003; Seoane et al., 2007), the total ghrelin secretion from rat stomach (Seoane et al., 2007), while demonstrates no effect on gastric ghrelin level (Qi et al., 2003). These findings indicate that growth hormone exerts a negative feedback action on

ghrelin production and secretion. Administration of recombinant human IGF-1 in severe malnutrition patients elevates plasma total ghrelin concentration (Grinspoon et al., 2004). Because IGF-1 always inhibits growth hormone secretion, it is reasonable to assume that IGF-1 may induce ghrelin secretion through the reduction of growth hormone.

### 11.2 Ghrelin and somatostatin

Study has showed that ghrelin is a functional antagonist of somatostatin (Tannenbaum et al., 2003). This finding is in conformity with early *in vitro* studies of the GHSs demonstrating that this effect is at the level of the pituitary gland (Conley et al., 1995). However, ghrelin effectively stimulates GH secretion in the absence of somatostatin, indicating that its GH-releasing activity is not dependent on inhibiting endogenous somatostatin release (Tannenbaum et al., 2003).

Infusion of somatostatin or somatostatin analog octreotide significantly decreases the plasma acyl and total ghrelin levels (Shimada et al., 2003), indicating that somatostatin probably inhibits ghrelin synthesis by directly acting on the somatostatin receptor present in rat stomach (Silva et al., 2005). Furthermore, somatostatin null mice display an increased ghrelin mRNA in stomach and serum total ghrelin without any alteration in hypothalamic and pituitary ghrelin mRNA and serum acyl ghrelin concentration (Luque et al., 2006). Since serum acylated ghrelin remains unchanged in the somatostatin knockout mice, somatostatin may only affect the transcription and translation of ghrelin, but have no effect on its post-translational modification.

### 11.3 Ghrelin and NPY/AgRP

Ghrelin receptor is expressed predominantly in NPY/AgRP neurons in the arcuate nucleus of the hypothalamus (Hahn et al., 1998; Willesen et al., 1999). The arcuate NPY/AgRP neurons have been shown to be essential in the control of food intake and body weight (Gropp et al., 2005; Luquet et al., 2005). Functional activation of these neurons by ghrelin increases expression of the orexigenic neuropeptides themselves, NPY and AgRP (Kamegai et al., 2001; Nakazato et al., 2001). NPY receptor antagonists decrease ghrelin induced increase in food intake (Asakawa et al., 2001b; Shintani et al., 2001; Bagnasco et al., 2003), while disruption of NPY and AgRP via targeted mutagenesis abolishes virtually all ghrelin-induced effects (Chen et al., 2004; Luquet et al., 2005). All these results suggest that the orexigenic effect of ghrelin is fully dependent on NPY/AgRP. However, effects of ghrelin on appetite and body weight are not compromised in mice with selective disruption of the NPY gene, suggesting that AgRP may be a sufficient mediator (Tschop et al., 2002). Additional studies have also demonstrated that ghrelin induces more AgRP mRNA expression than NPY mRNA (Kamegai et al., 2001; Tschop et al., 2002).

### 11.4 Ghrelin and melanocortin

Ghrelin, either originated from blood or from local ghrelin expressing neurons, inhibits melanocortin signaling both directly and indirectly, resulting in an increase of food intake (Cowley et al., 2003). The orexigenic effect of ghrelin has been demonstrated to be attenuated in the Mc3r/Mc4r double knockout mice. This finding suggests that ghrelin stimulates energy intake partly by suppressing hypothalamic melanocortin tone.



Immunohistochemistry and electrophysiology studies have shown that ghrelin acts on NPY neurons, which synapse on and inhibit POMC neurons directly (Cowley et al., 2001) or activates inhibitory GABAergic interneurons innervating POMC and MC4r neurons (Cowley et al., 1999), thereby inhibits melanocortin signaling indirectly.

### 11.5 Ghrelin and leptin

Leptin is commonly considered as an inhibitor of ghrelin synthesis. Reciprocal relationship has been found between serum concentrations of ghrelin and leptin. Leptin concentration in obese is significantly higher than lean control, whereas ghrelin is lower (Tschop et al., 2001b). Moreover, ghrelin mRNA increases in stomach during fasting whereas leptin and leptin mRNAs decrease (Zhao et al., 2008). Leptin dose-dependently inhibits ghrelin transcription *in vitro* (Zhao et al., 2008) and decreases ghrelin release from isolated rat stomach (Kamegai et al., 2004). Central leptin gene therapy decreases plasma leptin level, whereas increases ghrelin level significantly in the mouse fed with high-fat diet (Dube et al., 2002), indicating that leptin inhibits ghrelin secretion only in peripheral tissues. Thus, peripheral, especially gastric leptin, might repress ghrelin expression through its receptor in gastric mucosa cells.

In the CNS, 57% of neurons activated by peripheral ghrelin express the Ob-R (Traebert et al., 2002), suggesting the co-expression of GHS-R and the Ob-R in majority of neurons. It is therefore proposed that ghrelin and leptin exert their opposite effect on food intake by acting largely on the same population of hypothalamic neurons. Ghrelin-induced increase in food intake in the light phase has been demonstrated to be suppressed by ICV administration of leptin (Nakazato et al., 2001), or pretreatment with anti-NPY immunoglobulin (Nakazato et al., 2001), suggesting that ghrelin and leptin may act via the same cellular pathway.

Leptin has been long considered to cause satiety by depolarizing the POMC neurons, while hyperpolarizing NPY cells (Cowley et al., 2001). Ghrelin substantially blocks this reduction of feeding in rats pretreated with leptin (Nakazato et al., 2001). These results indicate that ghrelin may antagonize leptin action in the regulation of the NPY system.

### 11.6 Ghrelin and dopamine

When delivered directly into the VTA, ghrelin binds to the VTA neurons and produces a marked increase in food intake that resembles rebound feeding after fasting (Naleid et al., 2005; Abizaid et al., 2006). GHS-R has been detected in about 50–60% VTA dopamine cells. In addition, VTA dopamine cells are innervated by lateral hypothalamic hypocretin/orexin neurones, which are also sensitive to ghrelin (Toshinai et al., 2003). These results indicate that ghrelin might potentially influence the release of dopamine from these cells (Abizaid et al., 2006; Zigman et al., 2006). This concept is supported by numerous studies in which extracellular dopamine content in the nucleus accumbens of rats has been shown to be elevated by peripheral, i.c.v. and intra-VTA injections of ghrelin (Jerlhag et al., 2006; Jerlhag et al., 2007; Quarta et al., 2009). Further study suggests that ghrelin increases dopamine release by improving the dopamine cells excitability (Abizaid et al., 2006). In the presence of ghrelin, dopamine cells in the VTA increase their frequency of action potentials, which appears to be mediated by glutamatergic neurotransmission. These changes are

undetectable in dopamine cells from the VTA of GHS-R knockout mice (Abizaid et al., 2006). As in the hypothalamus, ghrelin lowers the threshold of activation of dopamine neurons through mechanisms that involve remodeling the ratio of excitatory versus inhibitory inputs onto these cells (Abizaid et al., 2006).

Peripheral injections of ghrelin increase dopamine turnover in the ventral striatum of mice and rats (Abizaid et al., 2006). Considering that the VTA is protected by the blood-brain barrier and less permeable than the arcuate nuclei to blood-borne substances, ghrelin might be transported into VTA through a saturable mechanism remained to be fully determined (Banks et al., 2002). Another possibility is the indirect effect of ghrelin on afferent neurons which innervate the VTA such as lateral hypothalamic neurons and laterodorsal tegmental nucleus (Guan et al., 1997; Geisler & Zahm, 2005).

### 11.7 Ghrelin and insulin

In human pancreatic islets, ghrelin receptor immunoreactivity partially overlaps with insulin-positive  $\beta$ -cells (Granata et al., 2007), indicating that human  $\beta$ -cells might also be responsive to ghrelin stimulation. In cultured islet cells, acyl-ghrelin suppresses both basal insulin secretion (Dezaki et al., 2004) and glucose-induced insulin release (Dezaki et al., 2006). The level of insulin released from the perfused pancreas is significantly increased by either blocking the GHS-R1a or immunoneutralizing the endogenous ghrelin. Furthermore, glucose-induced insulin release is greater in islets isolated from ghrelin-null mice than wild type littermates. All these data suggest that ghrelin regulates insulin secretion from the islet cells. The molecular mechanism by which ghrelin suppresses glucose-induced insulin release has been reported to be the attenuation of  $Ca^{2+}$  signaling in  $\beta$ -cells via  $G_{\alpha i2}$  and Kv channel (Dezaki et al., 2007).

Gastric artery perfusion of insulin significantly inhibits ghrelin release from isolated stomach tissue in rats (Kamegai et al., 2004). Central administration of insulin reduces serum total ghrelin concentration (Ueno et al., 2006), while maintaining euglycemia (Saad et al., 2002; Flanagan et al., 2003). Several clinical observations in humans also indicate that insulin may inhibit ghrelin secretion.

### 11.8 Ghrelin and glucagon

Both ghrelin and GHS-R1a have been identified in either human or rat pancreatic islets  $\alpha$  cells (Date et al., 2002). Ghrelin induces significant increase in glucagon secretion from the pancreas of diabetic rats rather than in normal rats (Adeghate & Parvez, 2002). A possible reason for this difference is that signal transduction involving the calcium pathway is impaired in diabetic rat pancreas.

Glucagon may stimulate the gene transcription of ghrelin as well. Glucagon might elevate the activity of ghrelin gene promoter by the mediation of the second messenger cAMP (Wei et al., 2005). Several studies suggest that glucagon may contribute to the pre-prandial surge of ghrelin. Glucagon receptor is present in endocrine cells in gastric mucosa (Katayama et al., 2007). Glucagon concentration increases during fasting, and plasma acyl ghrelin concentration rises after administration of glucagon in rats. In addition, ghrelin released from the rat stomach is augmented by glucagon (Kamegai et al., 2004).

### 11.9 Ghrelin and estrogen

Numerous studies report that estrogen up-regulates ghrelin level. The effects of estrogen to stimulate food intake and growth hormone secretion might therefore be partially mediated through ghrelin. Plasma total ghrelin concentration in female patients with anorexia nervosa is significantly elevated (Grinspoon et al., 2004), while ghrelin mRNA level rises dramatically in cultured stomach cells (Sakata et al., 2006) after estrogen administration. Discrepant results on the effect of estrogen on ghrelin have been reported. Precise mechanism for the discrepancy remains unknown, but may relate to the distinction of age (Matsubara et al., 2004), physiological status (Chu et al., 2006) and variation in methods used for estrogen application (Kellokoski et al., 2005; Chu et al., 2006). Estrogen replacement therapy in post-menopausal women has been reported to induce increase (Kellokoski et al., 2005; Lambrinoudaki et al., 2008), no significant change, or even decreases (Chu et al., 2006) in serum total and acyl ghrelin secretion. In female rats, ovariectomy induces a transient augment in plasma acyl ghrelin, ghrelin expressing cells and ghrelin mRNA in stomach (Matsubara et al., 2004).

## 12. Conclusion

Since its discovery in 1999, ghrelin has attracted a tremendous interest from both academy and industry. It has become one of the most extensively studied fields. This is due to its highly conserved sequence between species, its unique molecular structure, and the ubiquity of ghrelin and receptors which implicates its important physiological function during the development. The multiplicity of physiological functions of ghrelin are revealing gradually. Current evidences show that ghrelin affects GH release, food intake, energy and glucose homeostasis, gastrointestinal, cardiovascular and immune functions, cell proliferation and differentiation, and cognitive behavior. Ghrelin is therefore a critical hormone linking the gastrointestinal activities with organism functions.

## 13. Acknowledgement

This work was supported by grants from the National Natural Science Foundation of China (81030012, 81170795, 30890043, 30971434, 30871194, 30821001, and 30971085), and the Major National Basic Research Program of China (2010CB912504)

## 14. References

- Abizaid, A., Liu, Z.W., Andrews, Z.B., Shanabrough, M., Borok, E., Elsworth, J.D., Roth, R.H., Sleeman, M.W., Picciotto, M.R., Tschop, M.H., Gao, X.B. & Horvath, T.L. (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest*, 116,pp 3229-3239.
- Adeghate, E. & Parvez, H. (2002) Mechanism of ghrelin-evoked glucagon secretion from the pancreas of diabetic rats. *Neuro Endocrinol Lett*, 23,pp 432-436.
- Amar, S., Mazella, J., Checler, F., Kitabgi, P. & Vincent, J.P. (1985) Regulation of cyclic GMP levels by neurotensin in neuroblastoma clone N1E115. *Biochem Biophys Res Commun*, 129,pp 117-125.

- Andrews, Z.B. (2011) The extra-hypothalamic actions of ghrelin on neuronal function. *Trends Neurosci*, 34,pp 31-40.
- Andrews, Z.B., Liu, Z.W., Wallingford, N., Erion, D.M., Borok, E., Friedman, J.M., Tschop, M.H., Shanabrough, M., Cline, G., Shulman, G.I., Coppola, A., Gao, X.B., Horvath, T.L. & Diano, S. (2008) UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature*, 454,pp 846-851.
- Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K. & Nakao, K. (2001) Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab*, 86,pp 4753-4758.
- Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., Meguid, M.M. & Kasuga, M. (2005) Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut*, 54,pp 18-24.
- Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Fujimiya, M., Katsuura, G., Makino, S., Fujino, M.A. & Kasuga, M. (2001a) A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology*, 74,pp 143-147.
- Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., Makino, S., Fujimiya, M., Niiijima, A., Fujino, M.A. & Kasuga, M. (2001b) Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology*, 120,pp 337-345.
- Avallone, R., Demers, A., Rodrigue-Way, A., Bujold, K., Harb, D., Anghel, S., Wahli, W., Marleau, S., Ong, H. & Tremblay, A. (2006) A growth hormone-releasing peptide that binds scavenger receptor CD36 and ghrelin receptor up-regulates sterol transporters and cholesterol efflux in macrophages through a peroxisome proliferator-activated receptor gamma-dependent pathway. *Mol Endocrinol*, 20,pp 3165-3178.
- Bagnasco, M., Tulipano, G., Melis, M.R., Argiolas, A., Cocchi, D. & Muller, E.E. (2003) Endogenous ghrelin is an orexigenic peptide acting in the arcuate nucleus in response to fasting. *Regul Pept*, 111,pp 161-167.
- Baldanzi, G., Filigheddu, N., Cutrupi, S., Catapano, F., Bonisconi, S., Fubini, A., Malan, D., Baj, G., Granata, R., Broglio, F., Papotti, M., Surico, N., Bussolino, F., Isgaard, J., Deghenghi, R., Sinigaglia, F., Prat, M., Muccioli, G., Ghigo, E. & Graziani, A. (2002) Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. *J Cell Biol*, 159,pp 1029-1037.
- Bang, A.S., Soule, S.G., Yandle, T.G., Richards, A.M. & Pemberton, C.J. (2007) Characterisation of proghrelin peptides in mammalian tissue and plasma. *J Endocrinol*, 192,pp 313-323.
- Banks, W.A., Tschop, M., Robinson, S.M. & Heiman, M.L. (2002) Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther*, 302,pp 822-827.
- Barazzoni, R., Bosutti, A., Stebel, M., Cattin, M.R., Roder, E., Visintin, L., Cattin, L., Biolo, G., Zanetti, M. & Guarnieri, G. (2005) Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle. *Am J Physiol Endocrinol Metab*, 288,pp E228-235.

- Barim, A.O., Aydin, S., Colak, R., Dag, E., Deniz, O. & Sahin, I. (2009) Ghrelin, paraoxonase and arylesterase levels in depressive patients before and after citalopram treatment. *Clin Biochem*, 42, pp 1076-1081.
- Barreiro, M.L., Gaytan, F., Caminos, J.E., Pinilla, L., Casanueva, F.F., Aguilar, E., Dieguez, C. & Tena-Sempere, M. (2002) Cellular location and hormonal regulation of ghrelin expression in rat testis. *Biol Reprod*, 67, pp 1768-1776.
- Beaumont, N.J., Skinner, V.O., Tan, T.M., Ramesh, B.S., Byrne, D.J., MacColl, G.S., Keen, J.N., Bouloux, P.M., Mikhailidis, D.P., Bruckdorfer, K.R., Vanderpump, M.P. & Srai, K.S. (2003) Ghrelin can bind to a species of high density lipoprotein associated with paraoxonase. *J Biol Chem*, 278, pp 8877-8880.
- Bednarek, M.A., Feighner, S.D., Pong, S.S., McKee, K.K., Hreniuk, D.L., Silva, M.V., Warren, V.A., Howard, A.D., Van Der Ploeg, L.H. & Heck, J.V. (2000) Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. *J Med Chem*, 43, pp 4370-4376.
- Bennett, P.A., Thomas, G.B., Howard, A.D., Feighner, S.D., van der Ploeg, L.H., Smith, R.G. & Robinson, I.C. (1997) Hypothalamic growth hormone secretagogue-receptor (GHS-R) expression is regulated by growth hormone in the rat. *Endocrinology*, 138, pp 4552-4557.
- Binn, M., Albert, C., Gougeon, A., Maerki, H., Coulie, B., Lemoyne, M., Rabasa Lhoret, R., Tomasetto, C. & Poitras, P. (2006) Ghrelin gastrokinetic action in patients with neurogenic gastroparesis. *Peptides*, 27, pp 1603-1606.
- Blake, A.D. & Smith, R.G. (1991) Desensitization studies using perfused rat pituitary cells show that growth hormone-releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> stimulate growth hormone release through distinct receptor sites. *J Endocrinol*, 129, pp 11-19.
- Bockaert, J. & Pin, J.P. (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J*, 18, pp 1723-1729.
- Bodart, V., Bouchard, J.F., McNicoll, N., Escher, E., Carriere, P., Ghigo, E., Sejlitz, T., Sirois, M.G., Lamontagne, D. & Ong, H. (1999) Identification and characterization of a new growth hormone-releasing peptide receptor in the heart. *Circ Res*, 85, pp 796-802.
- Bodart, V., Febbraio, M., Demers, A., McNicoll, N., Pohankova, P., Perreault, A., Sejlitz, T., Escher, E., Silverstein, R.L., Lamontagne, D. & Ong, H. (2002) CD36 mediates the cardiovascular action of growth hormone-releasing peptides in the heart. *Circ Res*, 90, pp 844-849.
- Bondensgaard, K., Ankersen, M., Thogersen, H., Hansen, B.S., Wulff, B.S. & Bywater, R.P. (2004) Recognition of privileged structures by G-protein coupled receptors. *J Med Chem*, 47, pp 888-899.
- Botto, J.M., Guillemare, E., Vincent, J.P. & Mazella, J. (1997) Effects of SR 48692 on neurotensin-induced calcium-activated chloride currents in the *Xenopus* oocyte expression system: agonist-like activity on the levocabastine-sensitive neurotensin receptor and absence of antagonist effect on the levocabastine insensitive neurotensin receptor. *Neurosci Lett*, 223, pp 193-196.

- Bowers, C.Y., Alster, D.K. & Frentz, J.M. (1992) The growth hormone-releasing activity of a synthetic hexapeptide in normal men and short statured children after oral administration. *J Clin Endocrinol Metab*, 74,pp 292-298.
- Bowers, C.Y., Momany, F., Reynolds, G.A., Chang, D., Hong, A. & Chang, K. (1980) Structure-activity relationships of a synthetic pentapeptide that specifically releases growth hormone in vitro. *Endocrinology*, 106,pp 663-667.
- Bowers, C.Y., Momany, F.A., Reynolds, G.A. & Hong, A. (1984) On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology*, 114,pp 1537-1545.
- Bozou, J.C., Rochet, N., Magnaldo, I., Vincent, J.P. & Kitabgi, P. (1989) Neurotensin stimulates inositol trisphosphate-mediated calcium mobilization but not protein kinase C activation in HT29 cells. Involvement of a G-protein. *Biochem J*, 264,pp 871-878.
- Brand, M.D., Affourtit, C., Esteves, T.C., Green, K., Lambert, A.J., Miwa, S., Pakay, J.L. & Parker, N. (2004) Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic Biol Med*, 37,pp 755-767.
- Bresson-Bepoldin, L. & Dufy-Barbe, L. (1994) GHRP-6 induces a biphasic calcium response in rat pituitary somatotrophs. *Cell Calcium*, 15,pp 247-258.
- Brighton, P.J., Szekeres, P.G. & Willars, G.B. (2004) Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol Rev*, 56,pp 231-248.
- Camina, J.P., Carreira, M.C., El Messari, S., Llorens-Cortes, C., Smith, R.G. & Casanueva, F.F. (2004) Desensitization and endocytosis mechanisms of ghrelin-activated growth hormone secretagogue receptor 1a. *Endocrinology*, 145,pp 930-940.
- Capella, C., Solcia, E. & Vassallo, G. (1969) Identification of six types of endocrine cells in the gastrointestinal mucosa of the rabbit. *Arch Histol Jpn*, 30,pp 479-495.
- Carlini, V.P., Gaydou, R.C., Schioth, H.B. & de Barioglio, S.R. (2007) Selective serotonin reuptake inhibitor (fluoxetine) decreases the effects of ghrelin on memory retention and food intake. *Regul Pept*, 140,pp 65-73.
- Carlini, V.P., Ghersi, M., Schioth, H.B. & de Barioglio, S.R. (2010a) Ghrelin and memory: differential effects on acquisition and retrieval. *Peptides*, 31,pp 1190-1193.
- Carlini, V.P., Martini, A.C., Schioth, H.B., Ruiz, R.D., Fiol de Cuneo, M. & de Barioglio, S.R. (2008) Decreased memory for novel object recognition in chronically food-restricted mice is reversed by acute ghrelin administration. *Neuroscience*, 153,pp 929-934.
- Carlini, V.P., Monzon, M.E., Varas, M.M., Cragolini, A.B., Schioth, H.B., Scimonelli, T.N. & de Barioglio, S.R. (2002) Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem Biophys Res Commun*, 299,pp 739-743.
- Carlini, V.P., Perez, M.F., Salde, E., Schioth, H.B., Ramirez, O.A. & de Barioglio, S.R. (2010b) Ghrelin induced memory facilitation implicates nitric oxide synthase activation and decrease in the threshold to promote LTP in hippocampal dentate gyrus. *Physiol Behav*, 101,pp 117-123.
- Carlini, V.P., Varas, M.M., Cragolini, A.B., Schioth, H.B., Scimonelli, T.N. & de Barioglio, S.R. (2004) Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem Biophys Res Commun*, 313,pp 635-641.

- Carreira, M.C., Camina, J.P., Smith, R.G. & Casanueva, F.F. (2004) Agonist-specific coupling of growth hormone secretagogue receptor type 1a to different intracellular signaling systems. Role of adenosine. *Neuroendocrinology*, 79,pp 13-25.
- Carvajal, P., Carlini, V.P., Schioth, H.B., de Barioglio, S.R. & Salvatierra, N.A. (2009) Central ghrelin increases anxiety in the Open Field test and impairs retention memory in a passive avoidance task in neonatal chicks. *Neurobiol Learn Mem*, 91,pp 402-407.
- Cassoni, P., Ghe, C., Marrocco, T., Tarabra, E., Allia, E., Catapano, F., Deghenghi, R., Ghigo, E., Papotti, M. & Muccioli, G. (2004) Expression of ghrelin and biological activity of specific receptors for ghrelin and des-acyl ghrelin in prostate neoplasms and related cell lines. *Eur J Endocrinol*, 150,pp 173-184.
- Cassoni, P., Papotti, M., Ghe, C., Catapano, F., Sapino, A., Graziani, A., Deghenghi, R., Reissmann, T., Ghigo, E. & Muccioli, G. (2001) Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. *J Clin Endocrinol Metab*, 86,pp 1738-1745.
- Chang, L., Ren, Y., Liu, X., Li, W.G., Yang, J., Geng, B., Weintraub, N.L. & Tang, C. (2004) Protective effects of ghrelin on ischemia/reperfusion injury in the isolated rat heart. *J Cardiovasc Pharmacol*, 43,pp 165-170.
- Chanoine, J.P. & Wong, A.C. (2004) Ghrelin gene expression is markedly higher in fetal pancreas compared with fetal stomach: effect of maternal fasting. *Endocrinology*, 145,pp 3813-3820.
- Chen, C., Zhang, J., Vincent, J.D. & Israel, J.M. (1990) Two types of voltage-dependent calcium current in rat somatotrophs are reduced by somatostatin. *J Physiol*, 425,pp 29-42.
- Chen, H.Y., Trumbauer, M.E., Chen, A.S., Weingarh, D.T., Adams, J.R., Frazier, E.G., Shen, Z., Marsh, D.J., Feighner, S.D., Guan, X.M., Ye, Z., Nargund, R.P., Smith, R.G., Van der Ploeg, L.H., Howard, A.D., MacNeil, D.J. & Qian, S. (2004) Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology*, 145,pp 2607-2612.
- Cheng, K., Chan, W.W., Barreto, A., Jr., Convey, E.M. & Smith, R.G. (1989) The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> on growth hormone (GH)-releasing factor-stimulated GH release and intracellular adenosine 3',5'-monophosphate accumulation in rat primary pituitary cell culture. *Endocrinology*, 124,pp 2791-2798.
- Cheng, K., Chan, W.W., Butler, B., Barreto, A., Jr. & Smith, R.G. (1991) Evidence for a role of protein kinase-C in His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>-induced growth hormone release from rat primary pituitary cells. *Endocrinology*, 129,pp 3337-3342.
- Chipman, J.J. (1993) Recent advances in hGH clinical research. *J Pediatr Endocrinol*, 6,pp 325-328.
- Chu, K.M., Chow, K.B., Leung, P.K., Lau, P.N., Chan, C.B., Cheng, C.H. & Wise, H. (2007) Over-expression of the truncated ghrelin receptor polypeptide attenuates the constitutive activation of phosphatidylinositol-specific phospholipase C by ghrelin receptors but has no effect on ghrelin-stimulated extracellular signal-regulated kinase 1/2 activity. *Int J Biochem Cell Biol*, 39,pp 752-764.

- Chu, M.C., Cospers, P., Nakhuda, G.S. & Lobo, R.A. (2006) A comparison of oral and transdermal short-term estrogen therapy in postmenopausal women with metabolic syndrome. *Fertil Steril*, 86,pp 1669-1675.
- Conley, L.K., Teik, J.A., Deghenghi, R., Imbimbo, B.P., Giustina, A., Locatelli, V. & Wehrenberg, W.B. (1995) Mechanism of action of hexarelin and GHRP-6: analysis of the involvement of GHRH and somatostatin in the rat. *Neuroendocrinology*, 61,pp 44-50.
- Corbetta, S., Peracchi, M., Cappiello, V., Lania, A., Lauri, E., Vago, L., Beck-Peccoz, P. & Spada, A. (2003) Circulating ghrelin levels in patients with pancreatic and gastrointestinal neuroendocrine tumors: identification of one pancreatic ghrelinoma. *J Clin Endocrinol Metab*, 88,pp 3117-3120.
- Cowley, M.A., Pronchuk, N., Fan, W., Dinulescu, D.M., Colmers, W.F. & Cone, R.D. (1999) Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron*, 24,pp 155-163.
- Cowley, M.A., Smart, J.L., Rubinstein, M., Cerdan, M.G., Diano, S., Horvath, T.L., Cone, R.D. & Low, M.J. (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, 411,pp 480-484.
- Cowley, M.A., Smith, R.G., Diano, S., Tschop, M., Pronchuk, N., Grove, K.L., Strasburger, C.J., Bidlingmaier, M., Esterman, M., Heiman, M.L., Garcia-Segura, L.M., Nillni, E.A., Mendez, P., Low, M.J., Sotonyi, P., Friedman, J.M., Liu, H., Pinto, S., Colmers, W.F., Cone, R.D. & Horvath, T.L. (2003) The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron*, 37,pp 649-661.
- Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E. & Weigle, D.S. (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, 50,pp 1714-1719.
- Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K. & Nakazato, M. (2000) Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology*, 141,pp 4255-4261.
- Date, Y., Nakazato, M., Hashiguchi, S., Dezaki, K., Mondal, M.S., Hosoda, H., Kojima, M., Kangawa, K., Arima, T., Matsuo, H., Yada, T. & Matsukura, S. (2002) Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes*, 51,pp 124-129.
- de Keyser, Y., Lenne, F. & Bertagna, X. (1997) Widespread transcription of the growth hormone-releasing peptide receptor gene in neuroendocrine human tumors. *Eur J Endocrinol*, 137,pp 715-718.
- De Winter, B.Y., De Man, J.G., Seerden, T.C., Depoortere, I., Herman, A.G., Peeters, T.L. & Pelckmans, P.A. (2004) Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. *Neurogastroenterol Motil*, 16,pp 439-446.
- Deghenghi, R., Papotti, M., Ghigo, E. & Muccioli, G. (2001) Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GHS) receptors of human pituitary gland. *J Endocrinol Invest*, 24,pp RC1-3.



- Dehlin, E., Liu, J., Yun, S.H., Fox, E., Snyder, S., Gineste, C., Willingham, L., Geysen, M., Gaylann, B.D. & Sando, J.J. (2008) Regulation of ghrelin structure and membrane binding by phosphorylation. *Peptides*, 29,pp 904-911.
- Delhanty, P.J., van der Eerden, B.C., van der Velde, M., Gauna, C., Pols, H.A., Jahr, H., Chiba, H., van der Lely, A.J. & van Leeuwen, J.P. (2006) Ghrelin and unacylated ghrelin stimulate human osteoblast growth via mitogen-activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K) pathways in the absence of GHS-R1a. *J Endocrinol*, 188,pp 37-47.
- Dezaki, K., Hosoda, H., Kakei, M., Hashiguchi, S., Watanabe, M., Kangawa, K. & Yada, T. (2004) Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca<sup>2+</sup> signaling in beta-cells: implication in the glycemic control in rodents. *Diabetes*, 53,pp 3142-3151.
- Dezaki, K., Kakei, M. & Yada, T. (2007) Ghrelin uses Galphai2 and activates voltage-dependent K<sup>+</sup> channels to attenuate glucose-induced Ca<sup>2+</sup> signaling and insulin release in islet beta-cells: novel signal transduction of ghrelin. *Diabetes*, 56,pp 2319-2327.
- Dezaki, K., Sone, H., Koizumi, M., Nakata, M., Kakei, M., Nagai, H., Hosoda, H., Kangawa, K. & Yada, T. (2006) Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes*, 55,pp 3486-3493.
- Diano, S., Farr, S.A., Benoit, S.C., McNay, E.C., da Silva, I., Horvath, B., Gaskin, F.S., Nonaka, N., Jaeger, L.B., Banks, W.A., Morley, J.E., Pinto, S., Sherwin, R.S., Xu, L., Yamada, K.A., Sleeman, M.W., Tschop, M.H. & Horvath, T.L. (2006) Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci*, 9,pp 381-388.
- Dixit, V.D., Schaffer, E.M., Pyle, R.S., Collins, G.D., Sakthivel, S.K., Palaniappan, R., Lillard, J.W., Jr. & Taub, D.D. (2004) Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest*, 114,pp 57-66.
- Dornonville de la Cour, C., Bjorkqvist, M., Sandvik, A.K., Bakke, I., Zhao, C.M., Chen, D. & Hakanson, R. (2001) A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. *Regul Pept*, 99,pp 141-150.
- Dube, M.G., Beretta, E., Dhillon, H., Ueno, N., Kalra, P.S. & Kalra, S.P. (2002) Central leptin gene therapy blocks high-fat diet-induced weight gain, hyperleptinemia, and hyperinsulinemia: increase in serum ghrelin levels. *Diabetes*, 51,pp 1729-1736.
- Dun, S.L., Brailoiu, G.C., Brailoiu, E., Yang, J., Chang, J.K. & Dun, N.J. (2006) Distribution and biological activity of obestatin in the rat. *J Endocrinol*, 191,pp 481-489.
- Echtay, K.S., Roussel, D., St-Pierre, J., Jekabsons, M.B., Cadenas, S., Stuart, J.A., Harper, J.A., Roebuck, S.J., Morrison, A., Pickering, S., Clapham, J.C. & Brand, M.D. (2002) Superoxide activates mitochondrial uncoupling proteins. *Nature*, 415,pp 96-99.
- Eden Engstrom, B., Burman, P., Holdstock, C. & Karlsson, F.A. (2003) Effects of growth hormone (GH) on ghrelin, leptin, and adiponectin in GH-deficient patients. *J Clin Endocrinol Metab*, 88,pp 5193-5198.

- Egido, E.M., Rodriguez-Gallardo, J., Silvestre, R.A. & Marco, J. (2002) Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. *Eur J Endocrinol*, 146,pp 241-244.
- Erdmann, J., Lippl, F. & Schusdziarra, V. (2003) Differential effect of protein and fat on plasma ghrelin levels in man. *Regul Pept*, 116,pp 101-107.
- Erdmann, J., Lippl, F., Wagenpfeil, S. & Schusdziarra, V. (2005) Differential association of basal and postprandial plasma ghrelin with leptin, insulin, and type 2 diabetes. *Diabetes*, 54,pp 1371-1378.
- Evans, B.E., Rittle, K.E., Bock, M.G., DiPardo, R.M., Freidinger, R.M., Whitter, W.L., Lundell, G.F., Veber, D.F., Anderson, P.S., Chang, R.S. & et al. (1988) Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *J Med Chem*, 31,pp 2235-2246.
- Falls, H.D., Dayton, B.D., Fry, D.G., Ogiela, C.A., Schaefer, V.G., Brodjian, S., Reilly, R.M., Collins, C.A. & Kaszubska, W. (2006) Characterization of ghrelin receptor activity in a rat pituitary cell line RC-4B/C. *J Mol Endocrinol*, 37,pp 51-62.
- Febbraio, M., Hajjar, D.P. & Silverstein, R.L. (2001) CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J Clin Invest*, 108,pp 785-791.
- Feighner, S.D., Howard, A.D., Prendergast, K., Palyha, O.C., Hreniuk, D.L., Nargund, R., Underwood, D., Tata, J.R., Dean, D.C., Tan, C.P., McKee, K.K., Woods, J.W., Patchett, A.A., Smith, R.G. & Van der Ploeg, L.H. (1998) Structural requirements for the activation of the human growth hormone secretagogue receptor by peptide and nonpeptide secretagogues. *Mol Endocrinol*, 12,pp 137-145.
- Filigheddu, N., Gnocchi, V.F., Coscia, M., Cappelli, M., Porporato, P.E., Taulli, R., Traini, S., Baldanzi, G., Chianale, F., Cutrupi, S., Arnoletti, E., Ghe, C., Fubini, A., Surico, N., Sinigaglia, F., Ponzetto, C., Muccioli, G., Crepaldi, T. & Graziani, A. (2007) Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells. *Mol Biol Cell*, 18,pp 986-994.
- Flanagan, D.E., Evans, M.L., Monsod, T.P., Rife, F., Heptulla, R.A., Tamborlane, W.V. & Sherwin, R.S. (2003) The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab*, 284,pp E313-316.
- Foster-Schubert, K.E., Overduin, J., Prudom, C.E., Liu, J., Callahan, H.S., Gaylinn, B.D., Thorner, M.O. & Cummings, D.E. (2008) Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *J Clin Endocrinol Metab*, 93,pp 1971-1979.
- Frascarelli, S., Ghelardoni, S., Ronca-Testoni, S. & Zucchi, R. (2003) Effect of ghrelin and synthetic growth hormone secretagogues in normal and ischemic rat heart. *Basic Res Cardiol*, 98,pp 401-405.
- Frohman, L.A. & Jansson, J.O. (1986) Growth hormone-releasing hormone. *Endocr Rev*, 7,pp 223-253.
- Fujino, K., Inui, A., Asakawa, A., Kihara, N., Fujimura, M. & Fujimiya, M. (2003) Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol*, 550,pp 227-240.
- Fukuda, H., Mizuta, Y., Isomoto, H., Takeshima, F., Ohnita, K., Ohba, K., Omagari, K., Taniyama, K. & Kohno, S. (2004) Ghrelin enhances gastric motility through direct

- stimulation of intrinsic neural pathways and capsaicin-sensitive afferent neurones in rats. *Scand J Gastroenterol*, 39,pp 1209-1214.
- Fukushima, N., Hanada, R., Teranishi, H., Fukue, Y., Tachibana, T., Ishikawa, H., Takeda, S., Takeuchi, Y., Fukumoto, S., Kangawa, K., Nagata, K. & Kojima, M. (2005) Ghrelin directly regulates bone formation. *J Bone Miner Res*, 20,pp 790-798.
- Gaytan, F., Barreiro, M.L., Caminos, J.E., Chopin, L.K., Herington, A.C., Morales, C., Pinilla, L., Paniagua, R., Nistal, M., Casanueva, F.F., Aguilar, E., Dieguez, C. & Tena-Sempere, M. (2004) Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. *J Clin Endocrinol Metab*, 89,pp 400-409.
- Geisler, S. & Zahm, D.S. (2005) Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *J Comp Neurol*, 490,pp 270-294.
- Ghigo, E., Broglio, F., Arvat, E., Maccario, M., Papotti, M. & Muccioli, G. (2005) Ghrelin: more than a natural GH secretagogue and/or an orexigenic factor. *Clin Endocrinol (Oxf)*, 62,pp 1-17.
- Gnanapavan, S., Kola, B., Bustin, S.A., Morris, D.G., McGee, P., Fairclough, P., Bhattacharya, S., Carpenter, R., Grossman, A.B. & Korbonits, M. (2002) The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab*, 87,pp 2988.
- Granata, R., Settanni, F., Biancone, L., Trovato, L., Nano, R., Bertuzzi, F., Destefanis, S., Annunziata, M., Martinetti, M., Catapano, F., Ghe, C., Isgaard, J., Papotti, M., Ghigo, E. & Muccioli, G. (2007) Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3',5'-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling. *Endocrinology*, 148,pp 512-529.
- Grinspoon, S., Miller, K.K., Herzog, D.B., Grieco, K.A. & Klibanski, A. (2004) Effects of estrogen and recombinant human insulin-like growth factor-I on ghrelin secretion in severe undernutrition. *J Clin Endocrinol Metab*, 89,pp 3988-3993.
- Gropp, E., Shanabrough, M., Borok, E., Xu, A.W., Janoschek, R., Buch, T., Plum, L., Balthasar, N., Hampel, B., Waisman, A., Barsh, G.S., Horvath, T.L. & Bruning, J.C. (2005) Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci*, 8,pp 1289-1291.
- Groschl, M., Knerr, I., Topf, H.G., Schmid, P., Rascher, W. & Rauh, M. (2003) Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. *J Endocrinol*, 179,pp 237-244.
- Grube, D. & Forssmann, W.G. (1979) Morphology and function of the entero-endocrine cells. *Horm Metab Res*, 11,pp 589-606.
- Gruendel, S., Otto, B., Garcia, A.L., Wagner, K., Mueller, C., Weickert, M.O., Heldwein, W. & Koebnick, C. (2007) Carob pulp preparation rich in insoluble dietary fibre and polyphenols increases plasma glucose and serum insulin responses in combination with a glucose load in humans. *Br J Nutr*, 98,pp 101-105.
- Gualillo, O., Caminos, J., Blanco, M., Garcia-Caballero, T., Kojima, M., Kangawa, K., Dieguez, C. & Casanueva, F. (2001) Ghrelin, a novel placental-derived hormone. *Endocrinology*, 142,pp 788-794.

- Guan, X.M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J., Smith, R.G., Van der Ploeg, L.H. & Howard, A.D. (1997) Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res*, 48,pp 23-29.
- Gutierrez, J.A., Solenberg, P.J., Perkins, D.R., Willency, J.A., Knierman, M.D., Jin, Z., Witcher, D.R., Luo, S., Onyia, J.E. & Hale, J.E. (2008) Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A*, 105,pp 6320-6325.
- Hahn, T.M., Breininger, J.F., Baskin, D.G. & Schwartz, M.W. (1998) Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci*, 1,pp 271-272.
- Hansen, T.K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J.S. & Jorgensen, J.O. (2002) Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol (Oxf)*, 56,pp 203-206.
- Hansson, C., Haage, D., Taube, M., Egecioglu, E., Salome, N. & Dickson, S.L. (2011) Central administration of ghrelin alters emotional responses in rats: behavioural, electrophysiological and molecular evidence. *Neuroscience*, 180,pp 201-211.
- Harada, T., Nakahara, T., Yasuhara, D., Kojima, S., Sagiya, K., Amitani, H., Laviano, A., Naruo, T. & Inui, A. (2008) Obestatin, acyl ghrelin, and des-acyl ghrelin responses to an oral glucose tolerance test in the restricting type of anorexia nervosa. *Biol Psychiatry*, 63,pp 245-247.
- Hardie, D.G. (2004) The AMP-activated protein kinase pathway--new players upstream and downstream. *J Cell Sci*, 117,pp 5479-5487.
- Hayashida, T., Nakahara, K., Mondal, M.S., Date, Y., Nakazato, M., Kojima, M., Kangawa, K. & Murakami, N. (2002) Ghrelin in neonatal rats: distribution in stomach and its possible role. *J Endocrinol*, 173,pp 239-245.
- Holst, B., Cygankiewicz, A., Jensen, T.H., Ankersen, M. & Schwartz, T.W. (2003) High constitutive signaling of the ghrelin receptor--identification of a potent inverse agonist. *Mol Endocrinol*, 17,pp 2201-2210.
- Holst, B., Egerod, K.L., Schild, E., Vickers, S.P., Cheetham, S., Gerlach, L.O., Storjohann, L., Stidsen, C.E., Jones, R., Beck-Sickinger, A.G. & Schwartz, T.W. (2007) GPR39 signaling is stimulated by zinc ions but not by obestatin. *Endocrinology*, 148,pp 13-20.
- Holst, B., Holliday, N.D., Bach, A., Elling, C.E., Cox, H.M. & Schwartz, T.W. (2004) Common structural basis for constitutive activity of the ghrelin receptor family. *J Biol Chem*, 279,pp 53806-53817.
- Holst, B., Lang, M., Brandt, E., Bach, A., Howard, A., Frimurer, T.M., Beck-Sickinger, A. & Schwartz, T.W. (2006) Ghrelin receptor inverse agonists: identification of an active peptide core and its interaction epitopes on the receptor. *Mol Pharmacol*, 70,pp 936-946.
- Holst, B. & Schwartz, T.W. (2006) Ghrelin receptor mutations--too little height and too much hunger. *J Clin Invest*, 116,pp 637-641.
- Hosoda, H., Kojima, M., Matsuo, H. & Kangawa, K. (2000a) Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun*, 279,pp 909-913.

- Hosoda, H., Kojima, M., Matsuo, H. & Kangawa, K. (2000b) Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. *J Biol Chem*, 275,pp 21995-22000.
- Hosoda, H., Kojima, M., Mizushima, T., Shimizu, S. & Kangawa, K. (2003) Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *J Biol Chem*, 278,pp 64-70.
- Howard, A.D., Feighner, S.D., Cully, D.F., Arena, J.P., Liberators, P.A., Rosenblum, C.I., Hamelin, M., Hreniuk, D.L., Palyha, O.C., Anderson, J., Paress, P.S., Diaz, C., Chou, M., Liu, K.K., McKee, K.K., Pong, S.S., Chaung, L.Y., Elbrecht, A., Dashkevich, M., Heavens, R., Rigby, M., Sirinathsinghji, D.J., Dean, D.C., Melillo, D.G., Patchett, A.A., Nargund, R., Griffin, P.R., DeMartino, J.A., Gupta, S.K., Schaeffer, J.M., Smith, R.G. & Van der Ploeg, L.H. (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science*, 273,pp 974-977.
- Huang, J., Zhou, H., Mahavadi, S., Sriwai, W., Lyall, V. & Murthy, K.S. (2005) Signaling pathways mediating gastrointestinal smooth muscle contraction and MLC20 phosphorylation by motilin receptors. *Am J Physiol Gastrointest Liver Physiol*, 288,pp G23-31.
- Iglesias, M.J., Pineiro, R., Blanco, M., Gallego, R., Dieguez, C., Gualillo, O., Gonzalez-Juanatey, J.R. & Lago, F. (2004) Growth hormone releasing peptide (ghrelin) is synthesized and secreted by cardiomyocytes. *Cardiovasc Res*, 62,pp 481-488.
- Iwase, M., Kanazawa, H., Kato, Y., Nishizawa, T., Somura, F., Ishiki, R., Nagata, K., Hashimoto, K., Takagi, K., Izawa, H. & Yokota, M. (2004) Growth hormone-releasing peptide can improve left ventricular dysfunction and attenuate dilation in dilated cardiomyopathic hamsters. *Cardiovasc Res*, 61,pp 30-38.
- Jeffery, P.L., Duncan, R.P., Yeh, A.H., Jaskolski, R.A., Hammond, D.S., Herington, A.C. & Chopin, L.K. (2005) Expression of the ghrelin axis in the mouse: an exon 4-deleted mouse proghrelin variant encodes a novel C terminal peptide. *Endocrinology*, 146,pp 432-440.
- Jerlhag, E., Eggecioglu, E., Dickson, S.L., Andersson, M., Svensson, L. & Engel, J.A. (2006) Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addict Biol*, 11,pp 45-54.
- Jerlhag, E., Eggecioglu, E., Dickson, S.L., Douhan, A., Svensson, L. & Engel, J.A. (2007) Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict Biol*, 12,pp 6-16.
- Jethwa, P.H., Smith, K.L., Small, C.J., Abbott, C.R., Darch, S.J., Murphy, K.G., Seth, A., Semjonous, N.M., Patel, S.R., Todd, J.F., Ghatei, M.A. & Bloom, S.R. (2006) Neuromedin U partially mediates leptin-induced hypothalamo-pituitary adrenal (HPA) stimulation and has a physiological role in the regulation of the HPA axis in the rat. *Endocrinology*, 147,pp 2886-2892.
- Jorgensen, J.O. & Christiansen, J.S. (1993) Brave new senescence: GH in adults. *Lancet*, 341,pp 1247-1248.

- Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H. & Oikawa, S. (2004) Effects of insulin, leptin, and glucagon on ghrelin secretion from isolated perfused rat stomach. *Regul Pept*, 119, pp 77-81.
- Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H. & Wakabayashi, I. (2001) Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes*, 50, pp 2438-2443.
- Kanamoto, N., Akamizu, T., Hosoda, H., Hataya, Y., Ariyasu, H., Takaya, K., Hosoda, K., Saijo, M., Moriyama, K., Shimatsu, A., Kojima, M., Kangawa, K. & Nakao, K. (2001) Substantial production of ghrelin by a human medullary thyroid carcinoma cell line. *J Clin Endocrinol Metab*, 86, pp 4984-4990.
- Kanamoto, N., Akamizu, T., Tagami, T., Hataya, Y., Moriyama, K., Takaya, K., Hosoda, H., Kojima, M., Kangawa, K. & Nakao, K. (2004) Genomic structure and characterization of the 5'-flanking region of the human ghrelin gene. *Endocrinology*, 145, pp 4144-4153.
- Kanehisa, M., Akiyoshi, J., Kitaichi, T., Matsushita, H., Tanaka, E., Kodama, K., Hanada, H. & Isogawa, K. (2006) Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. *Prog Neuropsychopharmacol Biol Psychiatry*, 30, pp 1403-1407.
- Katakami, H., Arimura, A. & Frohman, L.A. (1986) Growth hormone (GH)-releasing factor stimulates hypothalamic somatostatin release: an inhibitory feedback effect on GH secretion. *Endocrinology*, 118, pp 1872-1877.
- Katayama, T., Shimamoto, S., Oda, H., Nakahara, K., Kangawa, K. & Murakami, N. (2007) Glucagon receptor expression and glucagon stimulation of ghrelin secretion in rat stomach. *Biochem Biophys Res Commun*, 357, pp 865-870.
- Katugampola, S.D., Pallikaros, Z. & Davenport, A.P. (2001) [125I-His(9)]-ghrelin, a novel radioligand for localizing GHS orphan receptors in human and rat tissue: up-regulation of receptors with atherosclerosis. *Br J Pharmacol*, 134, pp 143-149.
- Kellokoski, E., Poykko, S.M., Karjalainen, A.H., Ukkola, O., Heikkinen, J., Kesaniemi, Y.A. & Horkko, S. (2005) Estrogen replacement therapy increases plasma ghrelin levels. *J Clin Endocrinol Metab*, 90, pp 2954-2963.
- Kim, M.S., Yoon, C.Y., Jang, P.G., Park, Y.J., Shin, C.S., Park, H.S., Ryu, J.W., Pak, Y.K., Park, J.Y., Lee, K.U., Kim, S.Y., Lee, H.K., Kim, Y.B. & Park, K.S. (2004) The mitogenic and antiapoptotic actions of ghrelin in 3T3-L1 adipocytes. *Mol Endocrinol*, 18, pp 2291-2301.
- Kishimoto, M., Okimura, Y., Nakata, H., Kudo, T., Iguchi, G., Takahashi, Y., Kaji, H. & Chihara, K. (2003) Cloning and characterization of the 5'(-)-flanking region of the human ghrelin gene. *Biochem Biophys Res Commun*, 305, pp 186-192.
- Kluge, M., Schussler, P., Schmid, D., Uhr, M., Kleyer, S., Yassouridis, A. & Steiger, A. (2009) Ghrelin plasma levels are not altered in major depression. *Neuropsychobiology*, 59, pp 199-204.
- Knerr, I., Groschl, M., Rascher, W. & Rauh, M. (2003) Endocrine effects of food intake: insulin, ghrelin, and leptin responses to a single bolus of essential amino acids in humans. *Ann Nutr Metab*, 47, pp 312-318.

- Kohno, D., Gao, H.Z., Muroya, S., Kikuyama, S. & Yada, T. (2003) Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca<sup>2+</sup> signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes*, 52,pp 948-956.
- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. & Kangawa, K. (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402,pp 656-660.
- Kola, B., Hubina, E., Tucci, S.A., Kirkham, T.C., Garcia, E.A., Mitchell, S.E., Williams, L.M., Hawley, S.A., Hardie, D.G., Grossman, A.B. & Korbonsits, M. (2005) Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J Biol Chem*, 280,pp 25196-25201.
- Koo, G.C., Huang, C., Camacho, R., Trainor, C., Blake, J.T., Sirotina-Meisher, A., Schleim, K.D., Wu, T.J., Cheng, K., Nargund, R. & McKissick, G. (2001) Immune enhancing effect of a growth hormone secretagogue. *J Immunol*, 166,pp 4195-4201.
- Korbonsits, M., Bustin, S.A., Kojima, M., Jordan, S., Adams, E.F., Lowe, D.G., Kangawa, K. & Grossman, A.B. (2001a) The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. *J Clin Endocrinol Metab*, 86,pp 881-887.
- Korbonsits, M., Kojima, M., Kangawa, K. & Grossman, A.B. (2001b) Presence of ghrelin in normal and adenomatous human pituitary. *Endocrine*, 14,pp 101-104.
- Kristensson, E., Sundqvist, M., Hakanson, R. & Lindstrom, E. (2007) High gastrin cell activity and low ghrelin cell activity in high-anxiety Wistar Kyoto rats. *J Endocrinol*, 193,pp 245-250.
- Lambrinoudaki, I.V., Christodoulakos, G.E., Economou, E.V., Vlachou, S.A., Panoulis, C.P., Alexandrou, A.P., Kouskouni, E.E. & Creatsas, G.C. (2008) Circulating leptin and ghrelin are differentially influenced by estrogen/progestin therapy and raloxifene. *Maturitas*, 59,pp 62-71.
- Larhammar, D. (1996) Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. *Regul Pept*, 62,pp 1-11.
- Lauwers, E., Landuyt, B., Arckens, L., Schoofs, L. & Luyten, W. (2006) Obestatin does not activate orphan G protein-coupled receptor GPR39. *Biochem Biophys Res Commun*, 351,pp 21-25.
- Levin, F., Edholm, T., Schmidt, P.T., Gryback, P., Jacobsson, H., Degerblad, M., Hoybye, C., Holst, J.J., Rehfeld, J.F., Hellstrom, P.M. & Naslund, E. (2006) Ghrelin stimulates gastric emptying and hunger in normal-weight humans. *J Clin Endocrinol Metab*, 91,pp 3296-3302.
- Li, L., Zhang, L.K., Pang, Y.Z., Pan, C.S., Qi, Y.F., Chen, L., Wang, X., Tang, C.S. & Zhang, J. (2006) Cardioprotective effects of ghrelin and des-octanoyl ghrelin on myocardial injury induced by isoproterenol in rats. *Acta Pharmacol Sin*, 27,pp 527-535.
- Li, W.G., Gavrila, D., Liu, X., Wang, L., Gunnlaugsson, S., Stoll, L.L., McCormick, M.L., Sigmund, C.D., Tang, C. & Weintraub, N.L. (2004) Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. *Circulation*, 109,pp 2221-2226.

- Lin, Y., Matsumura, K., Fukuhara, M., Kagiya, S., Fujii, K. & Iida, M. (2004) Ghrelin acts at the nucleus of the solitary tract to decrease arterial pressure in rats. *Hypertension*, 43,pp 977-982.
- Locatelli, V., Rossoni, G., Schweiger, F., Torsello, A., De Gennaro Colonna, V., Bernareggi, M., Deghenghi, R., Muller, E.E. & Berti, F. (1999) Growth hormone-independent cardioprotective effects of hexarelin in the rat. *Endocrinology*, 140,pp 4024-4031.
- Lopez, M., Lage, R., Saha, A.K., Perez-Tilve, D., Vazquez, M.J., Varela, L., Sangiao-Alvarellos, S., Tovar, S., Raghay, K., Rodriguez-Cuenca, S., Deoliveira, R.M., Castaneda, T., Datta, R., Dong, J.Z., Culler, M., Sleeman, M.W., Alvarez, C.V., Gallego, R., Lelliott, C.J., Carling, D., Tschop, M.H., Dieguez, C. & Vidal-Puig, A. (2008) Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab*, 7,pp 389-399.
- Lu, S., Guan, J.L., Wang, Q.P., Uehara, K., Yamada, S., Goto, N., Date, Y., Nakazato, M., Kojima, M., Kangawa, K. & Shioda, S. (2002) Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. *Neurosci Lett*, 321,pp 157-160.
- Luque, R.M., Gahete, M.D., Hochgeschwender, U. & Kineman, R.D. (2006) Evidence that endogenous SST inhibits ACTH and ghrelin expression by independent pathways. *Am J Physiol Endocrinol Metab*, 291,pp E395-403.
- Luquet, S., Perez, F.A., Hnasko, T.S. & Palmiter, R.D. (2005) NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science*, 310,pp 683-685.
- Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S.A., Anderson, J.G., Jung, S., Birnbaum, S., Yanagisawa, M., Elmquist, J.K., Nestler, E.J. & Zigman, J.M. (2008) The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci*, 11,pp 752-753.
- Luttrell, L.M. & Lefkowitz, R.J. (2002) The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J Cell Sci*, 115,pp 455-465.
- Makovey, J., Naganathan, V., Seibel, M. & Sambrook, P. (2007) Gender differences in plasma ghrelin and its relations to body composition and bone - an opposite-sex twin study. *Clin Endocrinol (Oxf)*, 66,pp 530-537.
- Masuda, Y., Tanaka, T., Inomata, N., Ohnuma, N., Tanaka, S., Itoh, Z., Hosoda, H., Kojima, M. & Kangawa, K. (2000) Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun*, 276,pp 905-908.
- Matsubara, M., Sakata, I., Wada, R., Yamazaki, M., Inoue, K. & Sakai, T. (2004) Estrogen modulates ghrelin expression in the female rat stomach. *Peptides*, 25,pp 289-297.
- Matsuda, K., Miura, T., Kaiya, H., Maruyama, K., Shimakura, S., Uchiyama, M., Kangawa, K. & Shioda, S. (2006) Regulation of food intake by acyl and des-acyl ghrelins in the goldfish. *Peptides*, 27,pp 2321-2325.
- Matsumura, K., Tsuchihashi, T., Fujii, K., Abe, I. & Iida, M. (2002) Central ghrelin modulates sympathetic activity in conscious rabbits. *Hypertension*, 40,pp 694-699.
- Mau, S.E., Witt, M.R., Bjerrum, O.J., Saermark, T. & Vilhardt, H. (1995) Growth hormone releasing hexapeptide (GHRP-6) activates the inositol (1,4,5)-trisphosphate/diacylglycerol pathway in rat anterior pituitary cells. *J Recept Signal Transduct Res*, 15,pp 311-323.



- Mazzocchi, G., Neri, G., Rucinski, M., Rebuffat, P., Spinazzi, R., Malendowicz, L.K. & Nussdorfer, G.G. (2004) Ghrelin enhances the growth of cultured human adrenal zona glomerulosa cells by exerting MAPK-mediated proliferogenic and antiapoptotic effects. *Peptides*, 25,pp 1269-1277.
- McKee, K.K., Palyha, O.C., Feighner, S.D., Hreniuk, D.L., Tan, C.P., Phillips, M.S., Smith, R.G., Van der Ploeg, L.H. & Howard, A.D. (1997a) Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. *Mol Endocrinol*, 11,pp 415-423.
- McKee, K.K., Tan, C.P., Palyha, O.C., Liu, J., Feighner, S.D., Hreniuk, D.L., Smith, R.G., Howard, A.D. & Van der Ploeg, L.H. (1997b) Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics*, 46,pp 426-434.
- McKeown, M. (1992) Alternative mRNA splicing. *Annu Rev Cell Biol*, 8,pp 133-155.
- Monteleone, P., Bencivenga, R., Longobardi, N., Serritella, C. & Maj, M. (2003) Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. *J Clin Endocrinol Metab*, 88,pp 5510-5514.
- Morello, J.P. & Bouvier, M. (1996) Palmitoylation: a post-translational modification that regulates signalling from G-protein coupled receptors. *Biochem Cell Biol*, 74,pp 449-457.
- Mori, K., Yoshimoto, A., Takaya, K., Hosoda, K., Ariyasu, H., Yahata, K., Mukoyama, M., Sugawara, A., Hosoda, H., Kojima, M., Kangawa, K. & Nakao, K. (2000) Kidney produces a novel acylated peptide, ghrelin. *FEBS Lett*, 486,pp 213-216.
- Mousseaux, D., Le Gallic, L., Ryan, J., Oiry, C., Gagne, D., Fehrentz, J.A., Galleyrand, J.C. & Martinez, J. (2006) Regulation of ERK1/2 activity by ghrelin-activated growth hormone secretagogue receptor 1A involves a PLC/PKC $\epsilon$  pathway. *Br J Pharmacol*, 148,pp 350-365.
- Muccioli, G., Tschop, M., Papotti, M., Deghenghi, R., Heiman, M. & Ghigo, E. (2002) Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. *Eur J Pharmacol*, 440,pp 235-254.
- Murata, M., Okimura, Y., Iida, K., Matsumoto, M., Sowa, H., Kaji, H., Kojima, M., Kangawa, K. & Chihara, K. (2002) Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. *J Biol Chem*, 277,pp 5667-5674.
- Murray, C.D., Martin, N.M., Patterson, M., Taylor, S.A., Ghatei, M.A., Kamm, M.A., Johnston, C., Bloom, S.R. & Emmanuel, A.V. (2005) Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut*, 54,pp 1693-1698.
- Nagaya, N., Kojima, M., Uematsu, M., Yamagishi, M., Hosoda, H., Oya, H., Hayashi, Y. & Kangawa, K. (2001a) Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regul Integr Comp Physiol*, 280,pp R1483-1487.
- Nagaya, N., Moriya, J., Yasumura, Y., Uematsu, M., Ono, F., Shimizu, W., Ueno, K., Kitakaze, M., Miyatake, K. & Kangawa, K. (2004) Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. *Circulation*, 110,pp 3674-3679.
- Nagaya, N., Uematsu, M., Kojima, M., Date, Y., Nakazato, M., Okumura, H., Hosoda, H., Shimizu, W., Yamagishi, M., Oya, H., Koh, H., Yutani, C. & Kangawa, K. (2001b)

- Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation*, 104,pp 2034-2038.
- Nagaya, N., Uematsu, M., Kojima, M., Ikeda, Y., Yoshihara, F., Shimizu, W., Hosoda, H., Hirota, Y., Ishida, H., Mori, H. & Kangawa, K. (2001c) Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation*, 104,pp 1430-1435.
- Nakahara, K., Nakagawa, M., Baba, Y., Sato, M., Toshinai, K., Date, Y., Nakazato, M., Kojima, M., Miyazato, M., Kaiya, H., Hosoda, H., Kangawa, K. & Murakami, N. (2006) Maternal ghrelin plays an important role in rat fetal development during pregnancy. *Endocrinology*, 147,pp 1333-1342.
- Nakashima, K., Akiyoshi, J., Hatano, K., Hanada, H., Tanaka, Y., Tsuru, J., Matsushita, H., Kodama, K. & Isogawa, K. (2008) Ghrelin gene polymorphism is associated with depression, but not panic disorder. *Psychiatr Genet*, 18,pp 257.
- Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K. & Matsukura, S. (2001) A role for ghrelin in the central regulation of feeding. *Nature*, 409,pp 194-198.
- Naleid, A.M., Grace, M.K., Cummings, D.E. & Levine, A.S. (2005) Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides*, 26,pp 2274-2279.
- Nishi, Y., Hiejima, H., Hosoda, H., Kaiya, H., Mori, K., Fukue, Y., Yanase, T., Nawata, H., Kangawa, K. & Kojima, M. (2005) Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. *Endocrinology*, 146,pp 2255-2264.
- Nogueiras, R., Tovar, S., Mitchell, S.E., Barrett, P., Rayner, D.V., Dieguez, C. & Williams, L.M. (2006) Negative energy balance and leptin regulate neuromedin-U expression in the rat pars tuberalis. *J Endocrinol*, 190,pp 545-553.
- Ogaya, M., Kim, J. & Sasaki, K. (2011) Ghrelin postsynaptically depolarizes dorsal raphe neurons in rats in vitro. *Peptides*, 32,pp 1606-1616.
- Olszanecka-Glinianowicz, M., Kocelak, P., Wikarek, T., Gruszka, W., Dabrowski, P., Chudek, J. & Zahorska-Markiewicz, B. (2010) Are plasma ghrelin and PYY concentrations associated with obesity-related depression? *Endokrynol Pol*, 61,pp 174-177.
- Ong, H., Bodart, V., McNicoll, N., Lamontagne, D. & Bouchard, J.F. (1998a) Binding sites for growth hormone-releasing peptide. *Growth Horm IGF Res*, 8 Suppl B,pp 137-140.
- Ong, H., McNicoll, N., Escher, E., Collu, R., Deghenghi, R., Locatelli, V., Ghigo, E., Muccioli, G., Boghen, M. & Nilsson, M. (1998b) Identification of a pituitary growth hormone-releasing peptide (GHRP) receptor subtype by photoaffinity labeling. *Endocrinology*, 139,pp 432-435.
- Otto, B., Cuntz, U., Fruehauf, E., Wawarta, R., Folwaczny, C., Riepl, R.L., Heiman, M.L., Lehnert, P., Fichter, M. & Tschop, M. (2001) Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol*, 145,pp 669-673.
- Ozawa, A., Cai, Y. & Lindberg, I. (2007) Production of bioactive peptides in an in vitro system. *Anal Biochem*, 366,pp 182-189.

- Palyha, O.C., Feighner, S.D., Tan, C.P., McKee, K.K., Hreniuk, D.L., Gao, Y.D., Schleim, K.D., Yang, L., Morriello, G.J., Nargund, R., Patchett, A.A., Howard, A.D. & Smith, R.G. (2000) Ligand activation domain of human orphan growth hormone (GH) secretagogue receptor (GHS-R) conserved from Pufferfish to humans. *Mol Endocrinol*, 14,pp 160-169.
- Pantel, J., Legendre, M., Cabrol, S., Hilal, L., Hajaji, Y., Morisset, S., Nivot, S., Vie-Luton, M.P., Grouselle, D., de Kerdanet, M., Kadiri, A., Epelbaum, J., Le Bouc, Y. & Amselem, S. (2006) Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest*, 116,pp 760-768.
- Papotti, M., Cassoni, P., Volante, M., Deghenghi, R., Muccioli, G. & Ghigo, E. (2001) Ghrelin-producing endocrine tumors of the stomach and intestine. *J Clin Endocrinol Metab*, 86,pp 5052-5059.
- Papotti, M., Ghe, C., Cassoni, P., Catapano, F., Deghenghi, R., Ghigo, E. & Muccioli, G. (2000) Growth hormone secretagogue binding sites in peripheral human tissues. *J Clin Endocrinol Metab*, 85,pp 3803-3807.
- Patchett, A.A., Nargund, R.P., Tata, J.R., Chen, M.H., Barakat, K.J., Johnston, D.B., Cheng, K., Chan, W.W., Butler, B., Hickey, G. & et al. (1995) Design and biological activities of L-163,191 (MK-0677): a potent, orally active growth hormone secretagogue. *Proc Natl Acad Sci U S A*, 92,pp 7001-7005.
- Patel, A.D., Stanley, S.A., Murphy, K.G., Frost, G.S., Gardiner, J.V., Kent, A.S., White, N.E., Ghatei, M.A. & Bloom, S.R. (2006) Ghrelin stimulates insulin-induced glucose uptake in adipocytes. *Regul Pept*, 134,pp 17-22.
- Pedretti, A., Villa, M., Pallavicini, M., Valoti, E. & Vistoli, G. (2006) Construction of human ghrelin receptor (hGHS-R1a) model using a fragmental prediction approach and validation through docking analysis. *J Med Chem*, 49,pp 3077-3085.
- Peeters, T.L. (2006) Potential of ghrelin as a therapeutic approach for gastrointestinal motility disorders. *Curr Opin Pharmacol*, 6,pp 553-558.
- Penicaud, L., Leloup, C., Fioramonti, X., Lorsignol, A. & Benani, A. (2006) Brain glucose sensing: a subtle mechanism. *Curr Opin Clin Nutr Metab Care*, 9,pp 458-462.
- Petersenn, S. (2002) Structure and regulation of the growth hormone secretagogue receptor. *Minerva Endocrinol*, 27,pp 243-256.
- Poinot-Chazel, C., Portier, M., Bouaboula, M., Vita, N., Pecceu, F., Gully, D., Monroe, J.G., Maffrand, J.P., Le Fur, G. & Casellas, P. (1996) Activation of mitogen-activated protein kinase couples neurotensin receptor stimulation to induction of the primary response gene Krox-24. *Biochem J*, 320 ( Pt 1),pp 145-151.
- Poitras, P., Polvino, W.J. & Rocheleau, B. (2005) Gastrokinetic effect of ghrelin analog RC-1139 in the rat. Effect on post-operative and on morphine induced ileus. *Peptides*, 26,pp 1598-1601.
- Pong, S.S., Chung, L.Y., Dean, D.C., Nargund, R.P., Patchett, A.A. & Smith, R.G. (1996) Identification of a new G-protein-linked receptor for growth hormone secretagogues. *Mol Endocrinol*, 10,pp 57-61.
- Prado, C.L., Pugh-Bernard, A.E., Elghazi, L., Sosa-Pineda, B. & Sussel, L. (2004) Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci U S A*, 101,pp 2924-2929.

- Purnell, J.Q., Weigle, D.S., Breen, P. & Cummings, D.E. (2003) Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *J Clin Endocrinol Metab*, 88,pp 5747-5752.
- Qi, X., Reed, J., Englander, E.W., Chandrashekar, V., Bartke, A. & Greeley, G.H., Jr. (2003) Evidence that growth hormone exerts a feedback effect on stomach ghrelin production and secretion. *Exp Biol Med (Maywood)*, 228,pp 1028-1032.
- Quarta, D., Di Francesco, C., Melotto, S., Mangiarini, L., Heidbreder, C. & Hedou, G. (2009) Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. *Neurochem Int*, 54,pp 89-94.
- Rindi, G., Necchi, V., Savio, A., Torsello, A., Zoli, M., Locatelli, V., Raimondo, F., Cocchi, D. & Solcia, E. (2002) Characterisation of gastric ghrelin cells in man and other mammals: studies in adult and fetal tissues. *Histochem Cell Biol*, 117,pp 511-519.
- Root, A.W. & Root, M.J. (2002) Clinical pharmacology of human growth hormone and its secretagogues. *Curr Drug Targets Immune Endocr Metabol Disord*, 2,pp 27-52.
- Rossi, F., Bertone, C., Petricca, S. & Santemma, V. (2007) Ghrelin inhibits angiotensin II-induced migration of human aortic endothelial cells. *Atherosclerosis*, 192,pp 291-297.
- Rouach, V., Bloch, M., Rosenberg, N., Gilad, S., Limor, R., Stern, N. & Greenman, Y. (2007) The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat. *Psychoneuroendocrinology*, 32,pp 693-702.
- Saad, M.F., Bernaba, B., Hwu, C.M., Jinagouda, S., Fahmi, S., Kogosov, E. & Boyadjian, R. (2002) Insulin regulates plasma ghrelin concentration. *J Clin Endocrinol Metab*, 87,pp 3997-4000.
- Sakata, I., Nakamura, K., Yamazaki, M., Matsubara, M., Hayashi, Y., Kangawa, K. & Sakai, T. (2002) Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract. *Peptides*, 23,pp 531-536.
- Sakata, I., Tanaka, T., Yamazaki, M., Tanizaki, T., Zheng, Z. & Sakai, T. (2006) Gastric estrogen directly induces ghrelin expression and production in the rat stomach. *J Endocrinol*, 190,pp 749-757.
- Sarret, P., Gendron, L., Kilian, P., Nguyen, H.M., Gallo-Payet, N., Payet, M.D. & Beaudet, A. (2002) Pharmacology and functional properties of NTS2 neurotensin receptors in cerebellar granule cells. *J Biol Chem*, 277,pp 36233-36243.
- Schanze, A., Reulbach, U., Scheuchenzuber, M., Groschl, M., Kornhuber, J. & Kraus, T. (2008) Ghrelin and eating disturbances in psychiatric disorders. *Neuropsychobiology*, 57,pp 126-130.
- Schwartz, T.W., Frimurer, T.M., Holst, B., Rosenkilde, M.M. & Elling, C.E. (2006) Molecular mechanism of 7TM receptor activation--a global toggle switch model. *Annu Rev Pharmacol Toxicol*, 46,pp 481-519.
- Sedlackova, D., Dostalova, I., Hainer, V., Beranova, L., Kvasnickova, H., Hill, M., Haluzik, M. & Nedvidkova, J. (2008) Simultaneous decrease of plasma obestatin and ghrelin levels after a high-carbohydrate breakfast in healthy women. *Physiol Res*, 57 Suppl 1,pp S29-37.

- Seoane, L.M., Al-Massadi, O., Barreiro, F., Dieguez, C. & Casanueva, F.F. (2007) Growth hormone and somatostatin directly inhibit gastric ghrelin secretion. An in vitro organ culture system. *J Endocrinol Invest*, 30,pp RC22-25.
- Shiiba, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M.S., Tanaka, M., Nozoe, S., Hosoda, H., Kangawa, K. & Matsukura, S. (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab*, 87,pp 240-244.
- Shimada, M., Date, Y., Mondal, M.S., Toshinai, K., Shimbara, T., Fukunaga, K., Murakami, N., Miyazato, M., Kangawa, K., Yoshimatsu, H., Matsuo, H. & Nakazato, M. (2003) Somatostatin suppresses ghrelin secretion from the rat stomach. *Biochem Biophys Res Commun*, 302,pp 520-525.
- Shimizu, Y., Nagaya, N., Isobe, T., Imazu, M., Okumura, H., Hosoda, H., Kojima, M., Kangawa, K. & Kohno, N. (2003) Increased plasma ghrelin level in lung cancer cachexia. *Clin Cancer Res*, 9,pp 774-778.
- Shintani, M., Ogawa, Y., Ebihara, K., Aizawa-Abe, M., Miyanaga, F., Takaya, K., Hayashi, T., Inoue, G., Hosoda, K., Kojima, M., Kangawa, K. & Nakao, K. (2001) Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes*, 50,pp 227-232.
- Silva, A.P., Bethmann, K., Raulf, F. & Schmid, H.A. (2005) Regulation of ghrelin secretion by somatostatin analogs in rats. *Eur J Endocrinol*, 152,pp 887-894.
- Smith, R.G., Cheng, K., Schoen, W.R., Pong, S.S., Hickey, G., Jacks, T., Butler, B., Chan, W.W., Chaung, L.Y., Judith, F. & et al. (1993) A nonpeptidyl growth hormone secretagogue. *Science*, 260,pp 1640-1643.
- Smith, R.G., Van der Ploeg, L.H., Howard, A.D., Feighner, S.D., Cheng, K., Hickey, G.J., Wyvrat, M.J., Jr., Fisher, M.H., Nargund, R.P. & Patchett, A.A. (1997) Peptidomimetic regulation of growth hormone secretion. *Endocr Rev*, 18,pp 621-645.
- Solcia, E., Capella, C., Vassallo, G. & Buffa, R. (1975) Endocrine cells of the gastric mucosa. *Int Rev Cytol*, 42,pp 223-286.
- Sun, Y., Wang, P., Zheng, H. & Smith, R.G. (2004) Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc Natl Acad Sci U S A*, 101,pp 4679-4684.
- Tack, J., Depoortere, I., Bisschops, R., Delpoort, C., Coulie, B., Meulemans, A., Janssens, J. & Peeters, T. (2006) Influence of ghrelin on interdigestive gastrointestinal motility in humans. *Gut*, 55,pp 327-333.
- Tack, J., Depoortere, I., Bisschops, R., Verbeke, K., Janssens, J. & Peeters, T. (2005) Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Aliment Pharmacol Ther*, 22,pp 847-853.
- Tacke, F., Brabant, G., Kruck, E., Horn, R., Schoffski, P., Hecker, H., Manns, M.P. & Trautwein, C. (2003) Ghrelin in chronic liver disease. *J Hepatol*, 38,pp 447-454.
- Tan, C.P., McKee, K.K., Liu, Q., Palyha, O.C., Feighner, S.D., Hreniuk, D.L., Smith, R.G. & Howard, A.D. (1998) Cloning and characterization of a human and murine T-cell orphan G-protein-coupled receptor similar to the growth hormone secretagogue and neurotensin receptors. *Genomics*, 52,pp 223-229.

- Tanaka, M., Hayashida, Y., Iguchi, T., Nakao, N., Nakai, N. & Nakashima, K. (2001a) Organization of the mouse ghrelin gene and promoter: occurrence of a short noncoding first exon. *Endocrinology*, 142,pp 3697-3700.
- Tanaka, M., Hayashida, Y., Nakao, N., Nakai, N. & Nakashima, K. (2001b) Testis-specific and developmentally induced expression of a ghrelin gene-derived transcript that encodes a novel polypeptide in the mouse. *Biochim Biophys Acta*, 1522,pp 62-65.
- Tannenbaum, G.S., Epelbaum, J. & Bowers, C.Y. (2003) Interrelationship between the novel peptide ghrelin and somatostatin/growth hormone-releasing hormone in regulation of pulsatile growth hormone secretion. *Endocrinology*, 144,pp 967-974.
- Taub, D.D. (2008) Novel connections between the neuroendocrine and immune systems: the ghrelin immunoregulatory network. *Vitam Horm*, 77,pp 325-346.
- Temel, Y., Boothman, L.J., Blokland, A., Magill, P.J., Steinbusch, H.W., Visser-Vandewalle, V. & Sharp, T. (2007) Inhibition of 5-HT neuron activity and induction of depressive-like behavior by high-frequency stimulation of the subthalamic nucleus. *Proc Natl Acad Sci U S A*, 104,pp 17087-17092.
- Tena-Sempere, M., Barreiro, M.L., Gonzalez, L.C., Gaytan, F., Zhang, F.P., Caminos, J.E., Pinilla, L., Casanueva, F.F., Dieguez, C. & Aguilar, E. (2002) Novel expression and functional role of ghrelin in rat testis. *Endocrinology*, 143,pp 717-725.
- Tesauro, M., Schinzari, F., Iantorno, M., Rizza, S., Melina, D., Lauro, D. & Cardillo, C. (2005) Ghrelin improves endothelial function in patients with metabolic syndrome. *Circulation*, 112,pp 2986-2992.
- Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueiras, R., Perez-Tilve, D., Pfluger, P., Castaneda, T.R., Muzzin, P., Schurmann, A., Szanto, I., Tschop, M.H. & Rohner-Jeanrenaud, F. (2006) Ghrelin action in the brain controls adipocyte metabolism. *J Clin Invest*, 116,pp 1983-1993.
- Tomasetto, C., Wendling, C., Rio, M.C. & Poitras, P. (2001) Identification of cDNA encoding motilin related peptide/ghrelin precursor from dog fundus. *Peptides*, 22,pp 2055-2059.
- Toshinai, K., Date, Y., Murakami, N., Shimada, M., Mondal, M.S., Shimbara, T., Guan, J.L., Wang, Q.P., Funahashi, H., Sakurai, T., Shioda, S., Matsukura, S., Kangawa, K. & Nakazato, M. (2003) Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology*, 144,pp 1506-1512.
- Toshinai, K., Yamaguchi, H., Sun, Y., Smith, R.G., Yamanaka, A., Sakurai, T., Date, Y., Mondal, M.S., Shimbara, T., Kawagoe, T., Murakami, N., Miyazato, M., Kangawa, K. & Nakazato, M. (2006) Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. *Endocrinology*, 147,pp 2306-2314.
- Traebert, M., Riediger, T., Whitebread, S., Scharrer, E. & Schmid, H.A. (2002) Ghrelin acts on leptin-responsive neurones in the rat arcuate nucleus. *J Neuroendocrinol*, 14,pp 580-586.
- Tremblay, F., Perreault, M., Klaman, L.D., Tobin, J.F., Smith, E. & Gimeno, R.E. (2007) Normal food intake and body weight in mice lacking the G protein-coupled receptor GPR39. *Endocrinology*, 148,pp 501-506.

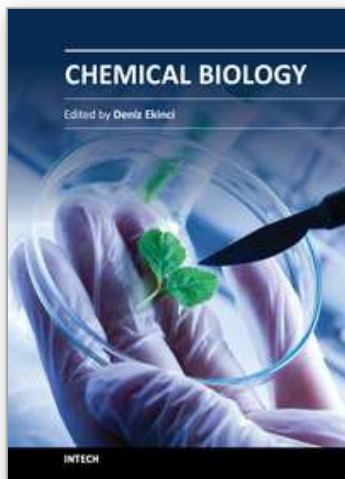
- Trudel, L., Tomasetto, C., Rio, M.C., Bouin, M., Plourde, V., Eberling, P. & Poitras, P. (2002) Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. *Am J Physiol Gastrointest Liver Physiol*, 282,pp G948-952.
- Tschop, M., Smiley, D.L. & Heiman, M.L. (2000) Ghrelin induces adiposity in rodents. *Nature*, 407,pp 908-913.
- Tschop, M., Statnick, M.A., Suter, T.M. & Heiman, M.L. (2002) GH-releasing peptide-2 increases fat mass in mice lacking NPY: indication for a crucial mediating role of hypothalamic agouti-related protein. *Endocrinology*, 143,pp 558-568.
- Tschop, M., Wawarta, R., Riepl, R.L., Friedrich, S., Bidlingmaier, M., Landgraf, R. & Folwaczny, C. (2001a) Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest*, 24,pp RC19-21.
- Tschop, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E. & Heiman, M.L. (2001b) Circulating ghrelin levels are decreased in human obesity. *Diabetes*, 50,pp 707-709.
- Tsolakis, A.V., Portela-Gomes, G.M., Stridsberg, M., Grimelius, L., Sundin, A., Eriksson, B.K., Oberg, K.E. & Janson, E.T. (2004) Malignant gastric ghrelinoma with hyperghrelinemia. *J Clin Endocrinol Metab*, 89,pp 3739-3744.
- Ueno, M., Carnevali, J.B., Oliveira, R.L., Velloso, L.A. & Saad, M.J. (2006) Circulating ghrelin concentrations are lowered by intracerebroventricular insulin. *Diabetologia*, 49,pp 2449-2452.
- Venkova, K., Fraser, G., Hoveyda, H.R. & Greenwood-Van Meerveld, B. (2007) Prokinetic effects of a new ghrelin receptor agonist TZP-101 in a rat model of postoperative ileus. *Dig Dis Sci*, 52,pp 2241-2248.
- Vincent, J.P., Mazella, J. & Kitabgi, P. (1999) Neurotensin and neurotensin receptors. *Trends Pharmacol Sci*, 20,pp 302-309.
- Volante, M., Allia, E., Gugliotta, P., Funaro, A., Broglio, F., Deghenghi, R., Muccioli, G., Ghigo, E. & Papotti, M. (2002) Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *J Clin Endocrinol Metab*, 87,pp 1300-1308.
- Wang, H.J., Geller, F., Dempfle, A., Schauble, N., Friedel, S., Lichtner, P., Fontenla-Horro, F., Wudy, S., Hagemann, S., Gortner, L., Huse, K., Renschmidt, H., Bettecken, T., Meitinger, T., Schafer, H., Hebebrand, J. & Hinney, A. (2004) Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J Clin Endocrinol Metab*, 89,pp 157-162.
- Wang, W.G., Chen, X., Jiang, H. & Jiang, Z.Y. (2008) Effects of ghrelin on glucose-sensing and gastric distension sensitive neurons in rat dorsal vagal complex. *Regul Pept*, 146,pp 169-175.
- Wei, W., Wang, G., Qi, X., Englander, E.W. & Greeley, G.H., Jr. (2005) Characterization and regulation of the rat and human ghrelin promoters. *Endocrinology*, 146,pp 1611-1625.
- Wierup, N., Svensson, H., Mulder, H. & Sundler, F. (2002) The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept*, 107,pp 63-69.

- Wierup, N., Yang, S., McEvelly, R.J., Mulder, H. & Sundler, F. (2004) Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *J Histochem Cytochem*, 52,pp 301-310.
- Willesen, M.G., Kristensen, P. & Romer, J. (1999) Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology*, 70,pp 306-316.
- Xu, G., Li, Y., An, W., Li, S., Guan, Y., Wang, N., Tang, C., Wang, X., Zhu, Y., Li, X., Mulholland, M.W. & Zhang, W. (2009) Gastric mammalian target of rapamycin signaling regulates ghrelin production and food intake. *Endocrinology*, 150,pp 3637-3644.
- Xu, G., Li, Y., An, W., Zhao, J., Xiang, X., Ding, L., Li, Z., Guan, Y., Wang, X., Tang, C., Zhu, Y., Wang, N., Li, X., Mulholland, M. & Zhang, W. (2010) Regulation of gastric hormones by systemic rapamycin. *Peptides*, 31,pp 2185-2192.
- Xu, X., Jhun, B.S., Ha, C.H. & Jin, Z.G. (2008) Molecular mechanisms of ghrelin-mediated endothelial nitric oxide synthase activation. *Endocrinology*, 149,pp 4183-4192.
- Xu, X., Pang, J., Yin, H., Li, M., Hao, W., Chen, C. & Cao, J.M. (2007) Hexarelin suppresses cardiac fibroblast proliferation and collagen synthesis in rat. *Am J Physiol Heart Circ Physiol*, 293,pp H2952-2958.
- Yabuki, A., Ojima, T., Kojima, M., Nishi, Y., Mifune, H., Matsumoto, M., Kamimura, R., Masuyama, T. & Suzuki, S. (2004) Characterization and species differences in gastric ghrelin cells from mice, rats and hamsters. *J Anat*, 205,pp 239-246.
- Yamagishi, S.I., Edelstein, D., Du, X.L., Kaneda, Y., Guzman, M. & Brownlee, M. (2001) Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem*, 276,pp 25096-25100.
- Yang, J., Brown, M.S., Liang, G., Grishin, N.V. & Goldstein, J.L. (2008a) Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell*, 132,pp 387-396.
- Yang, J., Zhao, T.J., Goldstein, J.L. & Brown, M.S. (2008b) Inhibition of ghrelin O-acyltransferase (GOAT) by octanoylated pentapeptides. *Proc Natl Acad Sci U S A*, 105,pp 10750-10755.
- Yeh, A.H., Jeffery, P.L., Duncan, R.P., Herington, A.C. & Chopin, L.K. (2005) Ghrelin and a novel preproghrelin isoform are highly expressed in prostate cancer and ghrelin activates mitogen-activated protein kinase in prostate cancer. *Clin Cancer Res*, 11,pp 8295-8303.
- Yoshimoto, A., Mori, K., Sugawara, A., Mukoyama, M., Yahata, K., Suganami, T., Takaya, K., Hosoda, H., Kojima, M., Kangawa, K. & Nakao, K. (2002) Plasma ghrelin and desacyl ghrelin concentrations in renal failure. *J Am Soc Nephrol*, 13,pp 2748-2752.
- Zhang, J.V., Ren, P.G., Avsian-Kretchmer, O., Luo, C.W., Rauch, R., Klein, C. & Hsueh, A.J. (2005) Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, 310,pp 996-999.
- Zhang, W., Chai, B., Li, J.Y., Wang, H. & Mulholland, M.W. (2008) Effect of des-acyl ghrelin on adiposity and glucose metabolism. *Endocrinology*, 149,pp 4710-4716.
- Zhang, W., Zhao, L., Lin, T.R., Chai, B., Fan, Y., Gantz, I. & Mulholland, M.W. (2004) Inhibition of adipogenesis by ghrelin. *Mol Biol Cell*, 15,pp 2484-2491.



- Zhang, W., Zhao, L. & Mulholland, M.W. (2007) Ghrelin stimulates myocyte development. *Cell Physiol Biochem*, 20,pp 659-664.
- Zhao, Z., Sakata, I., Okubo, Y., Koike, K., Kangawa, K. & Sakai, T. (2008) Gastric leptin, but not estrogen and somatostatin, contributes to the elevation of ghrelin mRNA expression level in fasted rats. *J Endocrinol*, 196,pp 529-538.
- Zhu, X., Cao, Y., Voogd, K. & Steiner, D.F. (2006) On the processing of proghrelin to ghrelin. *J Biol Chem*, 281,pp 38867-38870.
- Zigman, J.M., Jones, J.E., Lee, C.E., Saper, C.B. & Elmquist, J.K. (2006) Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol*, 494,pp 528-548.
- Zigman, J.M., Nakano, Y., Coppari, R., Balthasar, N., Marcus, J.N., Lee, C.E., Jones, J.E., Deysher, A.E., Waxman, A.R., White, R.D., Williams, T.D., Lachey, J.L., Seeley, R.J., Lowell, B.B. & Elmquist, J.K. (2005) Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J Clin Invest*, 115,pp 3564-3572.
- Zizzari, P., Longchamps, R., Epelbaum, J. & Bluet-Pajot, M.T. (2007) Obestatin partially affects ghrelin stimulation of food intake and growth hormone secretion in rodents. *Endocrinology*, 148,pp 1648-1653.

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## **Chemical Biology**

Edited by Prof. Deniz Ekinci

ISBN 978-953-51-0049-2

Hard cover, 444 pages

**Publisher** InTech

**Published online** 17, February, 2012

**Published in print edition** February, 2012

Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

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Ziru Li, Jie Luo, Yin Li and Weizhen Zhang (2012). Ghrelin, a Gastric Hormone with Diverse Functions, Chemical Biology, Prof. Deniz Ekinci (Ed.), ISBN: 978-953-51-0049-2, InTech, Available from: <http://www.intechopen.com/books/chemical-biology/ghrelin-a-gastric-hormone-with-diverse-functions>

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