

Ghrelin

Serdar Güler

Gül Gürsoy

Bekir Çakır

Ankara Education and Research Hospital, Department of Internal Medicine, Ankara, Turkey

Growth hormone secretagogues are synthetic peptidyl and non-peptidyl molecules that possess growth hormone releasing activity. Ghrelin is an endogenous natural ligand for the growth hormone secretagogue receptor that has recently been isolated from the rat stomach. Ghrelin administration stimulates growth hormone secretion but also causes weight gain by increasing food intake. Ghrelin's structural features, secretion patterns, secretion sites, mechanisms of action and interactions and its possible involvement in obesity are still investigated.

Key words: Ghrelin, growth hormone, growth hormone secretagogues

Ghrelin

The regulation of growth hormone (GH) release from the pituitary is influenced by a number of hypothalamic, pituitary and circulating factors (1). Growth hormone releasing hormone (GHRH) and somatostatin (SS) are the two classical hypothalamic stimulatory and inhibitory regulators of pulsatile GH release, but free fatty acids, acetylcholine, amino acids, opiates and glucocorticoids also have direct effects on GH release.

In 1977 Bowers and coworkers developed a series of small peptides which stimulated in vitro the release of GH from pituitary cells, although their potency was rather weak (2). Further studies led in 1984 to the development of several more potent peptides, including growth hormone releasing peptide-6 (GHRP-6) (3). This hexapeptide was shown to be a potent releaser of GH both in vivo and in vitro. Furthermore, this compound was shown to be active when administered by different routes such as intravenous, intraperitoneal, intramuscular and even by the oral route (4). Unfortunately, this

development was largely unnoticed by the scientific community, because GHRH was characterized in 1982. It soon became apparent that the mechanism of action of the effects of GHRP-6 on GH secretion could not be easily explained by the mechanisms about GHRH and SS (5). The initial comparative analysis of the effects of GHRH and GHRP-6 on GH secretion suggested that their mechanism of action was both different and complementary. Thus, while GHRH is a more potent releaser of GH in vitro than GHRP-6, the opposite occurs in vivo (4). Using maximally or submaximally effective doses of GHRH and GHRP-6, GH secretory responses in vivo were potentiated rather than additive. Finally, different mechanisms of action were confirmed through the identification of different receptors and intracellular signaling for both of the peptides (4). It was also shown that GH secretagogues functionally antagonize release of SS from the pituitary and hypothalamus (6).

After the first synthetic GHRP; GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-amide), other potent growth hormone secretagogues (GHS) were developed (6) including:

GHRP-1 AlaHis-D-(2')-Nal-Ala-Trp-D-Phe-Lys-NH₂

GHRP-2 D-Ala-D-(2')-Nal-Ala-TRP-Nal-Lys-NH₂

Hexarelin His-D-2-MeTrp-Ala-Trp-D-Phe-Lys-NH₂

Correspondence address:

Serdar Güler
Ulucanlar Caddesi 68/5
Cebeci 06590 Ankara / Turkey
Tel : +90-532-427 59 58
Fax: +90-312-323 49 23
e-mail: sgulers@yahoo.com

The low bioavailability (<1%) of the peptidyl GHSs stimulated a search for nonpeptide compounds mimicking the action of GHRP-6 in the pituitary. Shortly thereafter, one of the most potent synthetic GHS; MK-0677 with improved oral bioavailability, was reported (6).

The actions of GHSs (both peptides and nonpeptides) are mediated by a specific GHS receptor (GHSR). This receptor is present in the pituitary and hypothalamus of various mammalian species and it is distinct from the GHRH receptor. GHSR was also detected in other areas of the central nervous system including cerebral cortex (but not cerebellar), hippocampus, medulla oblongata, choroid plexus, thalamus, substantia nigra and in peripheral tissues such as adrenal and thyroid glands, gonads, arteries, liver, adipose tissues uterus, skin, lymph nodes, heart, lung, kidney and skeletal muscles (6-8).

GHS receptors belong to the family of G-protein coupled receptors. Their activation leads to depolarization and inhibition of potassium channels, to an increase in intracellular concentrations of inositol triphosphate and to a transient increase in the concentrations of intracellular calcium (7).

Despite intensive searches by different groups, the isolation of an endogenous ligand of the GHSR remained elusive until recently. At last, in 1999 the group, led by Kojima and Kangawa, who tested extracts from different tissues such as brain, lung, heart, kidney and stomach succeeded (9). Despite the fact that most people assumed that the greatest concentrations would be in the hypothalamus, surprisingly, Kojima et al. found that the highest GHSR activation was found in stomach extracts. They also stated that this molecule was easily biodegradable, it may have been the reason GHRH was isolated first. This peptide is nowadays called ghrelin (G) (ghre is the Proto-Indo-European root of the word "growth").

Molecular structure

G is a peptide of 28 amino acids, with a molecular weight of 3314. Its serine 3 residue was n-octanoylated; this n-octanoylation appears to be essential for its activity. It is possible that the n-octanoyl group, adding a hydrophobic property to N-terminus may facilitate its entry and distribution in the brain. Octanoylation had not been observed

previously in peptide modification and opens up a new field in protein chemistry and in the development of biologically active peptides and peptidomimetic compounds (4). In many studies deoctanoyl form of the hormone was found to be 100 times less active than the mother peptide (9). Matsumoto et al stated that not only ser (3) side chain modification but also, an L configuration of the third residue is critical for G activity (10). In a study performed by Bednarek et al it was shown that the entire sequence of G was not necessary but, only N-terminus tetrapeptide [Gly-Ser-Ser-(n-octaoil)-Phe] (6) was sufficient. Matsumoto et al showed that a smaller molecule, that is 5-aminopentanoil-Ser(Octyl)-Phe-Leu-aminoethylame was sufficient for activation (11).

Human G is homologous to rat G apart from two amino acids. Kojima et al also succeeded in isolating the gene coding for G (9). In both rats and humans the gene encodes a prepro-ghrelin of 117 amino acids. The gene is expressed in both the stomach and the hypothalamic arcuate nucleus (9).



Figure 1. Human Ghrelin.

More recently another ligand, showing the same activity has been purified from the rat stomach; it is a 27 amino acid peptide named des-Gln 14-ghrelin, the sequence of which is identical to G except for one glutamine at the 14th position. This peptide is not encoded by a gene distinct from G but is encoded by a messenger RNA created by an alternative splicing of G gene (12).

Secretion

Detailed studies of Date et al. have demonstrated that stomach G is present in a distinct cell type, X/A like cells or now designated "Gr cells", mainly located in the fundus of stomach and infrequently in the rest of the gastrointestinal tract, mostly in colon, while the number of hypothalamic G arcuate neurons is small. The Gr cells are round-ovoid type, having round compact electron dense granules in the oxyntic mucosa. These cells are

not in continuity with the stomach lumen but rather are closely associated with the capillary network of the lamina propria supporting an endocrine role (13). In rats, G mRNA levels increase with age. Food intake decreases and fasting increases G secretion in rats and humans. Ghrelin levels are reduced to 35% of those in normal controls in totally gastrectomized patients.

Recently G is detected in placenta (14) and kidney (15). In human and rat placenta G appears to be mainly expressed in the first half of pregnancy, whereas it could not be detected at term. This time related variation in the secretion of G is also detected in several members of the somatotrophic axis which are also secreted from placenta, such as GHRH, SS, leptin, and IGF-1 (14). Whether placenta derived G is involved in the modulation of fetal growth and maturation remains to be established.

Pre-pro-ghrelin gene is expressed in the kidney and glomerulus, it is also demonstrated that G is locally secreted in these cells (15). Furthermore pre-pro-ghrelin gene expression is observed in fibroblast like NRK-49F cells. Fibroblast accumulation and fibrosis have been well known to play important roles in tubulointerstitial renal diseases, which strongly affect renal function. Thus G might not only have physiological but also pathophysiological significance in the kidney.

G immunoreactive cells were identified at the periphery in a cells of pancreas. It has been shown that G increases the cytosolic free calcium concentration in β cells, and stimulates insulin secretion when it is added to isolated rat pancreatic islets or administered intravenously (16,17).

Effects on food intake

Wren et al. found that intracerebroventricular (icv) G or neuropeptide Y (NPY), administration stimulated food intake with a duration of 24 hours in rats. In this study icv GHRP-6 also had the same effect but a shorter duration (18). Moreover, after a single intraperitoneal (ip) injection of G or GHRP-6 to rats, food intake was immediately increased during the first hour. The orexigenic activity of G after peripheral administration was considerably important in that other hypothalamic peptides that alter food intake are ineffective by this route of

administration. In another study Wren et al also demonstrated that intravenous G administration stimulates appetite and food intake in human. G is the first circulating human hormone that is shown to increase food intake (19).

The mechanisms of GHRP/G in food intake are still investigated. The probable mediators may be GH, GHRH, NPY, an endogenous melanocortin receptor antagonist; agouti-related peptide (AGRP).

During once daily subcutaneously (sc) G administration to wild type mice for 2 weeks, body weight and fat mass increased without a change in food intake, locomotor activity, lean body mass and bone mass (20). Moreover, daily sc administration of G to GH deficient dwarf rats produced the same effects observed in the wild type mice. Even very low doses of G administered icv for 7 days to normal adult male rats produced a dose dependent weight gain and food intake, without an effect on locomotor activity. Because G releases GH and increases food intake, and because GH is lipolytic rather than lipogenic, these results are unexpected. As concluded, G induced a positive energy balance and body weight gain by decreasing fat utilization. It can be thought that the metabolic and GH effects of G can be dissociated or G's food intake stimulatory effect is not mediated by GH.

Given that GHRH is a potent orexigenic peptide, it is interesting to speculate that G increases food intake via GHRH. However the orexigenic effect KP-102 is not blocked by GHRH antagonist. Thus it is likely that G stimulates feeding behavior via mechanisms different from those of GHRH (21).

Bowers et al showed that G increased fat mass in NPY deficient mice. Plasma G levels increase in fasted rats and decrease in fed rats. It was stated that GHRP-2 produced similar effects. After chronic once daily administration of GHRP/G, desensitization of these metabolic effects was not induced. The authors were intrigued with the possibility that G may play the role of regulating energy balance via a direct hypothalamic action and proposed that hypothalamic G regulates positive energy balance with paracrine activity, and stomach G regulates GH secretion with endocrine activity. In this model daytime secretion of stomach G is suppressed by food intake and presumably is augmented by fasting during nighttime to regulate GH secretion.

In contrast daytime secretion of hypothalamic G is increased, and during the nighttime decreased to regulate food intake. The endocrine secretion of stomach G during the nighttime inhibits the paracrine action of hypothalamic G via a homologous desensitization action. During the daytime, the endocrine secretion of stomach G is suppressed and paracrine secretion and action of G on food intake is augmented (22).

GHSR mRNA is expressed in the pituitary gland and in several areas of brain, including hypothalamus. In the hypothalamus, GHSR mRNA is expressed in NPY, GHRH, SS and proopiomelanocortin neurons. AGRP is localized primarily in the arcuate nucleus (ARC) and the majority of the neurons containing AGRP in the ARC also express NPY mRNA. Immunoreactive cells to G were determined in ARC. It is demonstrated that only orexigenic AGRP (21) or AGRP and NPY mRNA expression was stimulated after intracerebroventricular acute or chronic G administration (23,24). Currently there is evidence for the existence of at least six functional NPY receptor subtypes (Y1-Y6). NPY Y1 receptor antagonists suppress endogenous and exogenous NPY-induced feeding suggesting that Y1 receptor is a major NPY receptor subtype for its orexigenic activity. Recently it was demonstrated that Y5 receptor subtype also takes part in regulation of food intake (23). When intracerebroventricular Y1 receptor antagonist was administered the orexigenic action of G was diminished; these results provide evidence that at least a portion of G's orexigenic activity is mediated by NPY.

When G's possible involvement in pathogenesis of human obesity has been investigated it was hypothesized that obese individuals would present elevated G levels. Contrary to this hypothesis it was found that obese subjects had lower plasma concentrations of G than age matched lean control subjects. In addition, plasma G concentrations were significantly lower in Pima Indians, a population with one of the highest reported rates of obesity and Type II diabetes in the world, than in Caucasians (25). These data seem to indicate that G is downregulated in human obesity. This downregulation may be a sequence of elevated insulin or leptin, because fasting plasma G levels are negatively correlated with fasting plasma levels of insulin and leptin. It was further speculated that

decreased secretion of G, could be responsible for decreased levels of circulating GH in obese individuals. The decreased plasma G concentrations observed in obesity may represent a physiological adaptation to the positive energy balance associated with obesity (25). Relevant to these results plasma G levels were found to be elevated in cachectic patients with chronic heart failure than in those without cachexia; these G levels were found to be correlated with GH and tumor necrosis factor α (26). Furthermore G levels were found to be increased in anorexia nervosa, and decreased after weight gain, with an increase of %14 in body mass index (27). The authors suggested that a G resistance, such as a possible impairment of intracellular G receptor signaling, might exist in cachectic states or in eating disorders. Contrary to these results Ravussin et al. measured plasma G concentration in monozygotic twins before and after an overfeeding or submission to a negative energy balance in the twin pairs over a 100 day period. In response to the 100 day intervention plasma G exhibited a non significant decrease with overfeeding and non significant increase with negative energy balance. They speculated that plasma G is not possibly involved in the etiology of human obesity (28).

It is demonstrated that the satiety effect of leptin is mediated through the activation of the hypothalamic melanocortin system. Several lines of evidence have suggested that hypothalamic NPY also mediates some aspects of leptin action. It has been reported that leptin receptor is expressed in the majority of arcuate NPY neurons. Fasting-induced refeeding is severely affected in Y1 deficient mice, suggesting that hypothalamic NPY/Y1 receptor pathway is activated in response to fasting when plasma leptin concentrations are reduced. It has been reported that leptin can antagonize the action of exogenously administered NPY. It can be hypothesized that the satiety effect of leptin is mediated at least partly through the inhibition of the hypothalamic NPY/Y1 receptor pathway (29).

Because the satiety effect of leptin is abolished by icv injection of G, it can be speculated that G may antagonize leptin's satiety effect (23). It is likely that G and leptin act on the same NPY neurons to regulate hypothalamic NPY neurons. Therefore it can be postulated that G and leptin share the hypothalamic NPY/Y1 pathway. Although the leptin

induced decrease in hypothalamic NPY mRNA expression is completely abolished by icv injection of G, the satiety effect of leptin is only partially reversed by G. These observations suggest the involvement of other orexigenic or anorexigenic systems in the antagonism of leptin action by G (23).

Effects on hypothalamo-pituitary system

The site of action for the GH release by GHSs is at both hypothalamic and pituitary levels, the former is more significant *in vivo*. However the involvement of GHRH in GHS action remains controversial and it has been suggested that GHSs may induce the release of another hypothalamic factor with GH releasing capability (U factor).

After it was demonstrated that iv G stimulates GH release in rats, for the first time in human Takaya et al. reported that G increases GH secretion, when given iv, icv and ip (29). In this study IV bolus dosages of 0.2, 1, and 5 µg/kg G were used. The amount of GH released was much greater than that induced by maximal dosages of GHRH. This effect was relevant to the effects of other GHRPs. Dosages calculated on molar weight basis indicated that G is more potent in releasing GH than the other GHRPs. Even at very high IV bolus doses of G there were no unusual clinical effects and the adverse effects were minor.

It is also shown that G stimulates adrenocorticotrophic hormone (ACTH), cortisol, aldosterone and prolactin (PRL) but has no effect on luteotrophic hormone (LH), follicle stimulating hormone (FSH) (18,29,30). It is reported that G has neutral (29) or decreasing effect (18) on thyroid stimulating hormone (TSH). The inhibition of TSH by icv G, may represent an adaptive response to promote feeding and reduce energy expenditure in times of limited nutrition. This effect is also seen with AGRP (18).

The mechanism of G's stimulatory effect on ACTH and cortisol is a matter of debate; corticotropin releasing hormone (CRH), vasopressin or NPY are possible candidates of mediators (22,29). GABAergic pathways may also be responsible (29). In human adrenal glands specific GHS binding regions were shown, G might also be effective at this level (30).

The PRL releasing activity of G can be explained by direct action on the pituitary mammosomato-

troph cells (30). IV bolus administration of G results in high blood levels and reflects a pharmacological rather than physiological action of the peptide that suggests at physiological concentrations G possibly will not increase ACTH or PRL. G at the low 0.2 µg/kg iv bolus dosage in humans, released a substantial amount of GH but only minor increase of serum ACTH and PRL (22). Some recently developed GHSs, such as ipamorelin show high GH secretion without any ACTH or PRL release (31). This invention may increase the therapeutic potency of G in human diseases.

Furuta et al. showed that after icv G injection pulsatile LH secretions were significantly suppressed for about 1 hour. The main parameter suppressed was pulse frequency not the pulse amplitude, suggesting the hypothalamus as the site of G action (32).

GH releasing activity of G and GHRPs are parallel to each other. A study of Kang Chang in rats supports the commonality of action of GHRP-2 and G (22). In rats, combined G and GHRP-2 at maximal dosages induced the same magnitude of GH release as when the peptides were administered alone (22) GHRH antiserum and a GHRH antagonist inhibited the GH releasing action of both GHRP-2 and G. Combined G or GHRP-2 with GHRH released GH synergistically. Increasing dosages of G or GHRP-2 equally attenuate the inhibition of SS on the GH response of these individual peptides. This attenuation is more effectively induced when G or GHRP-2 is combined with GHRH. GHRP-2+GHRH releases GH *in vitro* additively rather than synergistically and it seems probable that the response of G+GHRH will be the same. Thus, the synergistic action of these peptides on GH release is not explained by the direct pituitary action of the combined peptides. Nevertheless, because of the chemical difference of these molecules and the primary origin of G from the stomach rather than the hypothalamus one is very circumspect of whether the biological activities will be found to be completely the same. Even small biological differences may become significantly important to the physiological action of G. For example, after repeatedly administered it was found that desensitization of the G GH response was of shorter duration than that of GHRP-2.

The high probability that the actions of G and the GHRPs will closely parallel each other are basically of theoretical and practical importance. At the theoretical level, the conceptual models of GH regulation proposed for the GHRPs should be directly applicable to G. This includes not only the envisioned physiological role of a putative GHRP hormone, probably G but also the envisioned pathophysiological role of the natural hormone in the decreased secretion of GH especially in older men and women. At the practical level, the relationship between the actions of GHRP and GHRH seems applicable to G and GHRH. This includes the independent and dependent, the additive and synergistic, as well as the permissive relationships of these peptides to each other in the release of GH at the endocrine, anatomical and molecular levels in animals and humans. Probably applicable to G and GHRH are the relationships, interpretations and implications described for GHRP-2 and GHRH (22).

The advantage of G compared with GHRPs as a diagnostic agent so far is not apparent. It is a matter of debate, if IV bolus infusion of G+GHRH instead of GHRP+GHRH is more suitable in diagnosis of GH deficiency. It is not shown that G is more valuable than GHRPs. Until now at a maximal GH releasing dosage of 1 µgr/kg GHRH was administered with 0.1, 1.0 or 0.25 µgr/kg GHRP-2, GHRP-6 or hexarelin respectively (22). The greater safety, ease, and simplicity of performance, dose related response, reproducibility and more global action of hypothalamic pituitary unit together with isolation of G are cogent reasons for proposing and developing approach rather than using the current gold standard insulin tolerance test. A problem that arises in the utilization of the GHRP+GHRH or G+GHRH diagnostic test for GH deficiency hypothalamic origin concerns the unknowns that exist at both the approach and clinical disorder level.

Previously, the pathophysiology of idiopathic decreased GH secretion of hypothalamic origin was considered to be secondary to decreased GHRH secretion and/or increased SS release. With the availability of the GHRPs and G, indirect evidence from acute iv bolus studies with GHRP-2 and GHRH alone and together indicate that endogenous G deficiency alone or possibly even

more likely G plus GHRH may be responsible for the decreased GH secretion in normal older men and women with low serum insulin like growth factor 1 (IGF-1). From the results obtained, GHRH deficiency alone or excess SS secretion seems unlikely.

G augments the GH response of GHRH, like GHRP-2, and presumably GHRH will be found to augment the GH response of G. Additionally, G+GHRH more effectively attenuates the inhibition of GH secretion by SS than either peptide alone. Two more possible examples where G may play a role are in the decreased GH response in obesity and during the chronic administration of dexamethasone. Because food intake decreases G secretion from the stomach the decreased GHRH GH response in obesity may be due to suppressed synthesis and release of stomach G. Also because dexamethasone is known to decrease GHRH in the hypothalamic ARC, the GH releasing action of G on the hypothalamus may be attenuated and subsequently the GH releasing actions of GHRH may be impaired (22).

A series of studies in humans on continuous infusion of GHRPs demonstrating an increase in normal pulsatile GH secretion and an elevation of serum IGF-1 levels also presumably will reflect the action of endogenous G (22). Because of the in vivo and in vitro findings in rats and in humans that the GH response of the GHRPs rapidly and markedly becomes desensitized after repeated administration it is predicted that continuous G infusion as well as continuous endogenous G secretion also will induce sustained increases in pulsatile GH secretion and IGF-1 levels.

A GHRH antagonist markedly inhibits the GH response of GHRP-6 in normal men and a GHRH antagonist, as well as a GH antiserum markedly inhibits the GH response of G in rats. Because the GH releasing activity of G/GHRP in vivo is very dependent on endogenous GHRH and GH secretion is not maintained in the absence of GHRH, the primary regulator of GH secretion would be GHRH. G would be best considered as a helper and modulator of GHRH in the regulation of GH secretion. A GH role requires an explanation of how stomach or hypothalamic G or both might interact with GHRH as well as SS, GH, IGF-1 to regulate pulsatile secretion of GH (21).

G levels were found to be low in patients with GH deficiency; adult onset or developed in their childhood before the initiation of GH replacement, in comparison with healthy subjects in the control group. One year replacement therapy of GH did not change the circulating G levels, despite significant decreases in body fat mass. It is conceivable that the lack of G modifications after long term GH therapy was due to the reduction of adiposity and insulin on one hand and increased GH secretion on the other. However it is still possible that systemic G is involved in the development of obesity both in normal and GH deficient subjects (33).

It was shown that NPY/AGRP neurons had GH receptor mRNA and NPY/AGRP neurons were activated following systemic administration of GH. However the effects of two peptides on GH axis seem to be different. NPY inhibits GH secretion in rats when administered centrally, whereas central injection of an anti-NPY serum in food deprived rats augments GH secretion. Central administration of AGRP did not alter the GH secretion pattern. Whether the presence of NPY and/or AGRP in the ARC are necessary for the effect of G is not certain (24).

Cardiac effects

Coronary vasoconstriction or protective effect against ischemia depending on dosage and improvement of cardiac performance after myocardial infarction have been observed in rats, while an increase in the left ventricular ejection fraction has been reported in humans with petidyl, but not with nonpeptidyl GHSs (34). It is stated that GHRPs have a direct favorable cardioprotectant action on the heart independent of a GH effect (35).

Nagaya et al reported that after G administration in rats cardiac out-put increased significantly and left ventricular dysfunction improved (36). Furthermore, specific G binding sites in human vasculature including aorta, coronary, pulmonary and arcuate arteries in the kidney and saphenous veins were discovered. It was also shown that G binding was up regulated in atherosclerotic coronary arteries compared to normal vessels (37).

Effects on stomach

G stimulates gastric acid secretion and motility in rats and circulating G levels are correlated with

gastric emptying time in humans (38). The structural and effect related similarities between G and members of gastrointestinal hormone family; motilin/motilin related peptide are pointed out.

Effects on malignancies

Specific binding sites for GHRPs were detected in membranes from several types of breast carcinomas, whereas a negligible binding was found in fibroadenomas and mammary parenchyma. The highest binding activity was found in well differentiated invasive breast carcinomas and was progressively reduced in moderately to poorly differentiated tumors. In human breast carcinomas, estrogen dependent or independent, it was shown that G was bound in cell lines. It was also stated that both octanoylated and desoctanoylated human G was able to inhibit cell growth and thymidine incorporation in vitro at concentrations close to their binding affinity. Inhibition of breast cancer cell growth induced by desoctanoyl G which possesses no stimulatory effect on GH secretion theoretically may have potential clinical applications (39).

Like breast carcinomas, in thyroid carcinomas of follicular origin, an identical profile of GHS binding had been observed. Functionality of (probably overexpressed) GHS binding sites were maintained in better differentiated tumors of thyroid and was decreased in less differentiated neoplasm (40).

Kanamoto et al showed that G was produced in thyroid C cells as well as in medullary thyroid carcinoma (41). Papotti and his coworkers demonstrated that most gastric carcinoids and some intestinal carcinoids can produce G (42).

It was observed that G mRNA and peptide were expressed in normal and adenomatous pituitary tissue. Pituitary tumors arising from somatotroph, corticotroph, lactotroph, and gonadotroph cells as well as nonfunctioning adenomas also showed expression of G mRNA. G expression was relatively low in corticotroph adenomas and was relatively high in somatotroph adenomas compared with that in normal tissue (1). It can be speculated that locally produced G in the pituitary gland may have direct paracrine or/and autocrine effects on pituitary function (43).

Expression of GHSR and G was detectable in all immune cells regardless of the maturity and cell

types (normal or leukemic cell lines). But GH expression in human leukemic cell lines was observed mainly in B cell lines. It may act locally on its own receptors. Widespread distribution of G and GHSR in human immune cells may indicate unknown functions other than enhancing GH secretion in the immune system (44).

Glucose metabolism

Prolonged treatment with GHS had been followed by hyperglycemia in obese rats and this effect was supposed to reflect GHS induced enhancement in the activity of hypothalamus pituitary adrenal axis. Chronic treatment with MK-0677 in normal elderly but not obese subjects was coupled with hyperglycemia and hyperinsulinism, but this effect was supposed to reflect increased GH secretion (45). In another study it has been shown that during treatment with pegvisomant, a GH receptor antagonist in fed conditions, GHRP-6 induces hyperglycemia coupled with increased insulin levels. On the other hand prolonged treatment with hexarelin in elderly subjects did not induce any significant increase in glucose levels though in presence of a significant trend toward increase in HbA1c (46).

It is a known fact that after IV administration of G or after it is added to pancreatic cells, G induces an increase in cytosolic calcium concentrations in b cells and stimulates insulin secretion (16,17). Broglio and his coworkers showed that the acute administration of 1 µg/kg human G elicits prompt increase in glucose levels which was then followed by slight but significant decrease in insulin secretion likely allowing further increase in glucose levels. Increase in plasma glucose levels and decrease in insulin secretion persisted two hours after G administration (46). This time course of glucose and insulin variations following G administration may suggest that G has direct non-GH-mediated hyperglycemic effect. It could reflect glycogenolytic activity in the liver. It is also unlikely that G stimulates glucagon secretion while in turn should cause an increase in insulin secretion.

References

1. Korbonitz B, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, Kangawa K, Grossman AB. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. *JCEM* **86**(2): 881-887, 2001.
2. Bowers CY, Chang J, Momany F, Folkers F. Effects of enkefalins and enkefalin analogues on release of pituitary hormones in vitro. *Molecular Endocrinology* (Ed: MacIntyre I). Amsterdam/North Holland, Elsevier, 1977. 287-292.
3. Bowers CY, Momany F, Reynolds GA, Hong A. 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**: 1537-1545, 1984.
4. Dieguez C, Casaneuva FF. Ghrelin: a step forward in the understanding of somatotroph cell function and growth regulation. *European J Endocrinol* **142**: 413-417, 2000.
5. Ghigo E, Arvat E, Muccioli G, Camanni F. Growth hormone- releasing peptides. *European J Endocrinol* **136**: 445-460, 1997.
6. MA Bednarek, Feighner SC, Pong S, McKee KK, Hrenjuk DL, Silva MV, Warren VA, Howard AD, Van der Ploeg LHY. Structure-function studies on the new growth-hormone-releasing peptide, Ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretatogue receptor 1a. *J Med Chem* **43**: 4370-4376, 2000.
7. Pong S, Chaung L, Dean D, Nargund R, Patchett A, Smith R. Identification of a new G protein linked receptor for growth hormone segretagogues. *Mol Endocrinol* **10**: 57-61, 1996.
8. Guan X, Yu H, Palyha O, Mckee K, Feighner S, Sirinathsinghi D, Smith R, Van der Ploeg. Distribution of mRNA encoding the growth hormone segretagogue receptor in brain and peripheral tissues. *Mol Brain Res* **48**: 23-29, 1997.
9. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth hormone – releasing acylated peptide from stomach. *Nature* **402**: 656-660 1999.
10. Matsumoto M, Hosoda H, Kitajima Y, Morozumi N, Minamitake Y, Tanaka S, Matsuo H, Kojima M, Hayashi Y, Kangawa K. Structure activity relationship of ghrelin: pharmacological study of ghrelin peptides. *Biochem Biophys Res Commun* **287**(1): 142-146, 2001.
11. Matsumoto M, Kitajima Y, Ivanami T, Hayasi Y, Tanaka S, Minamitake Y, Hosoda H, Kojima M, Matsuo H, Kangawa K. Structural similarity of ghrelin derivatives to peptidyl growth hormone secretagogues. *Biochem Biophys Res Commun* **284**(3): 655-659, 2001.
12. Hosoda H, Kojima M, Matsuo H, Kangawa K. Purification and characterization of rat des-Gln 14-ghrelin, a second endogenous ligand for growth hormone secretagogue receptor. *J Biol Chem* **275**: 21995-22002, 2000.
13. Date Y, Kojima M, Matson H, Kangawa K. Gherelin, a novel growth hormone releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinol* **141**: 4255-4261, 2000.
14. Gualillo, Caminos E, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, Dieguez C, Casanueva FF.

- Ghrelin, a novel placental-derived hormone. *Endocrinol* **142** (2): 788-794, 2001.
15. Mori K, Yoshimoto A, Takaya K, Hosoda K, Ariyasu H, Yahata K, Mukoyama M, Sugawara A, Hosoda H, Kojima M, Kangawa K, Nakao K. Kidney produces a novel acylated peptide, ghrelin. *FEBS Letters* **486**: 213-216, 2000.
 16. Lee HM, Wang G, Englander EW, Kojima M, Greely Jr GH. Ghrelin a new gastrointestinal peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine and dietary manipulations. *Endocrinol* **143** (1): 185-90, 2002.
 17. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondol MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsujura S. Ghrelin is present in pancreatic a-cells of humans and rats and stimulates insulin secretion. *Diabetes* **51**(1): 124-129, 2002.
 18. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DGA, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinol* **141**(11): 4325-4328, 2000.
 19. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillon WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *JCEM* **86**(12): 5992, 2001.
 20. Tschöp M, Smiley DL, Behman ML. Ghrelin induces adiposity in rodents. *Nature* **47**: 908-913, 2000.
 21. Okada K, Ishii S, Minami S, Sigihara H, Shibasaki T, Wakabayashi I. Intracerebroventricular administration of growth hormone releasing peptide KP-102 increases food intake in free-feeding rats. *Endocrinol* **137**: 5155- 5159, 1996.
 22. Bowers CY. Unnatural growth hormone releasing peptide begets natural ghrelin. *JCEM* **86**(4): 1464- 1469, 2001.
 23. 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. 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**: 227- 232, 2001.
 24. Kamegai J, Tamura H, Shimizu T, Ishi S, Sugihara H, Wakabayashi I. Central effect of Ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinol* **141**(12): 4797-4800, 2000.
 25. Tschöp M, Weyer C, Tataranni A, Devanarayan V, Ravussin E, Heiman ML. Circulating Ghrelin levels are decreased in human obesity. *Diabetes* **50**: 707- 709, 2001.
 26. 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. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation* **104**(17): 2034-2038, 2001.
 27. Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschöp M. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* **145**(5): 669-673, 2001.
 28. Ravussin E, Tschöp M, Morales S, Bouchard C, Heiman ML. Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *JCEM* **86**(9): 4547-4551, 2001.
 29. Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Kamatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K. Ghrelin strongly stimulates Growth hormone release in humans. *JCEM* **85**(12): 4908-4911, 2000.
 30. Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, Papotti M, Muccioli G, Diguez C, Casaneuva FF, Deghenghi R, Camanni F, Ghigo E. Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans, comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *JCEM* **86**(3): 1169-1174, 2001.
 31. Raum K, Hansen BS, Johansen NL. Ipamorelin, the first selective growth hormone secretagogue. *Eur J Endocrinol* **139**: 552-561, 1998.
 32. Furuta M, Funabashi T, Kimura F. Intracerebroventricular administration of ghrelin rapidly suppresses pulsatile luteinizing hormone secretion in ovariectomized rats. *Biochem Biophys Res Commun* **288**(4): 780-785, 2001.
 33. Janssen JA, Van Der Toorn FM, Hofland LJ, Van Koetsveld P, Broglio F, Ghigo E. Systemic ghrelin levels in subjects with growth hormone deficiency are not modified by one year of growth hormone replacement therapy. *Eur J Endocrinol* **145**(6): 711-716, 2001.
 34. Papotti M, Ghe C, Cassoni P, Catapano F, Deghenghi R, Ghigo E, Muccioli G. Growth hormone secretagogue binding sites in peripheral human tissue. *JCEM* **85**(10): 3803-3807, 2000.
 35. Tivesten A, Bollano E, Caidahl K. The growth hormone secretagogue hexarelin improves cardiac function in rats after experimental myocardial infarction. *Endocrinol* **141**: 60-66, 2000.
 36. Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshira F, Shimizu W, Hosoda H, Hirota Y, Ishida H, Mori H, Kangawa K. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation* **104**(12): 1430-1435, 2001.
 37. Katugampola SD, Pallikaros Z, Davenport AP. 1251 -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**(1): 143-149, 2001.
 38. Folwaczny C, Chang JK, Tschöp M. Ghrelin and motilin: two sites of one coin. *E J Endocrinol* **144**: R1-R3, 2001.
 39. Casoni P, Papotti M, Ghe C, Catapano F, Sapino A, Graziani A, Deghenghi R, Reissmann T, Ghigo E, Muccioli G. 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. *JCEM* **86**(4): 1738-1745, 2001.

40. Casoni P, Papotti M, Catapano F. Specific binding sites for synthetic growth hormone in non-tumoral and neoplastic human thyroid tissue. *Endocrinol* **165**: 139-146, 2000.
41. 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. Substantial production of ghrelin by human medullary thyroid carcinoma cell line. *JCEM* **86**(10): 4984-4990, 2001.
42. Papotti M, Casoni P, Volante M, Deghenghi R, Muccioli G, Ghigo E. Ghrelin producing endocrine tumors of stomach and intestine. *JCEM* **86**(10): 5052-5059, 2001.
43. Kim K, Arai K, Sanno A, Osamura RY, Teramoto A, Shibasaki T. Ghrelin and growth hormone secretagogue receptor mRNA expression in human pituitary adenomas. *Clin Endocrinol* **54**(6): 759-768, 2001.
44. Hattori N, Saito T, Tagyu T, Jiang BH, Kitagawa K, Inagaki C. GH, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. *JCEM* **86**(9): 4284-4291, 2001.
45. Svensson J, Lonn L, Jansson JO. Two month treatment of obese subjects with the oral growth secretagogue MK 0677 increases GH secretion, fat-free mass and energy expenditure. *JCEM* **83**: 362-369, 1997.
46. Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, Van Der Lely AJ, Deghenghi R, Ghigo E. Ghrelin, natural GH secretagogue produced by the stomach induces hyperglycemia and reduces insulin secretion in humans. *JCEM* **86**(10): 5083-5087, 2001.