**Review**

**Ectopic secretion of growth hormone-releasing hormone (GHRH) in neuroendocrine tumors: Relevant clinical aspects**

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**Summary**

The aim of this article is to briefly review the physiology of growth hormone-releasing hormone (GHRH) and the diagnosis and treatment of GHRH-mediated acromegaly. Moreover, the role of GHRH and its antagonists in the pathogenesis and treatment of cancer will be reviewed. Hypothalamic GHRH is secreted into the portal system, binds to specific surface receptors of the somatotroph cell and elicits intracellular signals that modulate pituitary GH synthesis and/or secretion. GHRH-producing neurons have been well characterized in the hypothalamus by immunostaining techniques. Hypothalamic tumors, including hamartomas, choristomas, gliomas, and gangliocytomas, may produce excessive GHRH with subsequent GH hypersecretion and resultant acromegaly. GHRH is synthesized and expressed in multiple extrapituitary tissues. Excessive peripheral production of GHRH by a tumor source would therefore be expected to cause somatotroph cell hyperstimulation and increased GH secretion. The structure of hypothalamic GHRH was elucidated from material extracted from pancreatic GHRH-secreting tumors in two patients with acromegaly [1, 2]. Analysis of one tumor revealed a 44-amino acid GHRH residue [3]; the other contained 37-, 40-, and 44-amino acid forms [4]. GHRH [1-40] and GHRH (1-44) are both found in extracts derived from the human hypothalamus. GHRH is secreted from neurons in the hypothalamic arcuate nucleus.

**Introduction**

The aim of this article will be to briefly review the physiology of GHRH and the diagnosis and treatment of GHRH-mediated acromegaly. Moreover, the role of GHRH and its antagonists in the pathogenesis and treatment of cancer will be reviewed.

**Physiology of GHRH**

Hypothalamic GHRH was initially elucidated from two different patients from ectopic pancreatic GHRH-secreting tumors causing acromegaly [1, 2]. Analysis of one tumor revealed a 44-amino acid GHRH residue [3]; the other contained 37-, 40-, and 44-amino acid forms [4]. GHRH [1-40] and GHRH (1-44) are both found in extracts derived from the human hypothalamus. GHRH is secreted from neurons in the hypothalamic arcuate nucleus.
nucleus and premammillary area, with axons that project to the median eminence [5]. There is considerable structural homology between GHRH and several gut peptides. In fact, varying degrees of homology exist between GHRH and VIP, glucagon, secretin, and GIP [6]. All of these peptides stimulate GH secretion in various physiologic systems, but with lower potencies than GHRH.

GHRH binds to specific receptors on the somatotroph membrane, resulting in increased intracellular cAMP [7]. The GHRH receptor gene has been cloned and sequenced, encoding a 47-kDa protein of 423 amino acids [8]. GHRH has a selective action on GH release; it does not release other anterior pituitary or gut hormones, except for a modest stimulation of PRL secretion [9]. GHRH increases GH synthesis as well as secretion, and stimulates transcription of GH mRNA [10]. GHRH stimulates GH release from both stored and newly synthesized intracellular GH pools, with a greater effect on stored pools [11]. There are a number of heterogeneous GH pools, varying according to time of GH synthesis [12-14], molecular size [14, 15], and response to provocative stimuli [15]. Somatostatin suppresses both basal and GHRH-stimulated GH release, but does not affect GH biosynthesis [16].

GHRH intravenously administered to normal adults elicits a prompt increase in serum GH levels with higher levels occurring in female subjects [17, 18]. Most acromegalic patients retain an intact GH response to exogenously administered GHRH [19].

Anti-GHRH antibodies eliminate spontaneous GH surges in rats. In humans, GH pulsatility persists when GHRH is tonically elevated due to ectopic GHRH production by a tumor or during GHRH infusion [1, 20, 21].

GHRH hypersecretion: Sources and clinical implications

Hypothalamic

Hypothalamic GHRH is secreted into the portal system, binds to specific surface receptors of the somatotroph cell and elicits intracellular signals that modulate pituitary GH synthesis and/or secretion [22]. GHRH-producing neurons have been well characterized in the hypothalamus by immunostaining techniques. Hypothalamic tumors, including hamartomas, choristomas, gliomas, and gangliocytomas may produce excessive GHRH with subsequent GH hypersecretion and resultant acromegaly [23].

Careful immunostaining of both pituitary adenomas and nontumorous surrounding pituitary tissue in these patients has failed to reveal the presence of immunoreactive GHRH. These patients may harbor somatotrope hyperplasia, or even a pituitary GH-cell adenoma, supporting the notion that excess hypothalamic GHRH may lead to pituitary hyperplasia and subsequent adenoma formation.

Pituitary mammosomatotroph hyperplasia with no evidence of pituitary adenoma or an extrapituitary tumor source of GHRH has been described in a young child with gigantism [24]. Excess secretion of hypothalamic GHRH, which possibly began in utero, may explain the potent induction of pituitary hyperplasia and GH secretion. Alternatively, increased pituitary GHRH sensitivity may have occurred in this case.

Peripheral

GHRH is synthesized and expressed in multiple extrapituitary tissues [25, 26]. Therefore, excessive peripheral production of GHRH by a tumor source would be expected to cause somatotroph cell hyperstimulation and increased GH secretion. The structure of hypothalamic GHRH was in fact elucidated from material extracted from pancreatic GHRH-secreting tumors in two patients with acromegaly [1, 2]. Immunoreactive GHRH is present in several tumors, including carcinoid tumors, pancreatic cell tumors, small-cell lung cancers, adrenal adenomas, and pheochromocytomas that have been reported to secrete GHRH. Acromegaly in these patients, however, is uncommon. In a retrospective survey of 177 acromegalic patients only a single patient was identified with elevated plasma GHRH levels [27].

The association of acromegaly with carcinoid tumors had been widely recognized in several patients prior to the characterization of hypothalamic GHRH [28, 29]. Carcinoid tumors comprise most of the tumors associated with ectopic GHRH secretion, the majority bronchial in origin [30, 31]. Pancreatic cell tumors, small-cell lung cancers, adrenal adenoma, pheochromocytoma, medullary thyroid, endometrial and breast cancer have also rarely been described to express GHRH and cause acromegaly [32, 34]. Although most patients with carcinoid tumors are not clinically acromegalic, many of these tumors do in fact express immunoreactive GHRH [35] and manifest abnormal GH secretory dynamics [36]. About 25% of carcinoid tumor samples derived from the gastrointestinal tract, lung, and thymus stain positively for GHRH. Although the presence of immunoreactive GHRH in tumor tissue does not necessarily imply hypersecretion of the peptide, over half of patients with carcinoid do in fact exhibit abnormal GH secretory dynamics, including increased 24-hour GH secretion, failure to suppress GH level after glucose ingestion and inappropriate GH response after TRH administration [34]. The observed high incidence of GHRH expression and low incidence of true acromegaly in these patients may be due to disordered tissue processing of GHRH by some tumors, or to impaired bioactivity of circulating GHRH. Post-translational processing and proteolytic degradation of GHRH is tissue and tumor-specific, and peptidase activity in some tumors is similar to hypothalamic activity, while in others different proteolytic profiles are present [36].

Most carcinoid tumors are slowly growing malignan-
cies, with insidious development of acromegaly. These patients present with features of classical acromegaly, accompanied by elevated circulating GH and IGF-1 levels. Patients also often experience systemic effects, obvious metastatic disease, or other signs and symptoms of the carcinoid syndrome. Following surgical removal, GH levels fall and soft tissue signs of acromegaly regress. The pituitary often shows evidence of somatotrope hyperplasia, although occasionally a true GH-cell adenoma may also be present.

**GHRH-induced acromegaly: Diagnosis and treatment**

**Diagnosis**

Over 95% of acromegalic patients harbor a GH-secreting pituitary adenoma [37]. The rare diagnosis of extra pituitary acromegaly should, therefore, be considered in only a small number of patients. Nevertheless, distinction of pituitary vs. extrapituitary acromegaly is extremely important in planning effective management. Regardless of the cause, GH and IGF-1 are invariably elevated and GH levels fail to suppress (<1 μg/l) after an oral glucose load in all forms of acromegaly. Patients with true acromegaly, normal GH and IGF-1 levels, and no evidence for extrapituitary tumor, probably represent ‘burned out’ acromegaly associated with an infarcted pituitary adenoma, often with resultant empty sella. As further dynamic tests are not useful in diagnosis, if a pituitary mass is indeed present, these patients may benefit from surgical resection. In a recent series, 4% of consecutive patients with proven GH-cell adenomas had normal GH and elevated IGF-1 levels [38].

Dynamic pituitary tests are not helpful in distinguishing acromegalic patients with pituitary tumors from those harboring extrapituitary tumors [9]. GH responses to TRH do not distinguish the various forms of acromegaly, as GH levels are usually stimulated in most patients and its diagnostic use is not cost effective. GH responses to dopamine agonist and to GHRH administration do not provide useful information for identifying the source of excess GH secretion [7]. Plasma GHRH levels are usually elevated in patients with peripheral GHRH-secreting tumors, and are normal or low in patients with pituitary acromegaly [8]. Measuring GHRH plasma levels therefore provides a precise and cost-effective test for the diagnosis of ectopic acromegaly. Peripheral GHRH levels are not elevated in patients with hypothalamic GHRH-secreting tumors, supporting the notion that excess eutopic hypothalamic GHRH secretion into the hypophyseal portal system does not appreciably enter the systemic circulation.

Unique and unexpected clinical features in an acromegalic patient, including respiratory wheezing or dyspnea, facial flushing, peptic ulcers, or renal stones will sometimes be helpful in alerting the physician to diagnosing non pituitary endocrine tumors. Specific biochemical markers of an underlying ectopic tumor (including hypoglycemia, hyperinsulinemia, hypergastrinemia, and rarely hypercortisolism) are not usually encountered in pituitary acromegaly, and their presence should also alert the physician to search for an extrapituitary source of GH excess. Anatomic localization of the pituitary or extrapituitary tumor is achieved using imaging techniques, including magnetic resonance imaging (MRI) and computed tomography (CT) scanning. As routine abdominal or chest imaging of all acromegalic patients will yield a very low incidence of true positive cases of ectopic tumor, such screening of these patients is not recommended as being cost effective. Elevated circulating GHRH levels, a normal or small-size pituitary gland, or clinical and biochemical features of other tumors known to be associated with extrapituitary acromegaly, are all indications for extrapituitary imaging. An enlarged pituitary is, however, often found on MRI of patients with peripheral GHRH-secreting tumors, and the radiologic diagnosis of a pituitary adenoma may be difficult to exclude.

**Treatment**

Surgical resection of the tumor secreting ectopic GHRH should reverse the hypersecretion of GH, and pituitary surgery should not be necessary in these patients. Nonresectable, disseminated or recurrent carcinoid syndrome with ectopic GHRH secretion can also be managed medically with long-acting somatostatin analogs (octreotide and lanreotide). Administration of the analog lowers circulating GH and IGF-1 levels, and also suppresses ectopic tumor elaboration of GHRH [39]. The drug, therefore, suppresses both pituitary GH as well as the peripheral tumor source of GHRH, thus attenuating the deleterious effects of chronic hypersomatotropism. The somatostatin analogs provide an effective option for medical management of carcinoid patients, especially those with recurrent disease.

**GHRH and GHRH receptors in human cancers**

The presence of GHRH and its receptors in several extrahypothalamic tissues, including ovary, testis and the digestive tract, suggests that GHRH may have a regulatory role in these tissues [40]. As previously mentioned, biologically or immunologically active GHRH and mRNA encoding GHRH have been found in several human malignant tumors, including cancers of the breast, endometrium and ovary and their cell lines [40]. GHRH is also produced by small-cell lung cancer (SCLC) cell lines, and mRNA encoding GHRH was detected in glioma culture cells. It is possible that in these tumors GHRH might function as an autocrine/paracrine growth factor, involved in regulating IGF-1 and/or IGF-2 secretion through its receptors. *In vivo* studies suggest that GHRH antagonists inhibit the growth of prostatic, mammary, lung, colorectal and pancreatic cancers and glioblastomas, in part by a direct
suppression of IGF-1 and IGF-2 production in tumors [41, 42]. Recent in vitro studies reinforced the concept that GHRH antagonists can act directly on tumor cells.

In fact antagonists of GHRH inhibited the rate of proliferation of mammary, prostatic, pancreatic and colorectal cancer cell lines, and reduced the expression of IGF-2 in the cells and the concentration of IGF-2 secreted into the culture medium [43]. However, in some tumors, GHRH antagonists have been reported to block tumor growth without apparently affecting IGF-1 or IGF-2 secretion. Thus, an effect of GHRH antagonists on tumor growth by mechanisms independent of IGF-1 is also possible.

The peptide receptors on tumors that respond to GHRH and GHRH antagonists have not been identified, but appear to be different from pituitary GHRH receptors [40]. GH-RH belongs to the family of peptides that includes VIP, glucagon, secretin and pituitary adenylate cyclase-activating peptide. These peptides show significant amino acid sequence homology [6]. The human GHRH receptor is a member of the G-protein-coupled receptors, and shows 47% homology to the VIP receptor protein [8]. Thus, GHRH and its analogs might act on tumors through other receptors related to both these receptor proteins.

**GHRH antagonists**

**Design**

Because hGHRH [1–29] is the shortest, fully active fragment of human GHRH (hGHRH), this sequence has been used for the development of agonistic and antagonistic GHRH analogs [44]. Synthetic work was aimed at developing GHRH antagonists with increased receptor-binding affinity, enhanced enzymatic stability and protracted biological activity. The synthesis and evaluation of analogs with various modifications revealed that certain hydrophobic and helix-stabilizing amino acid substitution, can produce antagonists, with increased GH release inhibitory potencies and GHRH receptor-binding affinities in vitro. Replacement of the Arg residue in position 29 with agmatine (Agm29), combined with the N-terminal acylation of the analogs, produced increased enzymatic stability and more protracted antagonist activity in vivo, as compared with the standard antagonist. Two representative, potent GHRH antagonists have been reported to be MZ-4-71 and MZ-5-156 [45]. Subsequent work revealed that incorporation of certain positively charged, hydrophilic amino acid substitutions, such as arginine or homoarginine in position 9 (Arg9 or Har9), and a new, positively charged, enzymatically resistant sequence at the C-terminus, produces a further increase in the activity of the antagonist in vivo [46]. Highly potent and long-acting GH-RH antagonist were developed by incorporating these structural features.

**Effects of GHRH antagonists in experimental cancer**

Osteogenic sarcomas are the most frequent primary bone tumors in children and young adults. The growth of osteosarcomas is stimulated by IGF-1 and GH, and receptors for IGF-1 have been demonstrated on these tumors. In nude mice the growth of SK-ES-1 and MNNG/HOS osteosarcoma cells is significantly inhibited by a GHRH antagonist, as shown by a reduction in tumor volume and weight. The treatment of osteosarcoma-bearing animals with MZ-4-71 decreases IGF-1 levels in the serum and tumor tissue [47].

Lung carcinoma is the leading cause of deaths from malignancies in the Western world. Small-cell lung carcinoma (SCLC) is a neuroendocrine tumor that accounts for 20%–25% of all cases of lung cancer, and non-SCLC includes the remaining histological subtypes. Several human SCLC and non-SCLC cell lines secrete and respond to IGF-1 and IGF-2 and express IGF-1 and IGF-2 genes. The presence of receptors for IGF-1 and IGF-2 on lung carcinoma has been demonstrated [48, 49]. Growth of SCLC H69 and non-SCLC H157 cell lines in nude mice is significantly inhibited by the GHRH antagonists; in addition, the levels of IGF-1, but not IGF-2, in serum and liver tissue are also reduced. In cell cultures, the proliferation of H69 SCLC cells and the H157 non-SCLC cell line was inhibited by GHRH antagonists.

Renal cell carcinoma is the most frequent kidney tumor and it causes ~12,000 deaths per year in the USA alone. No effective treatment is available for the advanced stages of this tumor.

The effects of the GHRH antagonist MZ-4-71 on the proliferation of Caki-1 renal adenocarcinoma have been investigated. After four weeks of treatment, the final volume of Caki-1 tumors in nude mice treated with MZ-4-71 was significantly decreased. Treatment with this GH-RH antagonist also reduced tumor weight, serum levels of GH and IGF-1, liver concentrations of IGF-1 and tumor levels of IGF-1 and IGF-2 [50].

Carcinoma of the prostate is the most common malignant tumor in men, while breast cancer is the most common malignancy in women.

GHRH antagonists inhibit the growth of prostate and breast cancer probably by decreasing pituitary GH release and hepatic IGF-1 secretion, as well as through pathways involving suppression of the autocrine or paracrine production of IGF-1 and IGF-2 in the tumors [49, 51].

Interestingly, the antitumoral effect of the GHRH antagonists seems to be more pronounced in tumors known or suspected to be neuroendocrine in nature (SCLC, prostate cancer) or endocrine dependent (breast cancer). Concerning other types of solid cancers (brain, pancreatic and colorectal carcinomas), less convincing evidence exists to show a potential role of GHRH antagonists in the treatment of these tumors.

Only the earliest GHRH antagonist, (Ac-Tyr1,D-Arg2)hGHRH(1-29)NH2, has been evaluated clinically...
[32]. Large doses of this antagonist eliminated nocturnal GH secretion in normal subjects and inhibited the response to GHRH. This GHRH antagonist also reduced GH levels in a patient with acromegaly [53]. The GHRH antagonist could be used for the treatment of conditions caused by excess GH, such as acromegaly. In addition, in diabetic retinopathy and diabetic nephropathy GHRH antagonists might find a place. Based on the above-mentioned experimental evidences it is likely that the principal application of GHRH would be in the field of cancer [49]. However, to date no clinical data are available in this field.

GHRH antagonists appear to inhibit the growth of IGF-1 and IGF-2-dependent cancers through indirect and direct pathways [49]. The indirect mechanism operates through suppression of GH release from the pituitary and the resulting inhibition of the IGF-1 production in the liver. In fact GHRH antagonists decrease the level of IGF-1 in the serum of nude mice bearing xenografts of prostatic, breast and renal cancers, osteosarcomas, and SCLCs and non SCLCs [49]. These findings suggest that the inhibitory effect of GHRH antagonists on tumor growth in vivo could be produced in part by suppression of hepatic IGF-1 secretion. However, studies showing a major reduction in tumor IGF-2 concentration in renal carcinoma, prostate cancer, pancreatic cancer, colorectal cancer and non-SCLC xenografted into nude mice after treatment with GHRH antagonists, suggest direct effects of the compound on the tumor [49]. In addition, the decrease in tumor IGF-1 concentration could be the result of direct action.

Preliminary evidence also suggests that GHRH antagonists might inhibit tumor growth by mechanisms independent of IGFs. In addition GHRH antagonists could be used for suppression of tumors that do not express somatostatin receptors, such as human osteogenic sarcomas. Subtype 2 of somatostatin receptors, which is preferred for therapy with octapeptide somatostatin analogs, is also absent in human pancreatic and colorectal cancers [54].

Conclusions

We conclude that GHRH could be rarely implicated in the pathogenesis of acromegaly due to hypothalamic or ectopic hypersecretion. The endocrinologist, however, should bear in mind these rare causes of acromegaly since the diagnosis is relatively simple (based on imaging and/or peripheral sampling for GHRH), and treatment significantly differs from that of acromegaly determined by pituitary tumors. Interestingly, clinical and pathophysiological relevance of GHRH has been recently increased by the observation that this peptide is often hyperexpressed in several solid tumors and by the availability of newly synthetized GHRH antagonists. The review of experimental results with these substances are promising, although no clinical data are available yet. Finally, the advent of these antagonists has allowed a significant progress in the understanding of the role of the central and tissue GHRH-GH-IGF system in the pathogenesis of tumors.

References

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