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Regulation of Ghrelin Secretion and Action

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The pulsatile release of growth hormone (GH) from anterior pituitary gland is regulated by the interplay of at least two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin, via their engagement with specific cell surface receptors on the anterior pituitary somatotroph. Furthermore, release of GH in vivo may also be controlled by a third type of receptor, the growth hormone secretagogue receptor, a G-protein-coupled receptor, called GHS receptor type 1a (GHS-R1a), which was identified in the pituitary and the hypothalamus in humans using a nonpeptidyl growth hormone secretagogue (MK-0677). Ghrelin, the endogenous ligand for the GHS-R1a, is a 28-amino-acid peptide isolated from human stomach that is modified by a straight chain octanoyl group covalently linked to Ser^3, which is essential for its endocrine activity. This hormone, predominantly expressed and secreted by the stomach, has a dual action on GH secretion and food intake, showing interdependency between these actions. The finding that fasting and food intake, respectively, increase and decrease the secretion of ghrelin suggests that this hormone may be the bridge connecting somatic growth and body composition with energy metabolism, and appears to play a role in the alteration of energy homeostasis and body weight in pathophysiological states such as hypothyroidism and hyperthyroidism. Despite this, little is known about the intracellular signaling through which ghrelin exerts its regulatory actions. Activation of intracellular calcium mobilization is one of the earliest known cellular signals elicited by ghrelin. In HEK-293 cells expressing the GHS-R1a, ghrelin induces a biphasic cytosolic calcium elevation characterized by a spike phase of the response, which reflects $Ins(1,4,5)P_3$-dependent calcium mobilization of intracellular stores, and a sustained phase of the response, which is due to calcium influx across the plasma membrane triggered by aperture of capacitative calcium channels (store-operated calcium channels). Upon repeated administration, ghrelin showed a marked suppression of ghrelin-mediated elevations of intracellular calcium. This homologous desensitization represents an important physiological mechanism that modulates receptor responsiveness and acts as an information filter for intracellular signaling system. The discovery of ghrelin adds a new component to the complex machinery responsible for regulation of GH secretion in connection with the regulation of appetite and energy homeostasis.

Key Words: GH; growth hormone secretagogues; ghrelin.

Ghrelin: The Natural Ligand for GHS Receptor

The regulation of GH release from the pituitary appears to be mediated by the interplay of hypothalamic, pituitary, and circulating factors *(1)*. GHRH and somatostatin, respectively, are the two classical hypothalamic stimulatory and inhibitory regulators of pulsatile GH release. On the other hand, glucocorticoids, opiates, amino acids, free fatty acids, and other agents also have effects on the GH release *(2)*. In addition to this complex regulatory machinery, GH release may be elicited by a group of synthetic agents, known as GH secretagogues (GHSs). The action of GHSs is mediated through a specific seven-transmembrane G-protein-coupled receptor called GHS receptor type 1a (GHS-R1a), which was identified using a nonpeptidyl GHS (MK-0677) in the pituitary and the hypothalamus in humans *(3,4)*. The identification of an endogenous ligand for this receptor, a peptide isolated from the stomach and called ghrelin, symbolizes one of major milestones in endocrinology *(5)*. Ghrelin is a 28-amino-acid peptide with an n-octanoyl group covalently linked to the hydroxyl group of serine 3 (Ser^3) residue, which is essential for its biological activity. In this sense, ghrelin is the first known example of a secreted bioactive peptide in mammals modified by an acyl acid *(14)*. The discovery of this hormone is a good example of reverse

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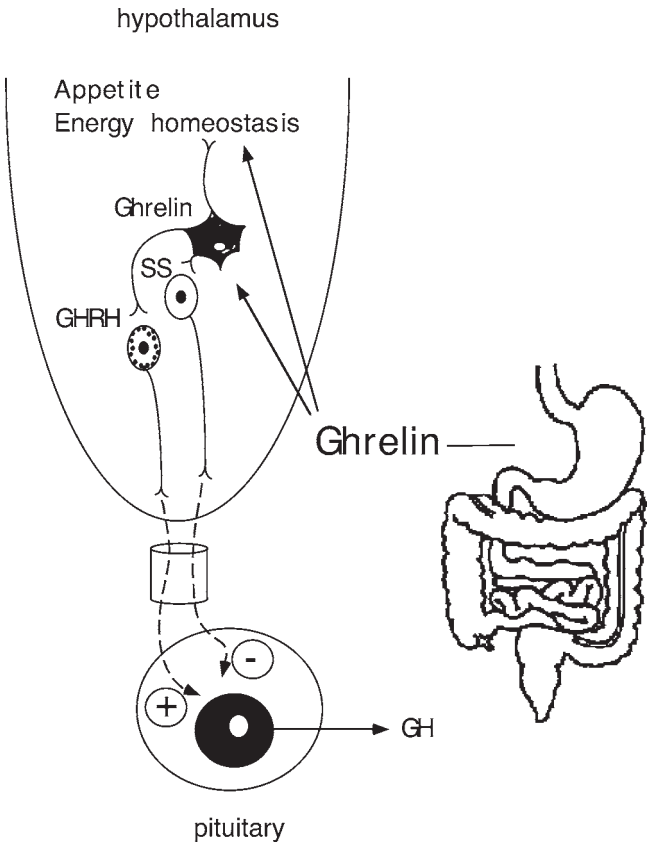

Fig. 1. Schematic representation of ghrelin action. Circulating ghrelin needs to reach the CNS to act in the afferent loop controlling energy homeostasis or GH secretion. In this sense, ghrelin appears as the link signal between general metabolism and energy homeostasis.

pharmacology, as starting with the synthetic analogs (GHSs) it was possible to arrive at the identification of the natural ligand, ghrelin, via the discovery of the GHS receptor *(5)*.

Ghrelin is expressed and secreted mainly by the stomach, with lower quantities by other segments of the intestine *(6)*. The X/A-like cells of the oxyntic gland located in the fundus of the stomach are the main source of this hormone *(6,13)*. These cells have no contact with the lumen of the oxyntic gland; rather they are closely associated with the capillary network, suggesting a secretion toward plasma and, thus, its endocrine role (Fig. 1). Other tissues expressing ghrelin are the pituitary *(7)*, hypothalamus *(4)*, heart, and kidney *(10)*, although the amount secreted and the physiological relevance of its presence in these tissues is undefined. Ghrelin has been detected in human and rat placenta showing a pregnancy-related time course of expression, although the physiological role of this hormone is not known at present *(8)*. More recently, ghrelin has been detected in rat testis *(9)* and ovary *(71)*. Testicular gene expression was demonstrated throughout postnatal development being detected in Leydig cells in adult testis. This expression appears to be under hormonal regulation and dependent on pituitary luteinizing hormone (LH) *(72)*, suggesting a possible involvement of ghrelin as mediator on LH actions in rat testis. The presence of ghrelin has been reported in the cyclic human ovary showing distinct but partially overlapping patterns of cellular location *(71)*. These findings appear to open a new field for ghrelin as a mediator of the reproductive axis.

This "new" hormone releases GH both in vitro and in vivo, and the stimulatory effect on GH secretion is more potent than that of either GHRH or GHSs *(11)*. Most probably ghrelin is involved in regulating the somatotroph system along with GHRH and somatostatin. However, ghrelin may be much more than a simply GH secretagogue, and other central and peripheral actions (i.e., corticotroph secretion, stimulation of lactotroph, influence on gastroenteropancreatic functions, energy homeostasis, sleep regulation, reproduction, and cardiovascular actions) are continuously emerging *(12)*.

Synthesis

The human ghrelin gene is located on chromosome 3, at the locus 3p25-26, and is made up of four exons and three introns (Fig. 2) (*15*). This genomic structure is identical to the ghrelin in rat and very similar to that of the mouse *(16–18)*. A common feature of mammalian ghrelin genes is the promoter region including the short noncoding first exon and its transcriptional regulation. Two types of ghrelin peptides are produced by alternative splicing of a single gene, ghrelin and a second, des-Gln 14-grelin, a similar molecule except for the deletion of Gln14 *(19)*. This alternative splicing is due to an intron between Gln13 and Gln14 of the ghrelin sequence. The 3'-end of the intron has two tandem CAG sequences, where AGs serve as the splicing signals. When the first AG is used for the splicing signal, preproghrelin mRNA is produced and the second CAG is translated into Gln14. On the other hand, when the second AG is used, prepro-des-Gln14-ghrelin mRNA is created to produce des-Gln14-ghrelin missing Gln14 *(16)*. In this way, ghrelin appears as the first example of the production of two different mature peptides from an alternative splicing mechanism in the peptide-coding region. Furthermore, multiple ghrelin-derived molecules produced by posttranslational processing have recently been identified *(75)*.

The testis-specific expression of ghrelin gene transcript in mouse, ghrelin gene-derived transcript (GGDT), has been reported *(18)*. GGDT was expressed only in the testis, and 5'-unique sequence of GGDT was identified between exons 3 and 4 of the ghrelin gene, indicating that GGDT was generated by alternative usage of the 68 bp exon as the testis-specific first exon. Furthermore, the expression of GGDT appears to be regulated in testis-specific and developmental stage-specific manners although, at present, the physiological functions have not been determined.

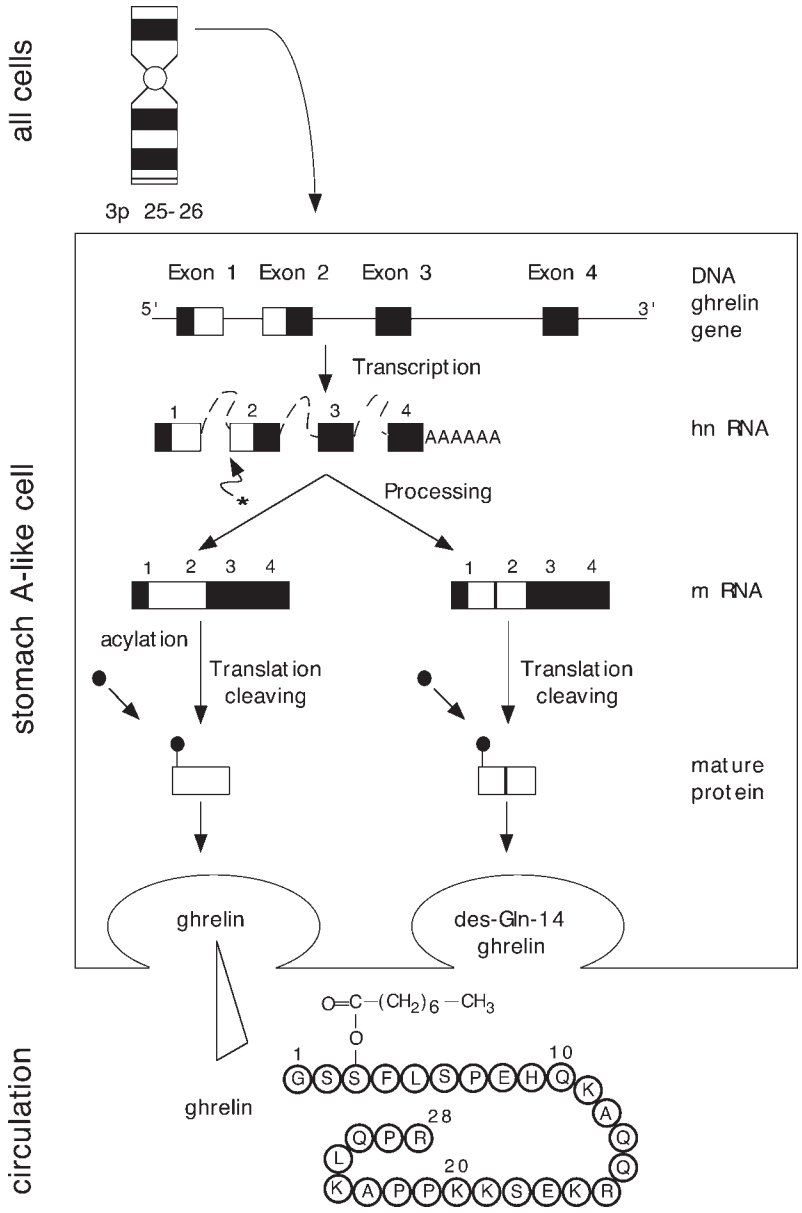

Fig. 2. Model for ghrelin synthesis in human. Two mature isoforms of mRNA for proghrelin are produced by alternative splicing from the gene: one for ghrelin and the other for des-Gln-14-ghrelin. The asterisk marks the boundary between the first intron and the second exon where the alternative splicing occurs. Before secretion, the peptide undergoes an esterification step catalyzed by unknown enzymatic machinery in the cytoplasm.

The human and rat ghrelin precursors are both composed of 117 amino acids, where the ghrelin sequence is followed by a signal peptide *(16)*. Before being secreted, the backbone peptide of ghrelin undergoes an esterification step catalyzed by unknown enzymatic machinery in the cytoplasm. After modification by n-octanoic acid, ghrelin and des-Gln14 ghrelin are secreted into blood vessels to circulate throughout the whole body. In adult human plasma samples, the ghrelin concentration is around 100–120 fmol/mL and close to 90% of the total content is desoctanoylated ghrelin, devoid of GH-releasing capacity *(20)*. The remaining 10%, with GH-releasing capacity, shows a short half-life as a bioactive hormone, mostly due to the esterases and proteases that cleave the octanoyl group and the peptide chain, respectively. Taking the high plasma level found for desoctanoylated ghrelin into account, it would seem possible to speculate about unknown, nonendocrine functions for it. In agreement with this hypothesis, desoctanoylated human ghrelin was found able to inhibit proliferation of breast carcinoma cells *(21)*.

Ghrelin Secretion

The regulation of ghrelin secretion is still largely unknown; however, it is already clear that circulating ghrelin and/or mRNA-expression levels are decreased by food intake *(22, 23)* and increased by food deprivation *(24)*. This point supports the concept of ghrelin as a potential regulator of energy homeostasis. In addition, ghrelin mRNA is up-regulated in hypoglycemia or leptin administration with a parallel increase of plasma ghrelin concentration *(25)*. Plasma ghrelin is reduced in obese compared with lean subjects *(26)*. On the contrary, plasma ghrelin levels are markedly elevated in patients with malnutrition due to anorexia nervosa *(27)*, returning toward normal after refeeding. Taking into account that ghrelin is a GH-releasing peptide, these data fit in well with the low GH values detected in obesity and with the high levels observed in fasting and malnutrition *(2)*, ghrelin secretion being enhanced in situations of negative energy balance, while the contrary occurs in situations of positive energy balance. However, this is not a general role, given that in hyperthyroid rats, a negative energy balance state, ghrelin levels are decreased *(28)*. Furthermore, ghrelin levels are increased in hypothyroidism, which is usually associated with decreased of food intake (Fig. 3). This appears to suggest that hypothyroidism leads to a state of resistance in relation to the orexigenic effects of ghrelin.

GHS Receptor

GH-releasing activity of ghrelin in the pituitary is mediated through a specific receptor belonging to the family of G-protein-coupled receptors, called GHS receptor (GHS-R) *(29)*. A single highly conserved gene, which is located at chromosomal location 3q26.2, expresses this receptor *(30)*. However, two types of cDNAs for GHS-R have been identified and designated as 1a and 1b *(31–33)*. The human GHS-R type 1a consists of 366 amino acids with seven transmembrane regions whose molecular mass is approx 41 kDa. On the other hand, type 1b consists of 289 amino acids with only five predicted transmembrane regions. The type 1b cDNA diverges in its nucleotide sequence and is fused to a short conserved reading frame of 24 amino acids. Type 1a was demonstrated to confer high affinity, specific binding of ghrelin and lead to intracellular calcium mobilization through stimulation of the G protein subunit G11. In contrast, type 1b failed to bind ghrelin. This binding affinity appears to be correlated with the GH stimulatory effect. Among all known GPCRs, GHS-R does not show significant homology with other known receptors; the closest relatives are the neurotensin receptor (59% similarity), the TRH receptor (56% similarity) and the motilin receptor (52% similarity) *(32)*.

GHS-R1a is confined in somatotroph pituitary cells and in a very limited region in the hipothalamic arcuate nucleus, which is known to control the appetite *(5,29,34–36)*. At much

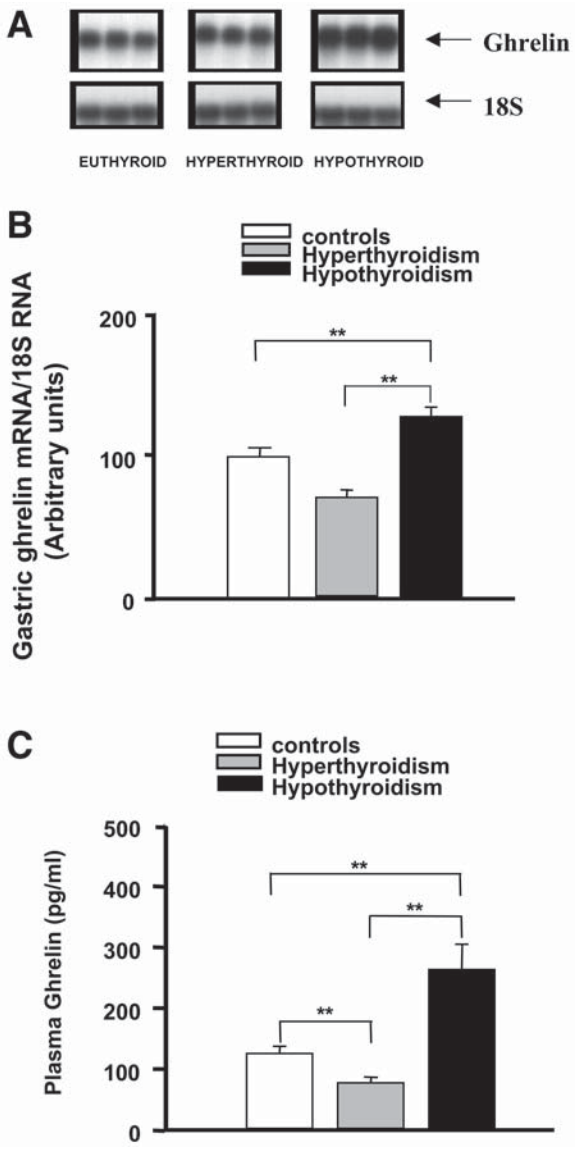

Fig. 3. Influence of thyroid status on ghrelin. In rats, hypothyroidism is associated with an increase in gastric ghrelin mRNA levels (**A,B**). In contrast, hyperthyroidism resulted in a decrease in gastric ghrelin mRNA levels (**A,B**). These results fit in well with the plasma ghrelin levels detected (**C**). From *28* with permission.

lower levels GHS-R1a is expressed in the thyroid gland, pancreas, spleen, myocardium and adrenal gland *(37,38)*. Recently, the expression of this receptor has been demonstrated in rat testis *(9,72,76)*, in ovary *(71)* and in prostate cancer cells *(74)* where co-expression of the GHS-R1a and ghrelin appears to provide evidence of autocrine/paracrine pathways in these systems. On the other hand, GHS-R1b expression is also widespread, showing high levels in skin, myocardium, pituitary, thyroid, pancreas, ileum, colon, somatotropinoma tumors, liver, breast, spleen, duodenum, placenta, lung, adrenal, buccal mucosa, stomach, lymph node, atrium lymphocytes and kidney *(38)*. The role of this

widespread expression is still unknown, although it is possible to think along the lines of a new possible ligand for this receptor with physiological effects in endocrine and non-endocrine tissues.

GHS-R1a transduces information provided by its own endogenous ligand, ghrelin and by the group of synthetic compounds, comprised as GHSs, although ghrelin is not related structurally to the synthetic GHSs. This aspect is related to the ongoing controversy about whether this receptor is the sole receptor for GHSs or just one of a group of receptors for such ligands. The existence of different receptors might be endorsed by the differences in the binding activities reported for ghrelin and different GHSs. However, site-directed mutagenesis and molecular modeling studies have demonstrated the existence of distinct regions suggesting an overlapping in the agonist-binding site *(30)*. Added to this complexity, GHS-R1a appears to show other binding site(s) different from the characterized GHS binding pocket, which was revealed by studies developed with adenosine as an agonist of this receptor *(39,40)*. In fact, adenosine does not possess a biological counterpart, being unable to stimulate GH secretion, consistent with the activation of GHS-R1a through a distinct binding site from the ghrelin-binding pocket. These findings support the idea that GHS-R1a activity might be modulated by different ligands acting through distinct biding pockets on the same receptor.

There is strong evidence suggesting the existence of different GHS-R subtypes. In fact, studies using a photoactivatable ligand suggest a second subtype in pituitary cells with a molecular mass of 57 kDa *(41)* and a third subtype in heart with a molecular mass of 84 kDa *(42)*. Although the cDNAs of these additional subtypes have not yet been isolated, characterization of new receptors might allow further insight into the regulation of GH secretion and additional physiological roles.

Intracellular Signaling

It is currently known that ghrelin activates a phosphatidylinositol-specific phospholipase C (PI-PLC) through a $G_{11\alpha}$ protein *(29–31)*. In HEK-293 cells expressing the GHS-R1a, this enzyme acts on phosphatidylinositol 4,5-biphosphate at the internal leaflet of the plasma membrane generating inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 triggers the release of calcium from IP_3 intracellular sensitive stores whereas DAG is responsible for the activation of PKC. The IP_3-sensitive calcium pools appear to be dynamically coupled to extracellular calcium and, in this way, when the calcium levels are reduced, a calcium influx is triggered by the aperture of capacitative calcium channels (or store operated channels) at the plasma membrane (Fig. 4). The exposure of GHS-R1a to either ghrelin or GHSs (as GHRP-6) results in a rapid attenuation of the receptor responsiveness (Fig. 5), originating from a group of cell biological events governing receptor signaling, desensitization, and resensitization. These regulatory mechanisms are fundamental for understanding the ability of cells to respond to ghrelin and, in this sense, the regulation of its intracellular signaling.

In somatotroph cells, GHS-R1a activation leads to a depolarization of the cell membrane potential, which in turn leads to an increase in transmembrane L- and T-type calcium currents via second messenger systems *(43)*. The ion channels involved in this depolarization have not been fully defined, although an inhibition of K^+ channels may play a role in this effect. It has been proposed that GHS-R1a activation leads to a first increase of intracellular calcium followed by a calcium influx due to an increase in membrane calcium permeability. The latter may be due to a membrane depolarization and the action of second messengers on calcium channels.

Of particular relevance are the recent data showing that ghrelin modulates the downstream of insulin signaling in hepatoma cells *(44)*. Ghrelin induces up-regulation of several insulin-induced activities including tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), association of the adapter molecule growth factor receptor-bound protein 2 with IRS-1, mitogen-activated protein kinase activity, and cell proliferation. Unlike insulin, ghrelin inhibits Akt kinase activity as well as up-regulating gluconeogenesis through the inhibition of Akt kinase activity.

Regulation of GH Secretion

In a first approach, the observation that ghrelin induced in vitro GH secretion in a dose-dependent manner *(5)* fits in well with the specific effect demonstrated in freely moving rats *(45)*. Therefore, it is possible that stomach-derived ghrelin may participate physiologically in GH regulation (Fig. 1). Ghrelin is a potent GH releaser in humans with no side-effects after its administration *(46–49)*. This GH releasing capability is higher than that of GHRH, and is comparable to that of GHSs *(50)*. The normal functioning of the GHRH receptor is necessary for ghrelin to be operative on GH secretion *(51,52)*, an action that is partially insensitive to the inhibitory action of either somatostatin or of metabolic compounds, such as glucose or free fatty acids *(53)*. However, somatostatin exerts a strong inhibition on ghrelin secretion that, as opposed to GH and insulin secretion, remains inhibited even after stopping the infusion of somatostatin *(73)*. On the other hand, the gender-based differences in the pattern of GH secretion do not appear to be mediated by ghrelin, as no gender-based differences in circulating ghrelin have been shown in humans *(27,54)*. Furthermore, adult patients with GH deficiency have ghrelin levels similar to control subjects, both before and after GH replacement *(55)*.

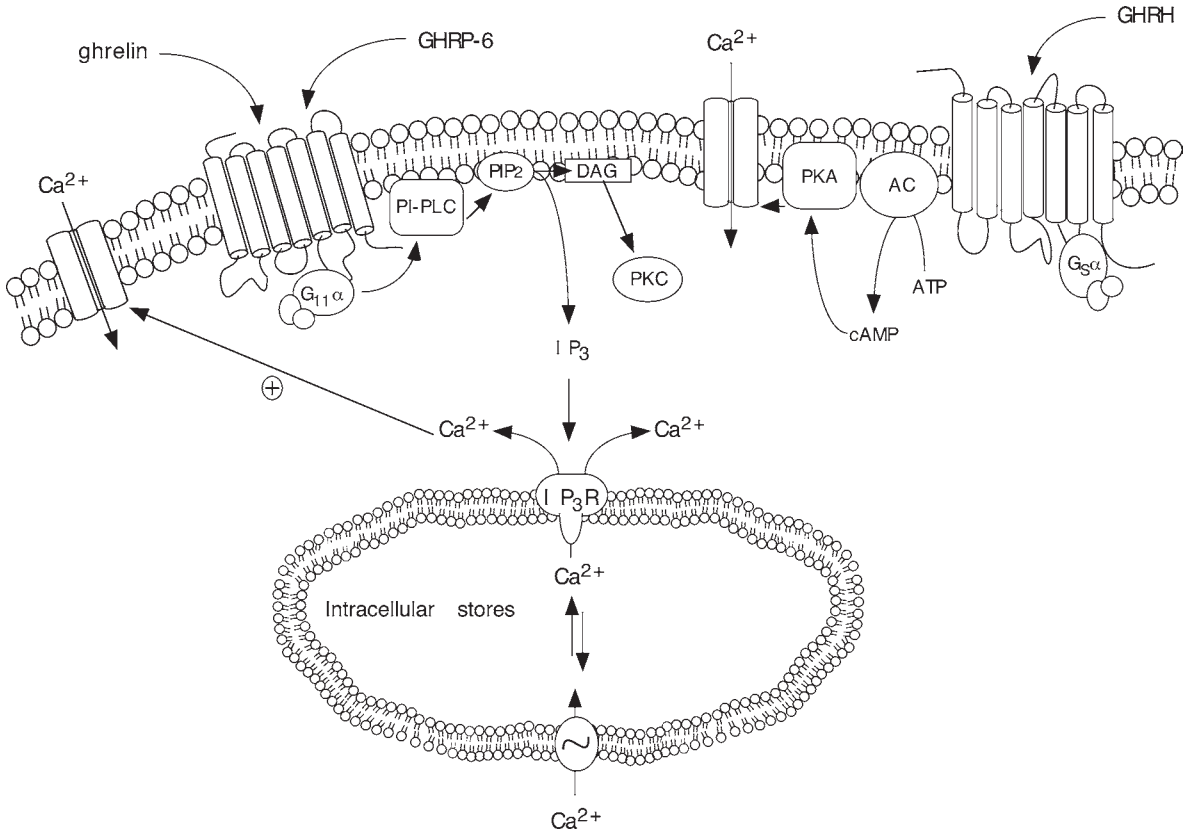

Fig. 4. Model for the signal transduction pathway used by ghrelin, GHRP-6, and GHRH to trigger intracellular calcium mobilization. The addition of ghrelin or GHS, as GHRP-6, to cells activates a phosphatidylinositol-specific phospholipase C (PI-PLC) through a $G_{11\alpha}$ protein-coupled GHS receptor. PI-PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) at the internal leaflet of the plasma membrane generating inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases calcium from intracellular IP_3-sensitive calcium pools. When the calcium levels in the IP_3-sensitive calcium pool are reduced after the activation of the IP_3-calcium pathway by ghrelin/GHRP-6, a calcium influx is triggered by aperture of capacitative calcium channels at the plasma membrane. The DAG generated activates PKC. In contrast, GHRH activates adenylate cyclase (AC) through a $G_{s\alpha}$-protein-coupled GHRH receptor resulting in a rise in intracellular cAMP concentrations. The cAMP generated stimulates protein kinase A (PKA), which activates voltage-sentitive calcium channels with the consequent influx of extracellular calcium.

The activity of ghrelin is not specific to GH and includes stimulatory effects on prolactin and ACTH/cortisol secretion *(47,56)*, without altering the secretion of LH, FSH, and TSH. These data appear to demonstrate that, at the pituitary level, only nontumoral somatotrope cells have functional GHS receptors, while, at the hypothalamic level and via other undefined pathways, ghrelin also activates ACTH and PRL pathways. Ghrelin-induced ACTH secretion depends on CNS-mediated mechanisms, which could include vasopressin-mediated actions, although the involvement of neuropeptide Y, GABA, or putative endogenous ligands *(57,58)* has been hypothesized. Nevertheless, these stimulatory effects have been observed with large doses of ghrelin, and it remains to be observed what happens after more physiological doses.

Regulation of Energy Homeostasis

The fact that ghrelin was an orexigenic agent received a powerful boost after the report that in rodents ghrelin stimulates food intake while reducing fat depot utilization *(59)*. This finding has been confirmed by other groups, indicating the involvement of ghrelin in the regulation of energy balance *(35,59–61)*. Unlike other orexigenic agents, such as neuropeptide Y, melanin-concentrating hormone, and AGRP, which are solely active when are centrally injected *(62–64)*, ghrelin exhibits orexigenic and adipogenic effects when it is administrated either centrally or peripherally *(60,65)*. In this sense, ghrelin is the most powerful stimulator of appetite of all known peptides, and this action is prevented by co-administration of neuropeptide Y blockers or AGRP antagonists.

The effects of ghrelin appear to be opposed to those of leptin and, in this sense, these hormones might be complementary players of one regulatory system that inform the central nervous system about the current status of acute and chronic energy balance *(59,66,70)*. Leptin has been reported to be able to reduce food intake and, at the same time, the fat mass without altering lean body mass, while ghrelin increases food intake and selectively enhances the fat mass.

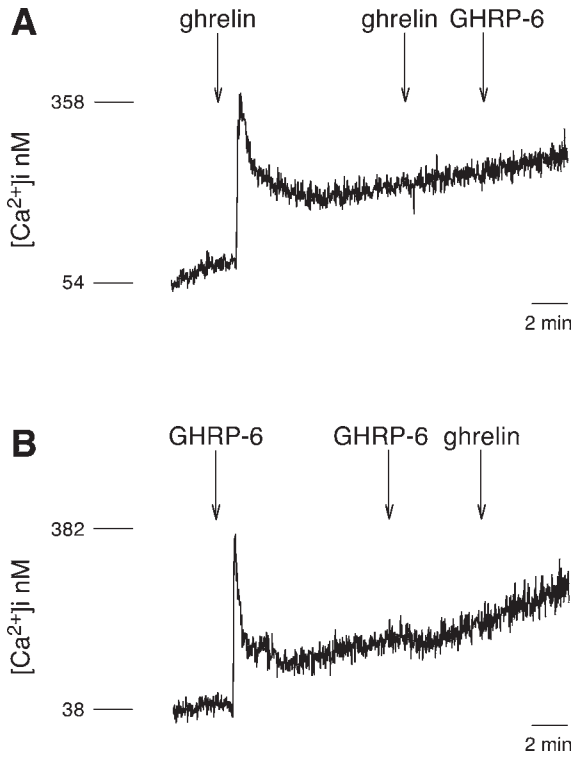

Fig. 5. Homologous desensitization of GHS-R1a. HEK-293 cells expressing the GHSR1a were stimulated with ghrelin (100 n*M*) and GHRP-6 (200 n*M*), and changes in the intracellular calcium were measured with the fluorescent probe fura-2. The repeated administration of a saturating dose of (**A**) ghrelin or (**B**) GHRP-6 caused homologous desensitization of GHS-R1a.

Furthermore, ghrelin can reverse the anorexigenic actions of leptin, probably through the activation of the NPY/Y1 receptor pathway *(67)*. In addition, a specific role for ghrelin might be to ensure the provision of calories that GH recruits for growth and repair.

Human plasma ghrelin levels undergo relevant changes in relation to food intake, rising nearly twofold shortly before each meal, and falling to basal levels within 1 h after eating *(22,23)*. This profile appears to be consistent with a physiological role for ghrelin in initiating individual food intake. The temporal patterns of ghrelin and insulin are reciprocal and are in phase with leptin. Ghrelin and leptin decrease after meals, although the amplitude of this decline was greater for ghrelin than for leptin *(22)*.

Circulating ghrelin levels are decreased in obesity *(26)*, while they are increased in states as malnutrition, cachexia, and anorexia nervosa *(27,68)*. Obese subjects showed lower plasma concentrations of ghrelin than lean control subjects. In addition, ghrelin plasma concentrations are significantly lower in Pima Indians, one of the populations with highest prevalence of obesity (*26)*. The decreased plasma ghrelin concentrations observed in obesity might represent a physiological adaptation to the positive energy balance associated with obesity. Considerable interest raised the possibility that ghrelin might be implicated in the etiology of obesity as mutations in the ghrelin gene are associated with obesity in humans *(69)*. In this sense, sequence variations in the preproghrelin might change the cleavage site of endoproteases, and would affect the mature ghrelin product. Conversely, in anorexia nervosa, fasting plasma levels of ghrelin are elevated, returning to normal after partial weight recovery *(27)*. These observations suggest the possible existence of ghrelin resistance in cachectic states, such as those caused by eating disorders. In any case, it would seem to be important to perform further prospective studies to better understand the physiological role of ghrelin for the regulation of body weight. At this point ghrelin appears as the unknown link connecting growth with metabolism and energy homeostasis *(77)*.

In conclusion, a third peptide component actively participates in the regulation of GH secretion, although no proof is available about a physiological role. In any case, ghrelin actions on the regulation of appetite and energy homeostasis are of paramount relevance and may well become the most active area of research in the field of GH secretagogues.

Acknowledgments

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References

1. Casanueva, F. F. (1992). *Endocrinol. Metab. Clin. N. Am.* **21,** 484–517.
2. Dieguez, C. and Casanueva, F. (1995). *Trends Endocrinol. Metab.* **6,** 55–59.
3. Casanueva, F. F. and Dieguez, C. (1999). *Trends Endocrinol. Metab.* **10,** 30–38.
4. Dieguez, C. and Casanueva, F. F. (2000). *Eur. J. Endocrinol.* **142,** 413–417.
5. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). *Nature* **402,** 656–660.
6. Date, Y., Kojima, M., Hosoda, H., et al. (2000). *Endocrinology* **141,** 4255–4261.
7. Korbonits, M., Bustin, S. A., Kojima, M., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 881–887.
8. Gualillo, O., Caminos, J., Blanco, M., et al. (2001). *Endocrinology* **142,** 788–794.
9. Tena-Sempere, M., Barreiro, M. L., Gonzalez, L. C., et al. (2002). *Endocrinology* **143,** 717–725.
10. Mori, K., Yoshimoto, A., Takaya, K., et al. (2000). *FEBS Lett.* **486,** 213–216.
11. Bowers, C. Y. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1464–1269.
12. Muccioli, G., Tschop, M., Papotti, M., Deghenghi, R., Heiman, M., and Ghigo, E. (2002). *Eur. J. Pharmacol.* **440,** 235–254.
13. Dornonville de la Cour, C., Bjorkqvist, M., Sandvik, A. K., et al. (2001). *Regul. Pept.* **99,** 141–150.
14. Resh, M. D. (1999). *Biochem. Biophys. Acta* **1451,** 1–16.

15. Wajnrajch, M. P., Ten, I. S., Gertner, J. M., and Leibel, R. L. (2000). *J. Endocr. Genet.* **1,** 231–233.
16. Kojima, M., Hosoda, H., Matsuo, H., and Kangawa, K. (2001). *Trends Endocrinol. Metab.* **12,** 118–122.
17. Tanaka, M., Hayashida, Y., Iguchi, T., Nakao, N., Nakai, N., and Nakashima, K. (2001). *Endocrinology* **142,** 3697–3700.
18. Tanaka, M., Hayashida, Y., Nakao, N., Nakai, N., and Nakashima, K. (2001). *Biochim. Biophys. Acta* **1522,** 62–65.
19. Hosoda, H., Kojima, M., Matsuo, H., and Kangawa, K. (2000). *J. Biol. Chem.* **275,** 21995–22000.
20. Ariyasu, H., Takaya, K., Tagami, T., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 4753–4758.
21. Cassoni, P., Papotti, M., Ghe, C., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1738–1745.
22. Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., and Weigle, D. S. (2001). *Diabetes* **50,** 1714–1719.
23. Tschop, M., Wawarta, R., Riepl, R. L., et al. (2001). *J. Endocrinol. Invest.* **24,** RC19–RC21.
24. Gualillo, O., Caminos, J. E., Nogueiras, R., et al. (2002). *Obes. Res.* **10,** 682–687.
25. Toshinai, K., Mondal, M. S., Nakazato, M., et al. (2001). *Biochem. Biophys. Res. Commun.* **281,** 1220–1225.
26. Tschop, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E., and Heiman, M. L. (2001). *Diabetes* **50,** 707–709.
27. Otto, B., Cuntz, U., Fruehauf, E., et al. (2001). *Eur. J. Endocrinol.* **145,** 669–673.
28. Caminos, J. E., Seoane, L. M., Tovar, S. A., Casanueva, F. F., and Dieguez, C. (2002). *Eur. J. Endocrinol.* **147,** 159–163.
29. Howard, A. D., Feighner, S. D., Cully, D. F., et al. (1996). *Science* **273,** 974–977.
30. Mckee, K. K., Palyha, O. C., Feighner, S. D., et al. (1997). *Mol. Endocrinol.* **11,** 415–423.
31. Smith, R. G., Van der Ploeg, L. H., Howard, A. D., et al. (1997). *Endocr. Rev.* **18,** 621–645.
32. Smith, R. G., Palyha, O. G., Feighner, S. D., et al. (1999). *Horm. Res.* **51,** 1–8.
33. Petersenn, S., Rasch, A. C., Penshorm, M., Beil, F. U., and Schulte, H. M. (2001). *Endocrinology* **142,** 2649–2659.
34. Yokote, R., Sato, M., Matsubara, S., et al. (1998). *Peptides* **19,** 15–20.
35. Shintani, M., Ogawa, Y., Ebihara, K., et al. (2001). *Diabetes* **50,** 227–232.
36. Bluet-Pajot, M. T., Tolle, V., Zizzari, P., et al. (2001). *Endocrine* **14,** 1–8.
37. Papotti, M., Ghe, C., Cassoni, P., et al. (2000). *J. Clin. Endocrinol. Metab.* **85,** 3803–3807.
38. Gnanapavan, S., Kola, B., Bustin, S. A., et al. (2002). *J. Clin. Endocrinol. Metab.* **87,** 2988–2991.
39. Tullin, S., Hansen, B. S., Ankersen, M., Moller, J., Von Cappelen, K. A., and Thim, L. (2000). *Endocrinology* **141,** 3397–3402.
40. Smith, R. G., Griffin, P. R., Xu, Y., et al. (2000). *Biochem. Biophys. Res. Commun.* **276,** 1306–1313.
41. Ong, H., McNicoll, N., Escher, E., et al. (1998). *Endocrinology* **139,** 432–435.
42. Bodart, V., Bouchard, J. F., McNicoll, N., et al. (1999). *Circ. Res.* **85,** 796–802.
43. Chen, C., Wu, D., and Clarke, I. J. (1996). *J. Endocrinol.* **148,** 381–386.
44. Murata, M., Okimura, Y., Iida, K., et al. (2002). *J. Biol. Chem.* **277,** 5667–5674.
45. Seoane, L. M., Tovar, S., Baldelli, R., et al. (2000). *Eur. J. Endocrinol.* **143,** R7–R9.
46. Arvat, E., Di Vito, L., Broglio, F., et al. (2000). *J. Endocrinol. Invest.* **23,** 493–495.
47. Peino, R., Baldelli, R., Rodriguez-Garcia, J., et al. (2000). *Eur. J. Endocrinol.* **143,** R11–R14.
48. Takaya, K., Ariyasu, H., Kanamoto, N., et al. (2000). *J. Clin. Endocrinol. Metab.* **85,** 4908–4911.
49. Hataya, Y., Akamizu, T., Takaya, K., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 4552–4555.
50. Bowers, C. Y. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1464–1469.
51. Inui, A. (2001). *Nature Rev. Neurosci.* **2,** 551–560.
52. Maheshwari, H. G., Rahim, A., Shalet, S. M., and Baumann, G. (1999). *J. Clin. Endocrinol. Metab.* **84,** 956–959.
53. Di Vito, L., Broglio, F., Benso, A., et al. (2002). *Clin. Endocrinol. (Oxf.)* **56,** 643–648.
54. Gualillo, O., Caminos, J. E., Kojima, M., et al. (2001). *Eur. J. Endocrinol*. **144,** 687–690.
55. Janssen, J. A., van Der Toorn, F. M., Hofland, L. J., et al. (2001). *Eur. J. Endocrinol.* **145,** 711–716.
56. Arvat, E., Maccario, M., Di Vito, L., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1169–1174.
57. Ghigo, E., Arvat, E., and Camanni, F. (1998). *Growth Horm. IGF Res.* **8,** 145–148.
58. Ghigo, E., Arvat, E., Giordano, R., et al. (2001). *Endocrine* **14,** 87–93.
59. Tschop, M., Smiley, D. L., and Heiman, M. L. (2000). *Nature* **407,** 908–913.
60. Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., and Wakabayashi, I. (2000). *Endocrinology* **141,** 4797–4800.
61. Wren, A. M., Small, C. J., Ward, H. L., et al. (2000). *Endocrinology* **141,** 4325–4328.
62. Kalra, S. P., Dube, M. G., Pu, S., Xu, B., Horvath, T. L., and Kalra, P. S. (1999). *Endocr. Rev.* **20,** 68–100.
63. Gehlert, D. R. (1999). *Neuropeptides* **33,** 329–338.
64. Tritos, N. A. and Maratos-Flier, E. (1999). *Neuropeptides* **33,** 339–349.
65. Hewson, A. K. and Dickson, S. L. (2000). *J. Neuroendocrinol.* **12,** 1047–1049.
66. Farooqi, I. S., Jebb, S. A., Langmack, G., et al. (1999). *N. Engl. J. Med.* **341,** 879–884.
67. Toogood, A. A. and Thorner, M. O. (2001). *Clin. Endocrinol.* **55,** 589–591.
68. Nagaya, N., Uematsu, M., Kojima, M., et al. (2001). *Circulation* **104,** 2034–2038.
69. Ukkola, O., Ravussin, E., Jacobson, P., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 3996–3999.
70. Popovic, V., Damjanovic, S., Dieguez, C., and Casanueva, F. F. (2001). *Pituitary* **4**, 7–14.
71. Gaytan, F., Barreiro, M. L., Chopin, L. K., et al. (2003). *J. Clin. Endocrinol. Metab.* **88,** 879–897.
72. Barreiro, M. L., Gaytan, F., Caminos, J. E., et al. (2002). *Biol. Reprod.* **67,** 1768–1776.
73. Broglio, F., Koetsveld, Pv. P., Benso, A., et al. (2002). *J. Clin. Endocrinol. Metab.* **87,** 4829–4832.
74. Jeffery, P. L., Herington, A. C., and Chopin, L. K. (2002). *J. Endocrinol.* **172,** R7–R11.
75. Hosoda, H., Kojima, M., Mizushima, T., Shimizu, S., and Kangawa, K. (2003). *J. Biol. Chem.* **278,** 64–70.
76. Barreiro, M. L., Suominen, J. S., Gaytan, F., et al. (2003). *Biol. Reprod*. **68,** 1631–1640.
77. Casanueva, F. F. and Dieguez, C. (2002). *Rev. Endocr. Metab. Disord.* **3,** 325–338.