Ghrelin: Much More than a Natural Growth Hormone Secretagogue

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Abstract

Ghrelin, a 28 amino acid acylated peptide predominantly produced by the stomach, displays strong growth hormone-releasing activity mediated by the hypothalamus-pituitary GH secretagogue receptors that were found to be specific for a family of synthetic, orally active GH secretagogues. The discovery of ghrelin brings us to a new understanding of the regulation of GH secretion. However, ghrelin is much more than simply a natural GH secretagogue. It also acts on other central and peripheral receptors and exhibits other actions, including stimulation of lactotroph and corticotroph secretion, orexigenia, influences gastroenteropancreatic functions, and has metabolic, cardiovascular and antiproliferative effects. Knowledge of the whole spectrum of biologic activities of this new hormone will provide new understanding of some critical aspects of neuroscience, metabolism and internal medicine. In fact, GHS were born more than 20 years ago as synthetic molecules, eliciting the hope that orally active GHS could be used to treat GH deficiency as an alternative to recombinant human GH. However, the dream did not become reality and the usefulness of GHS as an anabolic anti-aging intervention restoring the GH/IGF-I axis in somatopause is still unclear. Instead, we now face the theoretic possibility that GHS analogues acting as agonists or antagonists could become candidate drugs for the treatment of pathophysiologic conditions in internal medicine totally unrelated to disorders of GH secretion.

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Ghrelin, a 28 amino acid peptide predominantly produced by the stomach, displays strong growth hormone-releasing activity mediated by the activation of the GH secretagogue receptor 1a, which was shown to be specific for a family of synthetic, peptidyl and non-peptidyl GHS [1–6]. These synthetic molecules were invented more than 20 years ago, and one of the most important factors prompting research development in this field was their strong GH-releasing activity, even after oral administration, that suggested their potential clinical usefulness as a new tool for the diagnosis and treatment of GH deficiency in childhood and somatopause in aging [4,7]. For sure, the discovery of ghrelin represented a turning-point in our understanding of the control of the GH/IGF-I axis. However, it is already clear that ghrelin is much more than simply a natural GH secretagogue.

Ghrelin and synthetic GHS act via receptors concentrated in the hypothalamus-pituitary unit but are also distributed in other central and peripheral tissues [2–4]. While hypothalamus-pituitary receptors explain their stimulatory effect on GH as well as on prolactin and adrenocorticotropic hormone secretion [1,3–6,8,9], other central and peripheral specific binding sites explain other activities such as: a) the orexigenic effect coupled with the control of energy expenditure, b) control of gastric motility and acid secretion as well as influence on the endocrine pancreatic function and glucose metabolism, c) cardiovascular actions, and d) antiproliferative effects.

We present here an updated overview on the endocrine and non-endocrine actions of ghrelin, a new hormone possessing a wide spectrum of biologic activities which, in addition to its control of somatotroph function, will probably change our understanding of other critical aspects of neuroscience, metabolism and internal medicine.

Historical milestones: from GH-releasing peptides to ghrelin

Ghrelin was found in the stomach as a natural ligand of the orphan GHS-R1a which, in turn, was identified as a specific receptor for synthetic GHS [1–6]. Thus, the discovery of ghrelin is an example of reversed pharmacology – starting from synthetic analogues and leading to the natural ligand via the discovery of the natural receptor.

Synthetic GHS are a family including many peptidyl and non-peptidyl molecules [3,4]. The first molecules were non-natural peptides (GH-releasing peptides) that were invented – rather than isolated – by Bowers and Momany in the late 1970s as met-enkephalin derivatives devoid of any opioid activity [4,5]. GHRP-6

GH = growth hormone
GHS = GH secretagogues
GH/IGF-1 = growth hormone/insulin-like growth factor-I

GHS-R = GHS receptor
GHRP = GH-releasing peptide

was the first hexapeptide able to release GH *in vivo*, in humans even more than in animals, and one of its most remarkable properties was its strong GH-releasing activity even after oral administration – despite low bioavailability and short-lasting effect [3,4]. Aiming to select orally active molecules with better bioavailability and long-lasting effect, further studies led to the synthesis of other GHRPs and, above all, of orally active non-peptidyl molecules, the most representative of which is the spiroindoline MK-0677 [3,4]. MK-0677 possesses impressive bioavailability and long-lasting effect, being able to enhance 24 hour GH secretion and insulin-like growth factor-I levels after a single oral administration. This finding explains how it became the candidate drug for the treatment of GH deficiency in childhood and as an orally active anabolic anti-aging intervention in frail elderly subjects [3,4].

Apart from its clinical implications, MK-0677 allowed the discovery and cloning of the GHS receptor whose existence was indicated by binding studies [2–4]. Studies focusing on the GHS receptor distribution showed particular concentration of GHS receptors in the hypothalamus-pituitary area, but a remarkable presence of specific binding sites also in other central nervous system areas and peripheral, endocrine and non-endocrine animal and human tissues [2–4]. Indeed this GHS-R distribution explained not only its GH-releasing effect but also other endocrine and non-endocrine biologic activities [3,10]. Based on this knowledge, less than 2 years ago ghrelin was discovered by Japanese scientists involved in the cardiovascular field and attracted by the cardiovascular activities of synthetic GHS.

Ghrelin is a 28 amino acid peptide predominantly produced by the stomach, with substantially lower amounts deriving from bowel, pancreas, kidneys, placenta, pituitary and hypothalamus [1,6,11,12, and personal unpublished data]. Within the stomach ghrelin is produced by the enteroendocrine cells, probably the X/A-like cells, one of the major endocrine cell populations in the oxyntic mucosa whose hormonal product had not previously been clarified [6,11,12].

Ghrelin is the first peptide isolated from natural sources in which the hydroxyl group of one of its serine residues is acylated by n-octanoic acid [1,6]. The acylation of the peptide is considered crucial to cross the blood-brain-barrier, but it is also essential for binding the GHS-R1a and for its GH-releasing and other endocrine actions [1,6,13]. Interestingly, short peptides encompassing the first 4–5 residues (including acylated serine) of ghrelin have similarities with the old GHRPs and were found able to activate the GHS-R1a, indicating the core required for its efficient binding and activation [6]. However, non-acylated ghrelin, which circulates in an amount far greater than the acylated form, is not biologically inactive; it is able to exert some non-endocrine actions including cardiovascular and antiproliferative effects, probably by binding different GHS-R subtypes or receptor families [5,10, and personal unpublished results].

Another endogenous ligand of the GHS-R1a has also been isolated from the stomach, and is named Des-Gln14-ghrelin. It has the same acylation in Ser3 and is homologous to ghrelin except for one glutamine that is missing; it is the result of an alternative splicing of the ghrelin gene and possesses the same activity of ghrelin [5,6].

GHS-R is bound also by other molecules such as adenosine, which is unable to activate the receptor, and cortistatin, a neuropeptide homologous to somatostatin, which is unable to recognize that receptor [2,13,14]. It has been suggested that different molecules are able to bind different pockets of the GHS-R but not necessarily to activate it; however, further studies are required to clarify whether ghrelin is the sole ligand or one of a number of ligands activating the GHS-R, and whether the GHS-R used for ghrelin isolation is the sole receptor or one of a group of receptors for such ligands.

The GHS story led to the discovery of the motilin receptor, which is a member of the GHS-R family, having 52% identity [2]. Human ghrelin and motilin have 36% identity, and the pre-pro-motilin related peptide produced by the enteroendocrine cells of the stomach is fully identical with human pre-pro-ghrelin except that serine 26 is not octanoylated in pre-pro-motilin related peptide [1,6,15]. It is noteworthy that ghrelin and motilin share not only structural similarities but also biologic activities; however, although motilin is able to stimulate GH secretion and to exert an orexigenic effect, it does not bind the GHS-R1a [2,6,15]. Thus, ghrelin and motilin represent a novel family of gastrointestinal peptides that contribute to the regulation of diverse functions of the gut-brain axis [15].

Although the regulation of ghrelin secretion is still largely unknown, it is already clear that in humans, circulating ghrelin levels are decreased in chronic (obesity) and acute (feeding) states of positive energy balance [11,16,17], while plasma ghrelin levels are increased by fasting and in patients with anorexia nervosa [11]. Pre-meal elevation of circulating ghrelin levels suggests its role as a hunger signal triggering meal initiation, and this signal could be mediated by GHS-R subtypes [18].

Endocrine activities of GHS: clinical implications

GH-releasing activity

Ghrelin as well as synthetic GHS possess a strong and dose-related GH-releasing effect that is more marked in humans than in animals [1,3–6,8,9]. The GH response to GHS is strongly reduced, though not abolished, by hypothalamus-pituitary disconnection [3,4], which concurs with the assumption that the most important action of GHS occurs at the hypothalamic level [3,4].

GHS and GHRH have a synergistic effect indicating that they act, at least partially, via different mechanisms [3–5,19]. Nevertheless, GHS need GHRH activity to fully express their GH-releasing effect and probably act to trigger GHRH-secreting neurons [3–5,19]. In humans the GH response to GHS is strongly inhibited, though not abolished, by a GHRH antagonist as well as by hypothalamus-pituitary disconnection [3,4]. Moreover, in patients with GHRH-R deficiency, GHS lose their GH-releasing activity but maintain their stimulatory effect on PRL, ACTH and cortisol secretion [3]. GHS probably also act as functional somatostatin antagonists at both the pituitary and the hypothalamic level [3,19]. In humans, the GH response to GHS is not modified by substances acting via somatostatin inhibition (such as cholinergic agonists, arginine)

PRL = prolactin

which, in turn, truly potentiate the GHRH-induced GH rise [3]. Moreover, the GH-releasing activity of GHS is partially refractory to the inhibitory effect of substances acting via stimulation of hypothalamic somatostatin (such as cholinergic antagonists, beta-adrenergic agonists, glucose), which almost abolish the somatotroph responsiveness to GHRH [3]. Indeed, GHS are also partially refractory to the inhibitory effect of substances acting on somatotroph cells, such as free fatty acids, and even to exogenous somatostatin [3]. Moreover, GHS are partially refractory to the negative GH auto-feedback, whereas they exhibit sensitivity to the negative IGF-I feedback action [3]. The GH-releasing effect of GHS undergoes marked age-related variations – increasing at puberty, persisting in adulthood, and decreasing with aging [3]. The mechanisms underlying the age-related variations of the GH-releasing activity of GHS differ with each age. The enhanced GH-releasing effect of GHS at puberty reflects the positive influence of estrogens, which could trigger an increase in GHS-R expression [3]. However, estrogen insufficiency does not explain the reduced GH response to GHS in postmenopausal women [3]. In parallel to a reduction of hypothalamic GHRP receptors in the human aging brain, the GH response to hexarelin in elderly subjects is further increased but not restored by supramaximal doses [3]. The most important mechanism accounting for the reduced GH-releasing activity of GHS in aging is probably represented by age-related variations in the neural control of somatotroph function, including GHRH hypoactivity and somatostatinergic hyperactivity [3]. On the other hand, it has also been hypothesized that declining GH secretion may reflect an age-related decrease in the activity of the endogenous GHS ligand, i.e., ghrelin [4,5]. This hypothesis remains to be verified since we do not have any information about age-related variations in ghrelin secretion, yet.

Based on their strong and reproducible GH-releasing effect even after oral administration, GHS could theoretically have diagnostic and therapeutic usefulness. Particularly when combined with GHRH, GHS represent one of the most potent and reliable tests to evaluate the pituitary GH-releasable pool for the diagnosis of growth hormone deficiency, at least in adulthood [7]. Testing with GHS is as sensitive and specific as insulin tolerance test and GHRH+arginine, the two gold standard tests for the diagnosis of adult GH deficiency, provided that appropriate cut-off limits are assumed [7]. It is widely accepted that the diagnosis of GH deficiency in childhood is not simply assessed by the GH response to either classical or maximal provocative tests [3,7]. Indeed, a considerable number of short children with normal GH response to provocative tests show insufficient daily GH secretion, reflecting neurosecretory dysfunction and benefit from GH replacement [3,7]. Although a normal GH response to stimuli does not rule out GH insufficiency in childhood, potent and reproducible provocative tests of GH secretion such as GHS can provide definitive information about the maximal secretory capacity of somatotroph cells in short children suspected as GH-deficient [3,7].

The possibility that GH-releasing substances, particularly if orally active, could represent a therapeutic alternative approach to rhGH in GH-deficient patients has received considerable attention. Clearly, GH-releasing substances have no place as an alternative to rhGH for treatment of severe GH deficiency in patients with panhypopituitarism due to massive destruction of the pituitary gland and, consequently, of somatotroph cell population. On the other hand, isolated GH deficiency often reflects hypothalamic pathogenesis, as shown by clear GH responses to GHRH in many dwarf patients [3]. The hypothesis was that patients with GH deficiency could benefit from treatment with GHRH, preferably orally active GHS, which could have the advantage of restoring endogenous GH pulsatility and is, therefore, a more "physiologic" approach.

The therapeutic usefulness of GHRH has been demonstrated in children with isolated GH deficiency, but this parenteral treatment did not show any advantage over classical rhGH replacement [3]. The potential usefulness of GHS for treatment of short stature with isolated GH deficiency was suggested by some open studies, which reported an increase in height velocity in short children with idiopathic short stature or GH deficiency after chronic treatment with GHRPs given intranasally or subcutaneously [3,7]. When tested in a double-blind placebo-controlled trial in short children with GH deficiency, the efficacy of the most promising orally active GHS, i.e., MK-0677, was not comparable with that of rhGH [20]. Treatment with MK-0677 transiently increased height velocity (approximately 3 cm/year) in a dose-independent manner in children with partial GH deficiency but not in children with severe GH deficiency, who did not benefit at all from this treatment [20]. Thus, it is difficult to believe that GHS could replace rhGH in the treatment of GH deficiency in childhood. This evidence also refutes the hypothesis that isolated GH deficiency could reflect a defect in the activity of the endogenous GHS-like ligand, i.e., ghrelin. As a general comment on the failure of GHS as an alternative to rhGH for treatment of GH deficiency, it can be said that the patent dependence of GHS activity on the full integrity of hypothalamus-pituitary function could predict its failure in pathophysiologic conditions that, by definition, include some alteration in the hypothalamus-pituitary unit.

On the other hand, GHS could also represent an anabolic treatment in frail elderly subjects with somatopause. This hypothesis is based on the following evidence: a) the age-related reduction in the activity of GH/IGF-I axis probably accounts for changes in body composition, structure functions and metabolism in normal elderly subjects; these changes are remarkably similar to (but of lesser extent than) those in GH-deficient adults [3,7,21]; and b) the pituitary GH-releasable pool is still remarkable in aged subjects, thus GH-releasing substances would represent a more physiologic approach to restore endogenous GH pulsatility [3,7,21].

To date, the following results have been obtained by trials testing the effects of chronic treatment with MK-0677: a) in elderly subjects it restored IGF-I levels to the normal young range, indicating successful enhancement of somatotroph secretion [4,7]; b) in elderly subjects it increased REM sleep while decreasing REM latency, thus counteracting alterations in sleep pattern that are hallmarks of brain aging [22,23]; c) it reversed diet-induced catabolism in young volunteers, indicating an anabolic effect [24]

IGF = insulin-like growth factor

while increasing fat-free mass and energy expenditure in obese patients [25]; d) in a large population of postmenopausal osteoporotic women, one year treatment with MK-0677 alone and in combination with alendronate, a bisphosphonate, attenuated the indirect suppressive effect of alendronate on bone formation but did not translate into significant increases in bone mineral density at sites other than the femoral neck [26].

Overall, at present, there is no definitive evidence showing the therapeutic efficacy of GHS as an anabolic agent acting via rejuvenation of the GH/IGF-I axis in elderly subjects and further studies are needed to verify this hypothesis.

PRL and ACTH-releasing activity

The endocrine activity of both ghrelin and synthetic GHS is not fully specific for GH and includes stimulatory effects on both lactotroph and corticotroph secretion [3]. The stimulatory effect of GHS on PRL secretion in humans is slight, independent of both gender and age, and probably derives from direct stimulation of somatomammotroph cells [3]. The stimulatory effect of GHS on the activity of the hypothalamus-pituitary-adrenal axis in humans is significant and similar to that after naloxone, arginine-vasopressin and even corticotropin-releasing hormone; nonetheless, it is an acute neuroendocrine effect that probably vanishes during prolonged treatment [3,5].

The ACTH-releasing activity of GHS is independent of gender but shows peculiar age-related variations. It increases at puberty, shows a reduction in adulthood and, again, a trend toward increase in aging (when the GH response to these molecules is clearly reduced) [3]. This evidence suggests that these molecules act at different levels and/or on different receptor subtypes [3–5].

In physiologic conditions, the ACTH-releasing activity of GHS totally depends on CNS-mediated mechanisms [3]. These mechanisms could include CRH or AVP-mediated actions [3], though the possibility that they act via neuropeptide Y and/or GABA [3] cannot be ruled out. The ACTH response to GHS is generally sensitive to the negative feedback action of cortisol [3]. However, the stimulatory effect of GHS on corticotroph secretion is surprisingly exaggerated (and higher than that to hCRH) in patients with pituitary ACTH-dependent Cushing's disease [3,28]. Ghrelin and GHS-R are expressed in abnormal human pituitary as well as in other neuroendocrine tumors including ACTH-secreting tumors [27,28], and GHS stimulate ACTH release from human ACTH-secreting pituitary adenomas but not from normal human pituitary [3]. Thus, the exaggerated ACTH response in patients with Cushing's disease could reflect action at the pituitary level on tumoral corticotroph cells [3,28]. Parallel to the presence of ghrelin and GHS-R expression also in ectopic ACTH-secreting tumors [28,29], exaggerated ACTH and/or cortisol response to GHS has been observed also in patients with ectopic ACTH-dependent Cushing's syndrome [3]. This evidence reduces the possibility that testing with GHS could be useful to distinguish patients with pituitary from ectopic ACTH-dependent hypercortisolism.

CRH = corticotropin-releasing hormone
AVP = arginine-vasopressin

Non-endocrine activities of GHS: potential clinical implications

GHS receptors are concentrated in the hypothalamus-pituitary unit but are also distributed in other central and peripheral tissues [3,4,6]. Besides the potent GH-releasing effect and slight stimulation of PRL and ACTH secretion, ghrelin as well as synthetic GHS have other important activities including: a) orexant activity coupled with control of energy expenditure, b) influence on sleep, c) control of gastric motility and acid secretion, d) influence on the endocrine pancreatic function and glucose metabolism, e) cardiovascular actions, and f) antiproliferative effects in neoplastic thyroid, breast and lung cell lines. These biologic activities often suggest potential clinical benefit.

Orexant activity

In agreement with previous reports on the effects of synthetic GHS, several studies have unequivocally shown that ghrelin is involved in the regulation of energy balance [11,12]. Exogenous ghrelin induces a marked weight gain in rodents by increasing food intake and reducing fat utilization [11,12]. These activities are GH-independent and are most likely mediated by a specific central network of neurons that is also modulated by leptin [11,12]; ghrelin and leptin might in fact be complementary players of one regulatory system that has been developed to inform the central nervous system about the status of the energy balance [11,12]. It should be noted that in obesity, leptin levels are elevated while ghrelin levels are decreased, suggesting their adaptation to the positive energy balance rather than an involvement in the etiology of obesity [11,12].

In humans, circulating ghrelin levels are decreased in chronic (obesity) and acute (feeding) [11,16,17] states of positive energy balance, while plasma levels of ghrelin are increased by fasting and in patients with anorexia nervosa [11]. Pre-meal rise of circulating ghrelin levels suggests its role as a hunger signal triggering meal initiation [18]. Interestingly, the orexigenic effect of ghrelin could be mediated by GHS-R subtypes, as indicated also by the evidence that GHS analogs devoid of any GH-releasing effect stimulate food intake [11].

Peripheral ghrelin is mainly produced in the gastrointestinal tract [1,11,12, and personal unpublished data] and could reach GHS receptors in the CNS, namely the hypothalamus, through the general circulation, to regulate food intake and energy homeostasis [5,11,12,15]. Ghrelin-containing cells are also present in the mediobasal hypothalamus where GHRH-secreting neurons and the neuroendocrine network regulating energy balance are located [1,6,11]. Indeed, NPY1-receptor antagonists as well as melanocortin agonists and antisera to both NPY and agouti-related protein interfere with the orexant effect of ghrelin [11], which is however preserved in NPY-ko mice, suggesting a key role for AGRP [11].

The stimulatory effect of ghrelin and synthetic GHS on food intake raises obvious interest. Synthetic GHS analogues acting as agonists or antagonists on the appetite may have potential for drug intervention in eating disorders.

NPY = neuropeptide Y

Influence on sleep

Alterations in the sleep pattern are the hallmark of brain aging, likely reflecting age-related changes in neurotransmitters and neuropeptides [23]. The potential influence of GH and IGF-I on sleep pattern has also been suggested, based on studies in GH-deficient subjects; and it had been hypothesized that the sleep pattern in aged subjects could reflect an age-related decrease in the activity of the GH/IGF-I axis [23]. On the other hand, some studies reported that the acute administration of synthetic peptidyl GHS is able to modify the sleep pattern in normal subjects [30]. It has also been reported that prolonged treatment with oral MK-0677 (25 mg once daily) in elderly subjects is able to increase REM sleep while decreasing REM latency [22].

Gastroenteropancreatic and metabolic actions

It is not surprising that being a gastric hormone, ghrelin acts at the gastroenteropancreatic level where GHS-R mRNA expression has been demonstrated [3,31, and personal unpublished data]. It is noteworthy that ghrelin shows its highest homology with motilin, whose receptor is related to the GHS-R [4,11,12]; the similarity between activities of ghrelin and motilin has also been emphasized [15].

In rats, ghrelin stimulates gastric contractility and acid secretion [32]. These actions are mediated by the cholinergic system and are abolished by muscarinic blockade; interestingly the acetylcholine-mediated stimulatory effect of ghrelin on gastric acid secretion occurs, at least partially, at the central level [32].

Ghrelin is produced by about 20% of the rat and human neuroendocrine cell population of the stomach (A-like cells) [1,11,12]. The endocrine cell population of the stomach may undergo proliferative phenomena of both a hyperplastic and neoplastic nature. Gastric carcinoids (and also neuroendocrine cell hyperplastic conditions) often synthesize ghrelin, which thus could be the product of many apparently silent gastric carcinoids generally associated with atrophic gastritis. These might represent conditions of ghrelin hypersecretion, while gastrectomy could result in ghrelin insufficiency, although preliminary data indicate a reduction of only 50% in circulating ghrelin levels after gastrectomy [11]. The clinical impact of ghrelin hyper- and hyposecretion is still unknown.

Ghrelin and GHS-R mRNA are also present in normal and neoplastic endocrine pancreas [personal unpublished data], and we have recently demonstrated that ghrelin but not peptidyl GHS induces significant hyperglycemia that is surprisingly followed by a reduction in insulin secretion in humans [33]. Coupled with the observation that chronic treatment with MK-0677 induced hyperglycemia in a large number of elderly subjects [4,7], this evidence points to ghrelin as a gastroenteropancreatic hormone that plays a very significant role in glucose metabolism. These actions would also agree with the hypothesis that ghrelin has a major role in managing the neuroendocrine and metabolic response to starvation.

Cardiovascular actions

Specific binding sites for GHS are present at the peripheral level in both endocrine and non-endocrine human tissues, particularly in the cardiovascular system [3,34]. Moreover, the presence of GHS-R 1a mRNA has recently been demonstrated in animal and human cardiac tissues [6, and personal unpublished results]. Considerable specific ^{125}I-Tyr-Ala-Hexarelin binding is detectable in ventricles, atria, aorta, coronaries, carotid, endocardium and vena cava, with values often higher than those found in the pituitary [3,34]. This binding is inhibited by unlabeled Tyr-Ala-Hexarelin and hexarelin as well as by other peptidyl GHS but not by MK-0677, a non-peptidyl GHS, and even by ghrelin [3]. Thus, these binding sites, which are not recognized by classical cardioactive substances either, are likely GHS-R subtypes specific for peptidyl GHS only [3,34].

The first studies addressing the cardiovascular activities of GHS were performed with synthetic molecules. The researchers showed that prolonged treatment with peptidyl GHS markedly protects against cardiovascular damage in aged rats as well as in GH-deficient rats with post-ischemic ventricular dysfunction [3]. GHS were also reported to improve cardiac performances in rats after myocardial infarction [35], to protect against diastolic dysfunction of myocardial stunning in isolated perfused rabbit heart [36], and to enhance left ventricular contractility in pigs with heart failure [37]. On the other hand, acute administration of high dose peptidyl GHRP was reported to induce a clear though transient coronary vasoconstriction in perfused rat heart [34]. These effects of synthetic GHS have never been confirmed by studies on the effects of ghrelin. Particularly, the protectant activity against ischemia seemed exerted only by peptidyl GHS.

In humans, the acute administration of peptidyl GHS such as hexarelin was shown to increase left ventricular ejection fraction in normal young volunteers as well as in hypopituitary patients with severe GH deficiency without any variations of mean blood pressure, heart rate and catecholamine levels [38].The effect in GH deficiency further pointed toward GH-independent cardiac actions of GHS. The same positive effect of hexarelin was seen in patients with post-ischemic but not with idiopathic dilated cardiomyopathy [38, and personal unpublished results].

Recently, it was demonstrated that ghrelin also has hemodynamic activity in humans; ghrelin administration in normal young volunteers was followed by a reduction of cardiac afterload and an increase of cardiac output without any change in the heart rate [39]. This evidence indicates that the positive influence of GHS on cardiac contractility is likely mediated by the GHS-R1a. On the other hand, ghrelin as well as peptidyl and non-peptidyl GHS and even GHS analogs devoid of any GH-releasing activity have been shown to: a) prevent cell death of cultured cardiomyocytes and endothelial cells induced by either doxorubicin, serum withdrawal or activation of FAS; and b) stimulate survival intracellular signaling pathways, including tyrosine phosphorylation of intracellular proteins and activation of ERK1/2 and Akt [3]. Interestingly, the same effects of acylated ghrelin are shared by the non-acylated molecule, thus indicating that the acylation of the peptide is needed for the endocrine actions only and that even the non-acylated ghrelin is a biologically active peptide [Graziani, unpublished data]. Since non-acylated ghrelin is generally unable to bind the GHS-R1a, this evidence would imply the existence of another GHS-R subtype that mediates an anti-apoptotic effect in the cardiovascular system [1,3,6,14].

The cardiovascular activities of natural and synthetic GHS suggest potential pharmacotherapeutic implications. Theoretically, GHS analogs could be designed to protect against coronary ischemia and/or to prevent dilated cardiomyopathy and thus improve cardiac performances and/or reduce the progression of endothelial dysfunction and microangiopathy.

Anti-proliferative activity

GHS binding sites have also been found in neoplastic endocrine and non-endocrine tissues; interestingly GHS-R subtypes have been found in tumoral tissues from organs that do not express these receptors in physiologic conditions, e.g., the breast [10].

Both physiologic and neoplastic thyroid tissues express GHS receptors [3]. All follicular-derived thyroid tumors (but not the parafollicular-derived medullary carcinomas) show specific GHS binding sites, even more than the normal thyroid does [3]. Specific GHS binding sites are also present in thyroid tumor cell lines (follicular, papillar and anaplastic tumoral cell lines) [3]. Ghrelin as well as synthetic GHS inhibit the incorporation of ^{3}H-thymidine and inhibit cell proliferation, at least at the earliest time of treatment in all the cell lines [3, and personal unpublished results]. Specific GHS receptors have also been shown in breast tumor but not in fibroadenomas or normal mammary parenchyma [10]. In breast tumors the highest binding activity is present in well-differentiated invasive breast carcinomas and is progressively reduced in moderately to poorly differentiated tumors [10]. Specific GHS binding sites are also present in different human breast (estrogen-dependent and independent) carcinoma cell lines (MCF7, T47D, MDA-MB231) in which ghrelin as well as synthetic GHS inhibit cell proliferation at concentrations close to their binding affinity [10,40]. As in the cardiovascular system, the same effects of acylated ghrelin are shared by the non-acylated molecule, further indicating that even non-acylated ghrelin is a biologically active peptide possessing antiproliferative actions [10]. Since non-acylated ghrelin is generally unable to bind the GHS-R1a, this evidence could imply the existence of another GHS-R subtype that mediates anti-proliferative effects on breast cancer cells [1,6,14].

Preliminary data in our laboratory indicate that neuroendocrine as well as non-endocrine lung tumors exhibit specific binding sites, which are expressed also by a human lung tumor cell line (CALU-1) whose proliferation is inhibited by both peptidyl and non-peptidyl GHS [submitted].

The antiproliferative effects of ghrelin and synthetic GHS further show their multiple biologic activities and suggest the possibility that GHS analogs could exert an anti-neoplastic action. Particular attention should be paid to designing GHS analogues devoid of stimulatory effect on the activity of GH/IGF-I axis because of the well-known positive influence of growth factors on tumor cell proliferation. Once again, the potential clinical benefits are obvious.

Conclusions

GH secretagogues were born more than 20 years ago as synthetic molecules possessing strong GH-releasing activity, and evoked the vision that GH deficiency could be treated by orally active GHS as an alternative to rhGH. Not only did the dream not become reality but their usefulness as an anabolic anti-aging intervention in somatopause is still unclear. Despite the demise of that dream, the GHS story led to the discovery of ghrelin as a natural ligand of the specific receptors mediating the activities of synthetic GHS. It was not by chance that ghrelin was discovered by a group active in the cardiovascular field. In fact, it had already been shown that synthetic GH secretagogues were more than simply molecules stimulating GH release and were, for instance, endowed with cardiotropic actions. We now know that GHS were mimicking the activity of ghrelin, which is much more than a natural GH secretagogue. Other neuroendocrine, metabolic and non-endocrine actions have contributed to the profile of a new hormone that is shifting us from neuroendocrinology to the heart of internal medicine.

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