Introduction

The increasing prevalence of Metabolic Syndrome (MetS) and the consequent cardiovascular diseases, like atherosclerotic disease and type 2 diabetes has stimulated an active search for novel risk factors. The hormones regulating energy balance are of special interest as potential risk factors for MetS and Type 2 diabetes ***(Ukkola, 2011).***Ghrelin is a natural endogenous ligand of the growth hormone (GH) secretagogue receptor and initially identified as a strong stimulant for the release of GH. Subsequent research has shown that ghrelin and its various receptors are ubiquitous in many other organs and tissues. Moreover they participate in the regulation of appetite, energy, body weight, metabolism of glucoseand fat,as well as modulation of gastrointestinal, cardiovascular, pulmonary, immune functions and cell proliferation/apoptosis. Increasing evidence has demonstrated that Ghrelin has a close relationship with cardiovascular system***(Gaigai et al., 2010).*** Endothelial dysfunction is considered to be one of the earliest events of the atherosclerosis development. In patients with endothelial dysfunction, plasma Ghrelin levels decrease ***(Tajtakova et al., 2005).***The cardioprotective effects of ghrelin are independent of GH release and likely involve binding to cardiovascular receptors, a process that is upregulated during ischemia/reperfusion ***(Chang et al., 2004).***

Studies have suggested multi-protective effects of ghrelin in heart failure. Animal experiments show that Ghrelin can evoke significant decrease in mean arterial pressure (MAP) in normal, chronic heart failure (CHF) and GH deficient rats and similar effects are observed in humans. In healthy volunteers and patients with CHF, Ghrelin can decrease vascularresistance, increase cardiac index and stroke volume ***(Nagaya et al., 2001)***.This action may be related to its direct vasodilatory effects, as well as inhibition of the sympathetic activity. Moreover, in both normal and hypertrophied myocardial cells, Ghrelin inhibits contraction, relaxation and premature relaxation so ghrelin may also improve myocardial function by reducing myocardial oxygen consumption ***(Gaigai et al., 2010).***

Aim of The work

Is to study the relationship between serum levels of ghrelin in patients withType 2 diabetes and in patients with chronic heart failure.

GHRELIN

With the discovery of the anorexigenic (appetite-inhibiting) and adipostatic hormone leptin, studies focused on defining neural circuits responsible for mediating leptin actions in the brain ***(Saper et al., 2002)***. The hypothalamus is the center for the integration of feeding and associated neuroendocrine, autonomic and gastrointestinal activities. Ghrelin, identified as an endogenous ligand for the growth hormone secretagogue (GHS) receptor ***(Kojima et al., 1999)***, functions as an orexigenic (appetite-stimulating) signal from the stomach when an increase in metabolic efficiency is necessary. Ghrelin regulates, in an antagonistic manner to leptin, synthesis and secretion of several neuropeptides in the hypothalamus that regulate feeding and associated hypothalamic functions ***(Asakawa et al., 2001)***.

Growth Hormone Secretagogues(GHSs):

Growth hormone (GH), a multifunctional hormone secreted from somatotrophs of the anterior pituitary, regulates overall body and cell growth, carbohydrate-protein-lipid metabolism, and water-electrolyte balance ***(Argetsinger and Carter-Su, 1996).***

The pulsatile release of growth hormone (GH) from the anterior pituitary gland is regulated by tight inter-play between the hypothalamic growth hormone-releasing hormone (GHRH) and somatostatin. GHRH stimulates GH synthesis and release whereas somatostatin inhibits the release. However, several other neurotransmitters and neuropeptides are assumed to play a role in the control of GH secretion ***(Smith et al., 1997)***.

In the past two decades, particular attention has been given to a new family of substances, named GH secretagogues (GHSs), which show a powerful GH-releasing effect. GHSs are non-natural, synthetic compounds developed by Bowers and co-workers that directly stimulate GH secretion ***(Bowers, 2001)***. The prototype GH-releasing peptide 6 (GHRP-6) has been used as a diagnostic tool since before the isolation of GHRH in 1982

(Guillemin et al., 1982). Orally active GHSs have been proposed as an alternative to GH, insulin-like growth factor I (IGF-I) and GHRH as a growth-promoting treatment in GH- deficient children, as well as an anabolic treatment in elderly patients with somatopause***(Ghigo et al., 2001)***.

GHSs act through specific G-protein-coupled receptor(s) distinct from that of GHRH and exert a powerful synergism when administered in addition to GHRH. The GHS receptor type Ia (GHS-RIa) transduces the GH-releasing effect of GHSs, whereas GHS-RIb is a nonspliced, nonfunctional receptor mRNA variant. GHS-RIa operates through activation of the phospholipase-IP3 pathway and inhibition of K channels, leading to a rise in intracellular Ca2. GHS-RIa is predominantly expressed in the pituitary and hypothalamus and at low levels in other brain regions such as ventral tegmental area, substantianigra, nucleus tractussolitarius, and hippocampus as well as peripheral tissues such as heart, lung, pancreas, intestine, kidney, and adipose tissue. It has been speculated that GHSs mimic an as yet unidentified endogenous hormone reflecting the presence of an additional neuroendocrine system regulating pulsatile GH secretion ***(Gnanapavan et al., 2002)***.

Purification and Identification of Ghrelin:

Recently, Kojima and Kangawa identified the 28 amino acid peptide ghrelin as an endogenous ligand for the "orphan" GHS receptor. ***Ghre*** is the proto-Indo-European root of the word ***growth***. Although most people assumed that the greatest concentrations would be in the hypothalamus, the highest GHS receptor activation was found in stomach extracts. A cultured cell line expressing the GHS-R was established and used to identify tissue extracts that could stimulate the GHS-R, as monitored by increases in intracellular Ca^{2+} levels. After screening several tissues, very strong activity was unexpectedly found in stomach extracts ***(Kojima et al., 1999)***.Indeed, Tomassetto and co-workers identified it separately from the stomach as the motilin-related

peptide ***(Tomasetto et al., 2000)***, with structural and effect-related similarities to the duodenal hormone motilin that has a key role in the regulation of gastrointestinal motility ***(Asakawa et al., 2001)***. Motilin was also known to have a GH-releasing effect ***(Samson et al., 1984)***. Based on structural and effect-related similarities, motilin and ghrelin are considered to represent a novel gastrointestinal hormone family in addition to gastrin, secretin, pancreatic polypeptide (PP), insulin, epidermal growth factor (EGF), and tachykinin families ***(Ahlman and Nilsson, 2001)***.

Two molecular forms of ghrelin are found in the stomach: the 28 amino acid ghrelin having n-octanoylated serine in position 3 and the 27 amino acid des-[Gln14)] ghrelin produced by alternative splicing of the ghrelin gene ***(Hosoda et al., 2000)***. The acylation appears to be essential for GH-releasing activity in both natural forms of ghrelin although des-acyl ghrelin may have some biological activities such as those acting as a survival factor on the cardiovascular system ***(Baldanzi et al., 2002)***. Other minor forms of ghrelin are present in human plasma and stomach, measured as the total immunoreactivity by the conventional radioimmunoassay based on the carboxyl- terminal fragment of ghrelin ***(Hosoda et al., 2003)***. Studies of structure activity show that amino-terminal fragments conserving the first five amino acids of the molecule display full functional activity ***(Bednarek et al., 2000)***.

It is known that structural heterogeneity among species can be of major importance for motilin***(Walsh et al., 1994)***. Five or more positions in the 22 amino acid structure of motilin may differ among mammalian species. The situation is more complex in rats in which motilin is not identified and exogenous motilin administration does not reproduce the gastrointestinal motor effect of the peptide. However, structural heterogeneity of ghrelin appears minor ***(Tomasetto et al., 2001)***, and human ghrelin is identical to rat ghrelin except for two residues ***(Kojima et al., 1999)***.

Distribution of Ghrelin:

A. Measurement of Ghrelin Concentration:

The active form of ghrelin is acyl-modified; this modification is easily cleaved during sample extraction. Moreover, peptide samples are easily digested by many proteases in cells. Thus, to measure ghrelin concentrations correctly in plasma and tissues, we have to inhibit protease digestion of the ghrelin peptide and cleavage of its acyl-modification.

To measure the plasma concentration of ghrelin, it is necessary to use EDTA and aprotinin when collecting blood samples ***(Hosoda et al., 2004)***. After the samples are centrifuged, the plasma fraction should be collected and treated with 1/10 volume of 1 N HCl. The treated plasma should be kept in the freezer (−20 to −80°C). These samples are stable for at least 6–12 mo.

To measure the tissue concentration of ghrelin, it is sufficient to inactivate proteases by boiling the tissues in water for 5–10 min ***(Sudoh et al., 1988)***. This simple method is sufficient to keep active ghrelin intact.

Two major forms of ghrelin are found in tissues and plasma: *n*-octanoyl-modified and des-acyl ghrelin ***(Hosoda et al., 2000)***. The normal ghrelin concentration of plasma samples in humans is 10–20 fmol/ml for *n*-octanoyl ghrelin and 100–150 fmol/ml for total ghrelin, including both acyl-modified and des-acyl ghrelins. Plasma ghrelin concentration is increased in fasting conditions and reduced after habitual feeding ***(Tschöp et al., 2001a)***, suggesting that ghrelin may be as an initiation signal for food intake or ghrelin secretion is controlled by some nutritional factors in blood. Plasma ghrelin levels were lower in obese subjects than the age-matched lean controls ***(Hansen et al., 2002)***. Moreover, plasma ghrelin concentrations were significantly lower in Pima Indians, who are prone to develop insulin resistance and obesity, than in Caucasians (87 ± 28 vs. 129 ± 34 fmol/ml; $P< 0.01$) ***(Tschöp et al., 2001a)***. However, it is unclear whether

changes in plasma ghrelin concentration can influence the characteristics of obese people or Pima Indians.

B. Stomach and Gastrointestinal Organs:

In all vertebrate species, ghrelin is mainly produced in the stomach ***(Ariyasu et al., 2001)***. In the stomach, ghrelin-containing cells are more abundant in the fundus than in the pylorus ***(Yabuki et al., 2004)***. In situ hybridization and immunohistochemical analyses indicate that ghrelin-containing cells are a distinct endocrine cell type found in the mucosal layer of the stomach (Fig. 1) ***(Rindi et al., 2002)***.

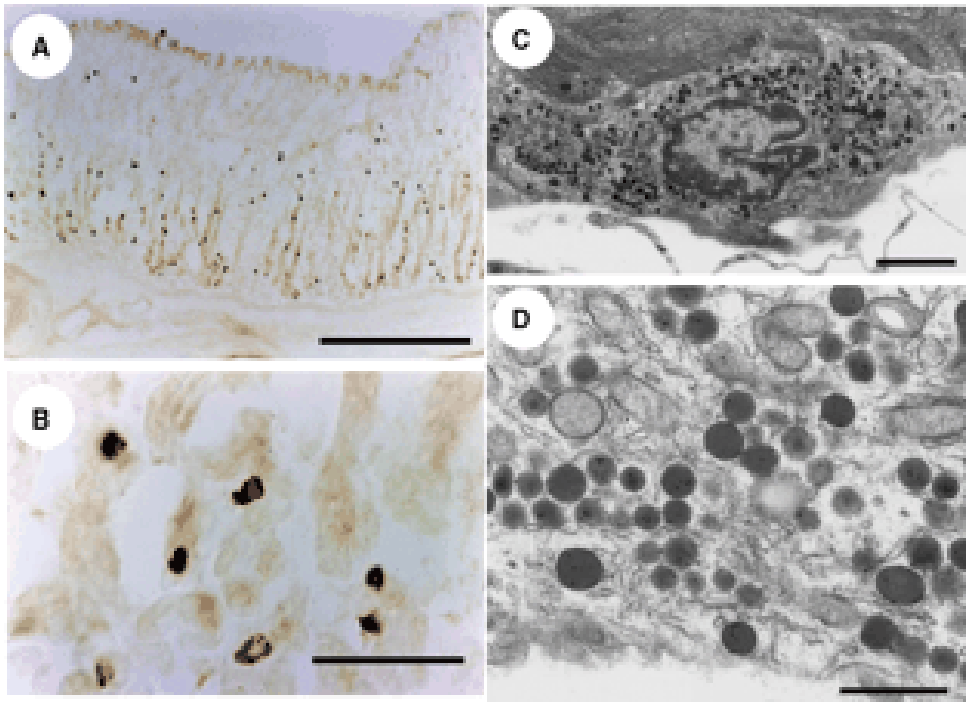

Fig. 1.: Ghrelin cells in the stomach. *A*: ghrelin-immunoreactive cells in the stomach are found from the neck to the base of the oxyntic gland. Scale bar, 400 μm. This distribution pattern is typical for gastric endocrine cells. *B*: high magnification of *A*. Scale bar, 40 μm. *C* and *D*: representative immunoelectron photographs of a ghrelin-producing cell in the oxyntic gland. *C*: this ovoid cell has many round, compact, electron-dense granules in its cytoplasm. Scale bar, 2 μm. *D*: high magnification of *C*. Scale bar, 500 nm. Granules in the cytoplasm are labeled with immunogold staining for ghrelin. [Adapted from Kojima et al. (1999) and Date et al. (2000).

C. Brain and Pituitary:

Since the ghrelin receptor GHS-R is mainly expressed in the hypothalamus and pituitary, its endogenous ligand has been thought to exist mainly in the hypothalamic regions ***(Guan et al., 1997)***. This is supported by the finding that another GH-releasing peptide, GHRH, is produced in the hypothalamus and is secreted into the hypophysial portal system to stimulate GH release from the pituitary somatotrophs. However, the ghrelin content of the brain is found to be very low ***(Hosoda et al., 2000)***.

Ghrelin has been found in the hypothalamic arcuate nucleus, an important region for controlling appetite ***(Lu et al., 2002)***. In addition, a recent study has reported the presence of ghrelin in previously uncharacterized hypothalamic neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei ***(Cowley et al., 2003)***. These ghrelin-containing neurons send efferent fibers to neurons that contain neuropeptide Y (NPY) and agouti-related protein (AgRP) and may stimulate the release of these orexigenic peptides. These localization patterns of ghrelin suggest a role in controlling food intake. In fact, injection of ghrelin into the cerebral ventricles of rats potently stimulates food intake.

GH-releasing somatotrophs in the pituitary gland are the target cells of ghrelin. In an in vivo assay, ghrelin stimulated primary pituitary cells and increased their intracellular Ca^{2+} concentration, indicating that the GHS-R is expressed in pituitary cells ***(McKee et al., 1997)***. Also, ghrelin has been found in the pituitary gland itself ***(Korbonits et al., 2001)***, where it may influence the release of GH in an autocrine or paracrine manner. The expression level of ghrelin in the pituitary is high after birth and declines with puberty. Pituitary tumors, such as adenomas, corticotroph tumors, and gonadotroph tumors contain ghrelin peptides.

D. Other Tissues:

Ghrelin mRNA is expressed in the kidney, especially in the glomeruli ***(Gnanapavan et al., 2002)***. Moreover, peptide extracts

from mouse kidney contain both *n*-octanoyl and des-acyl ghrelin peptides in significant amounts. The plasma ghrelin concentration was significantly correlated with the serum creatinine level and was increased 2.8-fold in patients with end-stage renal disease compared with those in patients with normal renal function ***(Yoshimoto et al., 2002)***. This result suggests that the kidney is an important site for clearance and/or degradation of ghrelin.

Ghrelin-immunoreactive cells were detectable in cytotrophoblast cells in first-trimester human placenta but were undetectable in third-trimester placenta ***(Gualillo et al., 2001)***. Ghrelin-containing cells were also detected in syncytiotrophoblast cells of the human placenta and in the cytoplasm of labyrinth trophoblasts of the rat placenta. Placental ghrelin mRNA was undetectable during early pregnancy, with a sharp peak of expression at *day 16* that decreases in the later stages of gestation.

Ghrelin immunoreactive cells have been identified in interstitial Leydig cells and in Sertoli cells ***(Tena-Sempere et al., 2002)***. However, ghrelin levels in Sertoli cells are very low. Moreover, the ghrelin receptor has been detected in germ cells, mainly in pachytene spermatocytes, as well as in somatic Sertoli and Leydig cells ***(Gaytan et al., 2004)***.

E. Ghrelin-Producing Cells:

Several cultured cell lines express ghrelin. Ghrelin is produced in TT cells, a human thyroid medullary carcinoma cell line ***(Kanamoto et al., 2001)***. TT cells express ghrelin mRNA, and both conditioned medium and cellular extracts of TT cells contain ghrelin peptides. As in the stomach, cellular extracts of TT cells contain both *n*-octanoyl ghrelin and des-acyl ghrelin. Other cultured cells that express ghrelin include 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)***.

Corebetta et al. (2003) reported a patient with a malignant neuroendocrine pancreatic tumor with ghrelin

immunoreactivityand a high circulating ghrelin level. A patient with a metastasizing gastric neuroendocrine tumor was also reported to have extremely high circulating levels of ghrelin ***(Tsolakis et al., 2004)***. In the latter case, the patient developed diabetes mellitus and hypothyroidism. However, it is not clear whether high ghrelin level had the pathophysiological role in these symptoms. In both cases, GH and IGF-I levels were within the normal range, and the patients had no clinical features of acromegaly.

Ghrelin Receptors:

A. The Ghrelin Receptor Family:

Ghrelin receptor, or GHS-R, is a typical GPCR with seven transmembrane domains (7-TM). Two distinct ghrelin receptor cDNAs have been isolated. The first, GHS-R type 1a, encodes a 7-TM GPCR with binding and functional properties consistent with its role as ghrelin's receptor. This type 1a receptor has features characteristic of a typical GPCR, including conserved cysteine residues in the first two extracellular loops, several potential sites for posttranslational modifications (*N*-linked glycosylation and phosphorylation), and an aromatic triplet sequence (E/DRY) located immediately after TM-3 in the second intracellular loop.

Another GHS-R cDNA, type 1b, is produced by an alternative splicing mechanism ***(Howard et al., 1996)***. The GHS-R gene consists of two exons; the first exon encodes TM-1 to TM-5, and the second exon encodes TM-6 to TM-7. Type 1b is derived from only the first exon and encodes only five of the seven predicted TM domains. The type 1b receptor is thus a COOH-terminal truncated form of the type 1a receptor and is pharmacologically inactive.

The GHS-R has several homologs, whose endogenous ligands are gastrointestinal peptides or neuropeptides. Figure 2 shows a dendrogram alignment of the ghrelin receptor superfamily. This superfamily contains receptors for ghrelin,

motilin, neuromedin U ***(Howard et al., 2000)***, and neurotensin***(Vincent et al., 1999)***. All of these peptides are found in gastrointestinal organs and regulate gastrointestinal movement and other functions. This family also contains an orphan receptor, GPR39, whose ligand is also likely to be a gastrointestinal peptide ***(McKee et al., 1997)***.

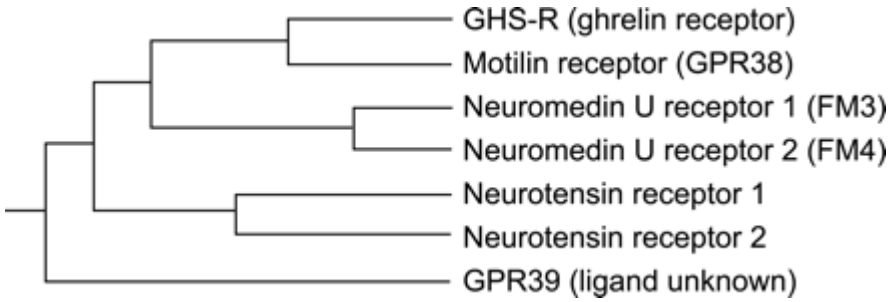

FIG. 2. Dendrogram alignment of ghrelin receptor (GHS-R) and other GPCRs. The ghrelin receptor is part of a GPCR superfamily that contains the motilin, neuromedin U and neurotensin receptors, and is most homologous to the motilin receptor. Because their endogenous ligands, ghrelin and motilin, have partly homologous amino acid sequences, the ghrelin and motilin systems may have evolved from a common ancestral system. This superfamily also contains an orphan receptor, GPR39, whose endogenous ligand is expected to be a peptide.

The ghrelin receptor is most homologous to the motilin receptor; the human forms share 52% identical amino acids ***(Smith et al., 2001)***. Moreover, their ligands, ghrelin and motilin peptides, have similar amino acid sequences. Preliminary studies have shown that motilin can stimulate the ghrelin receptor, albeit at a low level. In contrast, ghrelin does not activate motilin receptor ***(Dass et al., 2003)***.

The ghrelin receptor is well conserved across all vertebrate species examined, including a number of mammals, chicken, and pufferfish (Fugu) ***(Smith et al., 2001)***. This strict conservation suggests that ghrelin and its receptor serve important physiological functions.

It is suggested that a novel unidentified subtype of ghrelin receptor exists. Ghrelin binding activity is demonstrated in 3T3-L1 cells by radiolabeled ghrelin, although RT-PCR detected no

signal for the ghrelin receptor ***(Zhang et al., 2004)***. Moreover, both ghrelin and des-acyl ghrelin bind to H9c2 cardiomyocytes, which do not express the ghrelin receptor ***(Baldanzi et al., 2002)***. However, BLAST searches of the human genome using ghrelin receptor (GHS-R) cDNA as a search sequence have not revealed any ghrelin receptor homologs. Further study is required to search for an as-yet-unidentified ghrelin receptor subtype.

One case of familial short stature associated with a missense mutation in the ghrelin receptor has been reported. This mutation changed a single amino acid, resulting in a charge change at a highly conserved extracellular position. This mutated ghrelin receptor shows severely impaired ghrelin binding ***(Pantel et al., 2006)***.

B. Ghrelin Receptor Activation and Downstream Signal Transduction Pathways

Two endogenous GH-releasing peptides have been identified, ghrelin and GHRH. GHRH acts on the GHRH receptor to activate adenylatecyclase and increase intracellular cAMP, which serves as a second messenger to activate protein kinase A. This indicates that the GHRH receptor is coupled to a G_s subunit. On the other hand, ghrelin acts on the GHS-R and activates phospholipase C to generate IP_3 and diacylglycerol, resulting in an increase of intracellular Ca^{2+}, indicating that the ghrelin receptor is coupled to a G_q subunit.

The signal transduction pathway following ghrelin receptor activation was investigated using HepG2, a hepatoma cell line that responds to ghrelin ***(Murata et al., 2002)***. Ghrelin upregulates several activities that are also potentiated by insulin, including tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), association of the adaptor-molecule growth factor receptor-bound protein 2 with IRS-1 and stimulates mitogen-activated protein kinase activity. However, unlike insulin, ghrelin inhibits Akt kinase and partially reverses the downregulating

effect of insulin on phospho*enol*pyruvatecarboxykinase (PEPCK) mRNA expression, a rate-limiting enzyme of gluconeogenesis.

C. Ghrelin Receptor Distribution:

Ghrelin receptor mRNA is prominently expressed in the arcuate (ARC) and ventromedial nuclei (VMN) and in the hippocampus ***(Howard et al., 1996)***. The ghrelin receptor is highly sensitive to GH; its expression is increased in GH-deficient *dw*/*dw* dwarf rats, and treatment of these rats with GH decreases ghrelin receptor expression ***(Lawrence et al., 2002)***. GHS-R mRNA is also detected in multiple hypothalamic nuclei and in the pituitary, as well as the dentate gyrus, CA2, and CA3 regions of the hippocampus, the substantianigra, the ventral tegmental area, and the dorsal and median raphe nuclei.

RT-PCR analyses demonstrated ghrelin receptor mRNA expression in many peripheral organs, including heart, lung, liver, kidney, pancreas, stomach, small and large intestines, adipose tissue, and immune cells ***(Gnanapavan et al., 2002)***, indicating that ghrelin has multiple functions in these tissues ***(Broglio et al., 2003a)***.

The existence of ghrelin and its receptor in the hippocampus***(Lawrence et al., 2002)***, a region that is associated with learning and memory, suggest the role of ghrelin in memrory formation. In fact, intracerebroventricular injection of ghrelin induced c-Fos expression in the hippocampal C1, CA2, and C3 regions, indicating that the ghrelin receptor is active in that region ***(Nakazato et al., 2001)***. The involvement of ghrelin in memory was investigated using open-field, plus-maze, and step-down tests of inhibitory avoidance ***(Carlini et al., 2002)***. Ghrelin administration increased freezing in the open field and decreased the number of entries into open spaces and the time spent on the open arms in the plus-maze, indicating that ghrelin has an anxiogenic effect. Moreover, ghrelin increased in a dose-dependent manner the latency time in the step-down test, suggesting that it increases memory retention.

Physiological Functions of Ghrelin:

A. GH-Releasing Activity:

Ghrelin is a multifaceted peptide hormone. Ghrelin acts on the GHS-R, increasing intracellular Ca^{2+} concentration via IP_3 to stimulate GH release. In terms of both the area under the curve and mean peak GH levels, the GH-releasing activity of ghrelin is similar to that of GHRH when injected intravenously into rats ***(Takaya et al., 2000)***. However, the maximal stimulation effected by ghrelin is two to three times greater than that of GHRH ***(Arvat et al., 2000)***.

Ghrelin stimulates GH release both in vitro and in vivo in a dose-dependent manner (Fig. 3) ***(Arvat et al., 2000)***. Intravenous injection of ghrelin induces potent GH release both in rats and in humans. When anesthetized rats were injected intravenously with ghrelin, an increase in plasma GH concentration was observed [basal level: 12.0 ± 5.4 ng/ml; after ghrelin injection: 129.7 ± 11.3 (SE) ng/ml] ***(Kojima et al., 1999)***. GH release peaks at ~5–15 min after ghrelin injection and returns to basal levels 1 h later. A single intracerebroventricular administration of ghrelin also increased rat plasma GH concentration in a dose-dependent manner, with a minimum dose of only 10 pmol***(Date et al., 2000)***. Thus intracerebroventricular injection appears to be a more potent route of delivery than intravenous administration. Ghrelin has also been shown to induce GH release in nonmammalian vertebrates, including chicken ***(Baudet and Harvey, 2003)***, fish ***(Kaiya et al., 2003)***, and frog ***(Kaiya et al., 2001)***. Together, these in vivo assays confirmed that ghrelin is a potent GH-releasing peptide. In addition, high doses of ghrelin in humans increase ACTH, prolactin, and cortisol levels ***(Arvat et al., 2001)***.

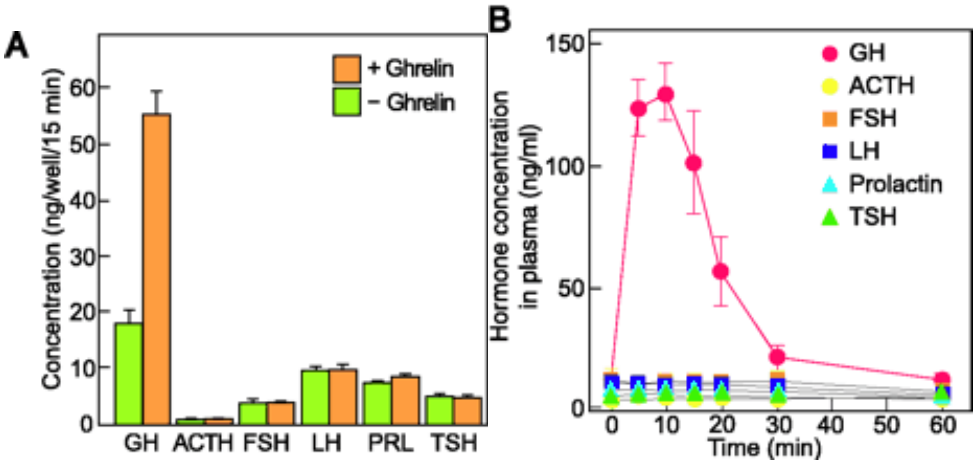

Fig. 3. Effects of ghrelin on pituitary hormone secretion in vitro and in vivo.*A*: effects of a high dose (10^{-6} M) of ghrelin on hormone secretion from rat primary pituitary cells in vitro. *B*: time courses of plasma hormone concentrations after intravenous injection of ghrelin into male rats in vivo. ACTH, adrenocorticotropin; FSH, follicle-stimulating hormone; LH, lutenizing hormone; PRL, prolactin; TSH, thyroid-stimulating hormone. [Adapted from Kojima et al. 1999]

Ghrelin stimulates GH release from primary pituitary cells, which indicates that ghrelin can act directly on the pituitary ***(Kojima et al., 1999)***. However, the involvement of the hypothalamus in ghrelin-mediated stimulation of GH release has been strongly suggested. Patients with organic lesions in the hypothalamic region showed insufficiency of GH release even when stimulated by ghrelin ***(Popovic et al., 2003)***. Moreover, when using primary pituitary cells, the ghrelin treatment only increased GH release by two to three times above the basal level ***(Kojima et al., 1999)***, which is lower than the level of induction seen when ghrelin is administered to rats in vivo. These facts suggest that other factors are involved in vivo in order for this maximal level of GH release to be achieved by ghrelin administration. One possibility is transmission via the vagus nerve. When the vagus nerve is cut, the induction of GH release after ghrelin injection is dramatically decreased ***(Date et al., 2002)***, indicating that the vagus nerve is needed for the maximal stimulatory effects of ghrelin. Another possibility is the lack of GHRH in primary pituitary cells. Coadministration of ghrelin and GHRH had a synergistic effect on GH secretion; that is,