

Biologic Activities of Growth Hormone Secretagogues in Humans

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Growth hormone secretagogues (GHSs) are synthetic peptidyl and nonpeptidyl molecules with strong, dose-dependent, and reproducible growth hormone (GH)-releasing activity even after oral administration. GHSs release GH via actions on specific receptors (GHS-R) at the pituitary and, mainly, at the hypothalamic levels. GHSs likely act as functional somatostatin antagonists and meantime enhance the activity of GH-releasing hormone (GHRH)-secreting neurons. The GH-releasing effect of GHSs is independent of gender but undergoes marked age-related variations. Estrogens play a major role in enhancing the GH response to GHSs at puberty, which GHRH hypoactivity, somatostatinergic hyperactivity and impaired activity of the putative GHS-like ligand and receptors probably explain the reduced GH-releasing effect of GHSs in aging. The activity of GHSs is not fully specific for GH. Their slight prolactin-releasing activity probably comes from direct pituitary action. In physiological conditions, the ACTH-releasing activity of GHSs is dependent on central actions; a direct action on GHS-R in pituitary ACTH-secreting tumors likely explains the peculiar ACTH and cortisol hyperresponsiveness to GHSs in Cushing disease. GHSs have specific receptor subtypes in other central and peripheral endocrine and nonendocrine tissues mediating GH-independent biologic activities. GHSs influence sleep pattern, stimulate food intake, and have cardiovascular activities. GHs have specific binding in normal and neoplastic follicular derived human thyroid tissue and inhibit the proliferation of follicular-derived neoplastic cell lines. The discovery of ghrelin, a 28 amino acid peptide synthesized in the stomach but also in other tissues, has opened new fascinating perspectives of research in this field.

Key Words: Growth hormone secretagogues; prolactin; adrenocorticotrophic hormone; cortisol; cardiovascular system; thyroid gland.

Introduction

Growth hormone secretagogues (GHSs) are synthetic peptidyl and nonpeptidyl molecules that possess strong, dose-dependent and reproducible growth hormone (GH)-releasing activity in vivo in several species and in humans after iv, sc, intranasal, and even oral administration (1–5).

Among members of the GHS family, those mostly studied in humans include peptidyl molecules (GH-releasing peptides [GHRPs]) such as GHRP-6 and its superanalogs GHRP-1, heptapeptide; GHRP-2 and hexarelin, two hexapeptides; Tyr-Ala-hexarelin, an octapeptide; ipamorelin, a pentapeptide; some tetra- and pseudo-tripeptides; as well as nonpeptidyl GHRP mimetics, such as MK-677, a spiroindoline that shows the most marked bioavailability and long-lasting effect after oral administration (1–7).

The activity of GHSs is not fully specific for GH (Fig. 1). In fact, they also possess significant prolactin (PRL)- and remarkable adrenocorticotrophic hormone (ACTH)/cortisol-releasing effects (2,8). Other central actions of GHSs include stimulation of food intake and influence on sleep pattern (9–11).

The activities of GHSs are mediated by specific receptors subtypes (GHS-R) that are mainly present at the pituitary and hypothalamic level but also in other areas of the central nervous system (CNS) and even at the peripheral level in both endocrine and nonendocrine human tissues (12–18) (Fig. 2). There is already evidence for peripheral targets of biologic activities of GHS such as the cardiovascular system and the thyroid (16–18).

Recently, the existence of a natural ligand of GHS-Rs—has been demonstrated a 29 amino acid peptide that is mostly synthesized in the stomach and has been named ghrelin (19).

Central and Peripheral Human GHS-Rs

A specific animal and human GHS-R has recently been cloned. It is encoded by a rare mRNA with a predicted open

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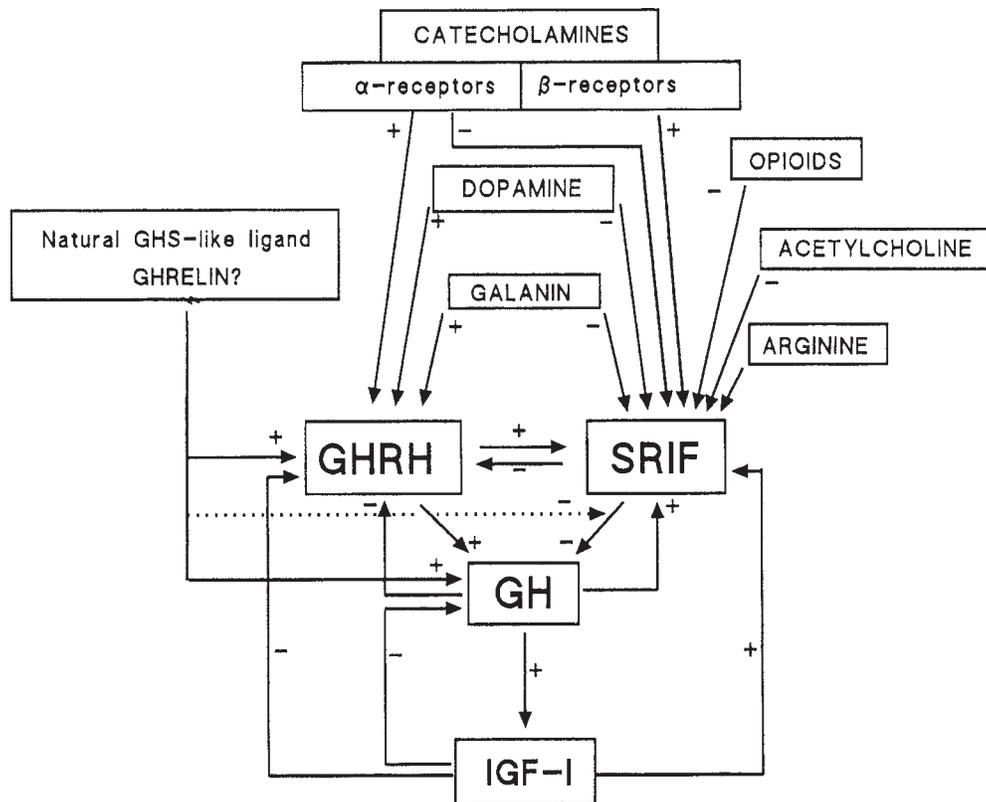


Fig. 1. Neural control of GH secretion in humans.

reading frame of 366 amino acids with a transmembrane topography typified by the G-protein-coupled receptor family. The receptor sequence does not show significant homology with other receptors known so far, and receptor transcripts are expressed in the pituitary and the hypothalamus (3,12). The existence of GHS-R subtypes has already been shown (14,20,21).

As in animals (1), the human hypothalamus and pituitary gland show the highest specific GHS binding, which is also present in other areas of the CNS, such as the cerebral cortex, hippocampus, medulla oblongata, and choroid plexuses but not in the cerebellum, thalamus, striatum, substantia nigra, and corpus callosum (13,15). GHS-Rs have also been demonstrated in fetal human pituitary and GHSs stimulate GH release from human fetal pituitary in vitro as well as in newborns (22,23).

The existence of specific GHS binding sites at the pituitary level and within the CNS explains the neuroendocrine and also the extraneuroendocrine activities of GHS, such as the control of sleep and food intake (9–11).

An endogenous ligand specific for GHS-Rs, named ghrelin and endowed with a specific stimulatory effect on GH secretion, has recently been identified in rat and human stomach. Ghrelin transcript as well as neurons immunoreactive for this peptide have been found in the hypothalamus, suggesting a neuroendocrine role of this substance in the regulation of GH secretion (19).

We studied the effects of sex and age on specific ^{125}I -Tyr-Ala-hexarelin binding sites in human pituitary gland, hypothalamus, and other areas of the CNS from subjects of both sexes (age ranging from 18 to 93 yr). GHS-R density did not vary as a function of sex in the pituitary, hypothalamus, and other areas of the human brain in agreement with evidence that the GH response to GHSs in men and women is similar (1–3,24). Age did not affect the binding of ^{125}I -Tyr-Ala-hexarelin to membranes from pituitary gland of middle-aged and elderly subjects. However, an age-related decrease in GHS-R density was observed in the hypothalamus of both middle-aged and elderly subjects, in agreement with evidence showing that the GH-releasing effect of GHSs undergoes an age-related reduction from adulthood to aging (1–3,24).

Our recent studies demonstrate that GHS-Rs are also present in peripheral tissues. In fact, a specific binding for Tyr-Ala-hexarelin was found in the adrenal gland, heart, ovary, testis, lung, and skeletal muscle and this was even more remarkable than or at least overlapping with that found in the pituitary and the hypothalamus (13,15). Significant GH-releasing peptide (GHRP) binding was also found in the kidney, epiphysis, and thyroid gland but not in the smooth muscle, parotid gland, spleen, and pancreas, but in the latter significant GHS-R mRNA has been found by others researchers (13,15–18,25).

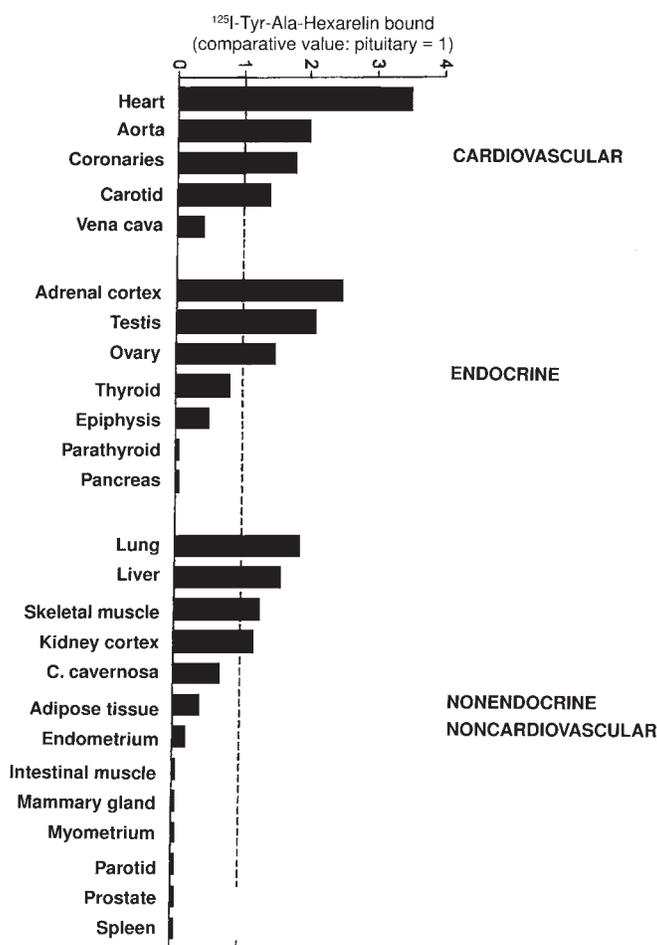


Fig. 2. Tissue distribution of GHRP receptors in humans.

Particular attention has been given to the characterization of specific binding sites in the human cardiovascular system and thyroid gland.

Considerable specific ^{125}I -Tyr-Ala-hexarelin binding was detected in the ventricles, atria, aorta, coronary and carotid arteries, endocardium, and vena cava with values that were higher than those found in the pituitary (except endocardium and vena cava). GHRP binding in the cardiovascular tissues was independent of gender (16).

The binding was inhibited by unlabeled Tyr-Ala-hexarelin and hexarelin as well as by GHRP-6, GHRP-1, and GHRP-2 but not by MK-677, a nonpeptidyl GHRP analog (3). Also, various cardioactive substances such as angiotensin II, endothelin-1, insulin-like growth factor-1 (IGF-1) epinephrine, norepinephrine, and acetylcholine did not inhibit the cardiac GHRP binding (16). The cardiovascular GHRP receptor has recently been characterized in animal tissue (17).

In the normal thyroid, there was clear ^{125}I -Tyr-Ala-hexarelin binding that was inhibited markedly by peptidyl GHSs and, to a lesser extent, by the nonpeptidyl MK-0677. Specific ^{125}I -Tyr-Ala-hexarelin binding was found also in follicular- but not in parafollicular-derived neoplastic hu-

man thyroid tissue. In follicular-derived papillary thyroid carcinoma as well as in normal thyroid tissue, the binding of radiolabeled Tyr-Ala-hexarelin was totally displaced by peptidyl GHSs and, to a lesser extent, by nonpeptidyl GHSs but not by both GHRH and somatostatin (18).

The existence of various GHS-R subtypes binding nonpeptidyl and peptidyl GHSs is very likely. The functional significance of peripheral GHS-Rs is still unknown even if recent findings suggest that at least in the cardiovascular system and in the thyroid gland they could mediate GH-independent activities of GHSs (6-18,26-28) (see Potential Targets of Nonendocrine Activities of GHSs).

Endocrine Activities of GHSs in Humans

GH-Releasing Activity

The GH response to GHSs is dose related and shows good intra-individual reproducibility (1-5). The GH-releasing activity of GHSs is higher in vivo than in vitro and is also clearly higher in humans than in animals (1-5). The GH response to GHSs is strongly reduced, though not abolished, by hypothalamopituitary disconnection (29-31), in agreement with the assumption that the most important action of GHSs takes place at the hypothalamic level (1-5).

GHSs and GHRH have a synergistic effect and even a very low dose of GHSs has been found able to potentiate strikingly the GHRH-induced GH rise, in agreement with data indicating that they act, at least partially, via different mechanisms (1–3). Nevertheless, GHSs need GHRH activity to fully express their GH-releasing effect and probably act triggering GHRH-secreting neurons. In fact, in humans the GH response to GHSs is strongly inhibited by a GHRH antagonist (32) as well as by hypothalamopituitary disconnection (31) and is lacking in patients with GHRH-R deficiency in whom other endocrine responses to GHSs are preserved (33).

GHSs do not reduce hypothalamic somatostatin release (34); nevertheless, they probably act also as functional somatostatin antagonists at both pituitary and the hypothalamic levels (1–4). In fact, in humans the GH response to GHSs is not modified by substances acting via somatostatin inhibition (such as cholinergic agonists, arginine), which, in turn, truly potentiate the GHRH-induced rise in GH (2,35,36). Moreover, the GH-releasing activity of GHSs is partially refractory to the inhibitory effect of substances acting via stimulation of hypothalamic somatostatin (such as cholinergic antagonists, β -adrenergic agonists, glucose), which, in turn, almost abolish the somatotroph responsiveness to GHRH (2,35–38). Above all, GHSs are also partially refractory to the inhibitory effect of substances acting on somatotroph cells such as free fatty acids and even to exogenous somatostatin (38,39). In addition, GHSs are partially refractory to the negative GH autofeedback (40,41) while showing peculiar sensitivity to the negative IGF-1 feedback action (42).

In adulthood the GH-releasing effect of GHSs is gender independent (24). On the other hand, the GH response to GHSs undergoes marked age-related variations different from those recorded after GHRH. In fact, the GH response to GHRH is maximal in newborns and then progressively decreases up to aging without any change at puberty; by contrast, the GH response to hexarelin is low at birth, strikingly increases at puberty, persists similarly in adulthood, and decreases thereafter, and in middle age is already similar to that in elderly subjects (24). The synergistic effect of hexarelin + GHRH undergoes similar age-related variations (24,43–45) but this has not been confirmed by other researchers (46).

The mechanisms underlying the age-related variations in the GH-releasing activity of GHSs differ age by age, probably reflecting changes in hypothalamic GHS-Rs.

In childhood, the GH response to hexarelin in prepubertal girls and boys is similar. Interestingly, the somatotroph responsiveness to hexarelin increases at puberty much more in girls than in boys (47). Moreover, in prepubertal children the GH response to hexarelin is increased to pubertal levels by short-term pretreatment with estradiol and testosterone but not with oxandrolone, a nonaromatizable androgen (48),

indicating that estrogens play a critical role in enhancing the somatotroph sensitivity to GHS, in agreement with some data in animals (1,49). However, this positive estrogenic influence is limited to puberty. In fact, as alluded to before, the GHSs effect in adult and elderly men and women is similar. Moreover, the low GH response to hexarelin in postmenopausal women is not modified by 3 mo of treatment with transdermal estradiol (50), indicating that the reduced stimulatory effect of GHSs on somatotroph secretion in aging does not depend on the decline in gonadal steroid levels.

In agreement with the hypothetical reduction in the activity of the natural GHS-like ligand and receptors in human aging brain (see before), the GH response to hexarelin in elderly subjects further increases with increasing dose but remains clearly lower than that elicited by the maximally effective dose in young adults (45).

However, the most important mechanism accounting for the reduced GH-releasing activity of GHSs in aging is probably represented by age-related variations in the neural control of somatotroph function including GHRH hypoactivity and somatostatinergic hyperactivity (51). In fact, in aged humans the GH response to hexarelin is increased, but not restored, by GHRH while arginine, which probably acts via inhibition of hypothalamic somatostatin release, restores the GH response to hexarelin and GHRH to young levels (24,43,45).

The GH-releasing effect of GHSs undergoes some desensitization, but, nevertheless, increased IGF-1 levels during chronic treatment with GHSs indicate that they are able to successfully enhance the activity of the GH/IGF-1 axis; this effect is clear when administering oral nonpeptidyl GHSs once daily, owing to their impressive bioavailability and long-lasting effect (1–3,52). Orally active GHSs have been proposed as an alternative to rhGH, rhIGF-1, and GHRH as growth-promoting treatment in GH-deficient children as well as anabolic treatment in elderly patients with somatopause or in critically ill patients (2,3,5,52,53). However, no definitive evidence of their clinical usefulness has been provided yet.

PRL-Releasing Activity

The stimulatory effect of GHSs on PRL secretion in humans is slight and dose dependent, being within the normal range of basal levels and markedly lower than that recorded after thyrotropin-releasing hormone, metoclopramide, or arginine administration (1–3). The lactotroph responsiveness to GHSs in humans is independent of both gender and age (24,54). The mechanism underlying the PRL-releasing activity of GHSs is not mediated by opioidergic, serotonergic, and histaminergic pathways (55–57), seems influenced by estrogens in animals but not in humans (2, 58), and probably reflects direct stimulation of somatomammotroph cells (59,60).

ACTH- and Cortisol-Releasing Activity

The stimulatory effect of GHSs on the activity of the hypothalamo-pituitary-adrenal (HPA) axis in humans is significant, overlapping with that after (Arg)⁸-vasopressin (AVP) or naloxone and even similar to that after corticotropin-releasing hormone (CRH) (55,61–63). The stimulatory effect of GHSs seems, however, to be an acute neuroendocrine effect, being lost during prolonged treatment (44).

The ACTH-releasing activity of GHSs is independent of gender but shows peculiar age-related variations (24). It increases at puberty, then shows a reduction in adulthood and, again, a trend toward increase in aging (54). The increased effect at puberty could depend on estrogens, and the rebounded effect in aging agrees with evidence showing HPA hyperactivity owing to neuroendocrine changes in the aging brain (64). The age-related pattern of ACTH response to GHSs is different from that to CRH, whose effect does not show any increase at puberty but shows a trend toward increase in aging (65). On the other hand, the age-related pattern of adrenocorticotroph responsiveness to GHSs is dissociated from that of somatotroph cells in aging. Taking also into account the age-independent effect of GHSs on lactotroph secretion, these findings indicate that GHSs act at different levels and on different receptors to induce different endocrine responses, in agreement with the existence of different GHS-R subtypes at both the pituitary and central levels (3,14).

The stimulatory effect of GHSs on cortisol secretion reflects their ACTH-releasing activity, which, in turn, totally depends on CNS-mediated mechanisms. In fact, the stimulatory effect of GHSs on the HPA axis is abolished by hypothalamopituitary disconnection and GHSs do not stimulate ACTH release from rat pituitary and human fetal pituitary (3,22,31).

Studies in animals have suggested CRH- and AVP-mediated actions for GHSs (63,66) which, in turn, could be mediated by NPY or the putative endogenous GHS-like ligand (12, 67). However, in humans the coadministration of hexarelin and AVP or CRH or naloxone has no interaction or an effect less than additive (55,61), in spite of the well-known synergistic effect of CRH and AVP on ACTH secretion (65); this finding suggested that the ACTH-releasing activity of GHSs could be, at least partially, independent of CRH and AVP. It has been shown that the GHS stimulatory effect on the HPA axis is not affected by serotonergic or histaminergic antagonists (56,57) whereas it is totally abolished by alprazolam, a benzodiazepine (68); this evidence suggested that GABAergic pathways could play a major role in the mechanisms underlying the ACTH-releasing activity of GHSs.

The ACTH response to GHSs is generally sensitive to the negative cortisol feedback mechanism in physiologic conditions. In fact, in normal subjects, the ACTH response

to hexarelin is inhibited by dexamethasone and enhanced by metyrapone, and it is abolished in patients with Cushing syndrome owing to adrenal adenoma and enhanced in patients with Addison disease (62,68,69). Surprisingly, the stimulatory effect of GHSs on corticotroph secretion is exaggerated in patients with ACTH-secreting pituitary microadenoma and, is, preserved in those bearing macroadenoma in spite of their hypercortisolism (70). Specific GHS-R mRNA is present in pituitary and ectopic ACTH-secreting tumors (71–74); moreover, GHSs stimulate ACTH release from pituitary ACTH-secreting human tumor cells in culture (73). Thus, the exaggerated ACTH response to GHSs in Cushing disease likely reflects peculiar stimulatory action on the pituitary tumor.

GHSs are also able to stimulate ACTH and cortisol release in some patients with ectopic ACTH-dependent Cushing syndrome (62,70). Thus, testing with GHSs does not have diagnostic usefulness for the differential diagnosis between pituitary and ectopic ACTH-dependent Cushing syndrome although it represents a new tool for investigating regulation of the HPA axis.

Potential Targets of Nonendocrine Activities of GHSs

Cardiovascular System

Specific binding sites for peptidyl GHSs are present in the human cardiovascular system. To clarify whether binding sites for peptidyl GHSs in the cardiovascular system mediate biologic activities, we investigated the cellular effects of hexarelin on H9c2 myocytes, a fetal cardiomyocyte-derived cell line in which specific GHRP binding sites have been found. It has been demonstrated that hexarelin promotes cell survival after treatment with cytotoxic agents such as tumor necrosis factor- α and doxorubicin. Preliminary data indicate that hexarelin activates Akt serine kinase, an enzyme that phosphorylates Bcl-2 and Bad and mediates the antiapoptotic signaling induced by several survival factors. Thus, GHRPs probably exert an antiapoptotic activity through a specific, receptor-mediated mechanism, that involves the activation of a well-known anti-apoptotic signaling pathway (16,75).

On the other hand, in both animals and humans there is already evidence showing that GHRPs possess direct cardiovascular activity. In fact, prolonged treatment with peptidyl GHSs dramatically protects against cardiovascular damage in aged rats as well as in GH-deficient rats with postischemic ventricular dysfunction (27,76–78); moreover, it increases cardiac contractility in rats after myocardial infarction (28). Interestingly, acute administration of high-dose peptidyl GHRP also induces clear though transient coronary vasoconstriction in perfused rat heart, and this effect is shared by a GHRP antagonist (17,79).

In humans, the acute administration of hexarelin has been found able to clearly increase the left ventricular ejec-

tion fraction (LVEF) in normal young volunteers as well as in hypopituitary patients with severe GH deficiency in the absence of any variations in mean blood pressure, heart rate, and catecholamine levels. Note that, as expected, this effect in GH deficiency took place in the absence of any stimulatory effect of hexarelin on GH secretion (80,81). By contrast, our preliminary results in patients with dilatative cardiomyopathy indicate that hexarelin has no effect on LVEF in spite of normal GH response (82). Taken together, all these findings indicate that GHSs, at least GHRPs, also possess GH-independent cardiovascular activities that are probably mediated by specific cardiovascular receptors.

Thyroid Gland

Specific binding sites for peptidyl and nonpeptidyl GHSs are present in normal and follicular- but not parafollicular-derived neoplastic human thyroid tissue (18).

To clarify whether these binding sites in the thyroid tissue mediate biologic activities, we studied the effect of GHSs on the proliferation of three follicular tumor cell lines (NPA, WRO, and ARO). A highly detectable specific binding of ^{125}I -Tyr-Ala-hexarelin was present in the three cell lines. The effect of GHRP-6, hexarelin, Tyr-Ala-hexarelin, and MK-0677 on cell proliferation was tested by thymidine incorporation in the NPA cell line (derived from a thyroid papillary carcinoma) and by growth curves in all NPA, WRO (follicular), and ARO (anaplastic) carcinoma cell lines. All the peptides tested inhibited the incorporation of ^3H -thymidine in NPA cells stimulated by fetal calf serum. By growth curves, the addition of both peptidyl and nonpeptidyl molecules to culture medium caused a significant inhibition of cell proliferation, which was evident at the earliest time of treatment in all the cell lines and at the latest time (96 h) only in well-differentiated carcinomas (NPA cell lines). In the ARO cell line, a trend toward rebound effect with delayed increase in cellular proliferation was recorded. These findings (unpublished results) suggest that GHSs could influence the cellular proliferation of follicular-derived neoplastic thyroid cell lines in vitro.

Conclusion

(GHSs) were invented more than 20 yr ago. The discovery of the GHS-R and mostly that of a natural ligand, ghrelin, has opened new fascinating perspectives in this field. The availability of ghrelin for basic and clinical studies will help clarify its physiologic role and, in the meantime, verify whether the endocrine and nonendocrine activities of GHSs described so far really belong to this endogenous ligand. If so, this could become a milestone not only in neuroendocrinology but also in internal medicine.

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References

- Bowers, C. Y., Veeraragavan, K., and Sethumadhavan, K. (1993). In: *Growth hormone II, basic and clinical aspects*. Bercu, B. R. and Walker, R. F. (eds.), Springer-Verlag: New York.
- Ghigo, E., Arvat, E., Muccioli, G., and Camanni, F. (1997). *Eur. J. Endocrinol.* **136**, 445–460.
- Smith, R. G., Van der Ploeg, L. X. T., Howard, A. D., et al. (1997). *Endocr. Rev.* **18**, 621–645.
- Deghenghi, R. (1993). In: *Growth hormone secretagogues*. Bercu, B. B., and Walker, R. F. (eds.). Serono Symposia: New York.
- Korbonits, M. and Grossman, A. B. (1995). *Trends Endocrinol. Metab.* **6**, 43–49.
- Raun, K., Hansen, B. S., Johansen, N. L., et al. (1998). *Eur. J. Endocrinol.* **139**, 552–561.
- Arvat, E., Di Vito, L., Lanfranco, F., et al. (1999). *J. Endocrinol. Invest.* **22**, 91–97.
- Ghigo, E., Arvat, E., and Camanni, F. (1998). *Growth Horm. & IGF Res.* **8**, 145–148.
- Locke, W., Kirgis, H. D., Bowers, C. Y., and Abdo, A. A. (1995). *Life Sci.* **56**, 1347–1352.
- Frieboes, R. M., Murck, H., Maier, P., Schier, T., Holsboer, F., and Steiger, A. (1995). *Neuroendocrinology* **61**, 584–589.
- Torsello, A., Luoni, M., Schweiger, F., et al. (1998). *Eur. J. Pharmacol.* **360**, 123–129.
- Howard, A. D., Feighner, S. D., Cully, D. F., et al. (1996). *Science* **273**, 974–977.
- Muccioli, G., Ghè, C., Ghigo, M. C., et al. (1997). *J. Endocrinol. Invest.* **20**, 52.
- Ong, H., McNicoll, N., Escher, E., et al. (1998). *Endocrinology* **139**, 432–435.
- Muccioli, G., Ghè, C., Ghigo, M. C., Papotti, M., Arvat, E., Boghen, M.F., et al. (1998). *J. Endocrinol.* **157**, 99–106.
- Muccioli, G., Broglio, F., Valetto, M., et al. (2000) *Annales d'Endocrinologie*, **61**, 21–31.
- Bodart, V., Bouchard, J. F., McNicoll, N., et al. (1999). *Circ. Res.* **85**, 796–802.
- Cassoni, P., Papotti, M., Catapano, F., Ghè C., Deghenghi R., Ghigo E., and Muccioli G. (2000) *J. Endocrinol.* **165**, 139–146.
- Kojima, M., Hosada, H., Data, Y., Nakazato, M., Matsuo, H. and Kankawa, K. (1999). *Nature.* **402**, 656–660.
- McKee, K. K., Tan, C. P., Palyha, O. C., et al. (1997). *Genomics* **46**, 426–434.
- Tan, C. P., McKee, K. K., Qingyun, L., et al. (1998). *Genomics.* **52**, 223–229.
- Shimon, I., Yan, X., and Melmed, S. (1998). *J. Clin. Endocrinol. Metab.* **83**, 174–178.
- Bartolotta, E., Bellone, J., Aimaretti, G., et al. (1997). *J. Pediatr. Endocrinol. Metab.* **10**, 491–497.
- Arvat, E., Giordano, R., Gianotti, L., et al. (1999). In: *Sex-steroid interactions with growth hormone*. Veldhuis, J. D. and Giustina, A. (eds.). Springer-Verlag: Serono Symposia USA.
- Guan, X., Yu, H., Palyha, O. C., et al. (1997). *Mol. Brain Res.* **48**, 23–29.
- Ong, H., Bodart, V., McNicoll, N., Lamontagne, D., and Bouchard, J. F. *Growth Horm. & IGF Res.* (1998). **8**: 137–140
- Berti, F., Muller, E., De Gennaro Colonna, V., and Rossoni, G. (1998). *Growth Horm. & IGF Res.* **8**, 149–152.

28. Tivesten, A., Bollano, E., Caidahl, K., et al. (2000). *Endocrinology*. **141**, 60–66.
29. Pombo, M., Barreiro, J., Penalva, A., Peino, R., Dieguez, C., and Casanueva, F. F. (1995). *J. Clin. Endocrinol. Metab.* **80**, 3180–3184.
30. Popovic, V., Damjanovic, S., Micic, D., Djurovic, M., Dieguez, C., and Casanueva, F. F. (1995). *J. Clin. Endocrinol. Metab.* **80**, 942–947.
31. Hickey, G. J., Drisko, J., Faidley, T., et al. (1996). *J. Endocrinol.* **148**, 371–380.
32. Pandya, N., De Mott-Friberg, R., Bowers, C. Y., Barkan, A. L., and Jaffe, C. A. (1998). *J. Clin. Endocrinol. Metab.* **83**, 1186–1189.
33. Maheshwari, H. G., Rahim, A., Shalet, S. M., and Baumann, G. (1999). *J. Clin. Endocrinol. Metab.* **84**, 956–959.
34. Korbonits, M., Little, J. A., Forsling, M. L., et al. (1999). *J. Neuroendocrinol.* **11**, 521–528.
35. Penalva, A., Carballo, A., Pombo, M., Casanueva, F. F., and Dieguez, C. (1993). *J. Clin. Endocrinol. Metab.* **76**, 168–171.
36. Arvat, E., Gianotti, L., Di Vito, L., et al. (1995). *Neuroendocrinology* **61**, 51–56.
37. Arvat, E., Gianotti, L., Ramunni, J., et al. (1996). *J. Endocrinol. Invest.* **19**, 25–29.
38. Maccario, M., Arvat, E., Procopio, et al. (1995). *Metabolism* **44**, 134–138.
39. Massoud, A. F., Hindmarsh, P. C., and Brook, C. G. D. (1997). *Clin. Endocrinol.* **47**, 537–547.
40. Massoud, A. F., Hindmarsh, P. C. and Brook, C.G. (1995). *Clin. Endocrinol.* **43**, 617–621.
41. Arvat, E., Di Vito, L., Gianotti, L., et al. (1997). *Metabolism* **46**, 83–88.
42. Ghigo, E., Gianotti, L., Arvat, E., et al. (1999). *J. Clin. Endocrinol. Metab.* **84**, 285–290.
43. Arvat, E., Gianotti, L., Grottoli, S., et al. (1994). *J. Clin. Endocrinol. Metab.* **79**, 1440–1443.
44. Chapman, I. M., Bach, M. A., Van Cauter, E., et al. (1996). *J. Clin. Endocrinol. Metab.* **81**, 4249–4257.
45. Arvat, E., Ceda, G. P., Di Vito, L., et al. (1998). *Pituitary*. **1**, 51–58.
46. Micic, D., Popovic, V., Doknic, M., Macut, D., Dieguez, C., and Casanueva, F. F. (1998). *J. Clin. Endocrinol. Metab.* **83**, 2569–2572.
47. Bellone, J., Aimaretti, G., Bartolotta, E., et al. (1995). *J. Clin. Endocrinol. Metab.* **80**, 1090–1094.
48. Loche, S., Colao, A., Cappa, M., et al. (1997). *J. Clin. Endocrinol. Metab.* **82**, 861–864.
49. Kamegai, J., Wakabayashi, I., Kineman, R. D., and Frohman, L.A. (1999). *J. Neuroendocrinol.* **11**, 299–306.
50. Arvat, E., Gianotti, L., Broglio, F., et al. (1997). *Eur. J. Endocrinol.* **136**, 483–487.
51. Ghigo, E., Arvat, E., Gianotti, L., et al. (1996). *J. Pediatr. Endocrinol. Metab.* **9**, 271–278.
52. Casanueva, F. F. and Dieguez, C. (1999). *Trends Endocrinol. Metab.* **10**, 30–38.
53. Ghigo, E., Arvat, E., Aimaretti, G., Broglio, F., Giordano, R. and Camanni, F. (1998). In: *Bailliere's Clinical Endocrinology and Metabolism*: Shalet, S. M. (ed.). Bailliere Tindall, London.
54. Arvat, E., Ramunni, J., Bellone, J., et al. (1997). *Eur. J. Endocrinol.* **137**, 635–642.
55. Korbonits, M., Trainer, P. J., and Besser, G. M. (1995). *Clin. Endocrinol.* **43**, 365–371.
56. Arvat, E., Maccagno B., Ramunni J., et al. (1997). *J. Endocrinol. Invest.* **20**, 122–127.
57. Arvat, E., Maccagno, B., Ramunni, J., et al. (1998). *J. Endocrinol. Invest.* **22**, 673–679.
58. Bennett, P. A., Thomas, G. B., Howard, A. D., et al. (1997). *Endocrinology* **138**, 4552–4557.
59. Ciccarelli, E., Grottoli, S., Razzore, P., et al. (1996). *Clin. Endocrinol.* **44**, 67–71.
60. Renner, U., Brockmeier, S., Strasburger, C.J., et al. (1994). *J. Clin. Endocrinol. Metab.* **78**, 1090–1096.
61. Arvat, E., Maccagno, B., Ramunni, J., et al. (1997). *Neuroendocrinology* **66**, 432–438.
62. Ghigo, E., Arvat, E., Ramunni, J., et al. (1997). *J. Clin. Endocrinol. Metab.* **82**, 2439–2444.
63. Korbonits, M., Kaltsas, G., Perry, L. A., et al. (1999). *J. Clin. Endocrinol. Metab.* **84**, 2489–2495.
64. Sapolsky, R. M., Krey, L. C., and McEwen, B. (1986). *Endocr. Rev.* **7**, 284–301.
65. Orth, D. N. (1992). *Endocr. Rev.* **13**, 164–191.
66. Thomas, G. B., Fairhall, K. M., and Robinson, I.C.A.F. (1997). *Endocrinology* **138**, 1585–1591.
67. Dickson, S. L. and Luckman, S. M. (1997). *Endocrinology* **138**, 771–777.
68. Arvat, E., Maccagno, B., Ramunni, J., et al. (1998). *Neuroendocrinology* **67**, 310–316.
69. Arvat, E., Ramunni, J., Maccagno, B., et al. (1999). *Neuroendocrinology* **70**, 200–206.
70. Arvat, E., Giordano, R., Ramunni, J., et al. (1998). *J. Clin. Endocrinol. Metab.* **83**, 4207–4211.
71. De Keyzer, Y., Lenne, F., and Bertagna, X. (1997). *Eur. J. Endocrinol.* **137**, 715–718.
72. Korbonits, M., Jacobs, R. A., Aylwin, S. J. B., Burrin, J. M., Dahia, P. L. M., Monson, J. P., et al. (1998). *J. Clin. Endocrinol. Metab.* **83**, 3624–3630.
73. Barlier, A., Zamora, A. J., Grino, M., et al. (1999). *J. Neuroendocrinol.* **11**, 491–502.
74. Jansson, J. O., Svensson, J., Bengtsson, B. A., et al. (1998). *Clin. Endocrinol. (Oxf.)* **48**, 243–250.
75. Graziani, A., Fubini, A., Filigheddu, N., et al. (1999). XXVIII National Congress of the Italian Society of Endocrinology, Turin, Italy.
76. De Gennaro Colonna, V., Rossoni, G., Bernareggi, M., Muller, E. E., and Berti, F. (1997). *Eur. J. Pharmacol.* **334**, 201–207.
77. Rossoni, G., De Gennaro Colonna, V., Bernareggi, M., Polvani, G. L., Muller, E. E., and Berti, F. (1998). *J. Cardiovasc. Pharmacol.* **32**, 260–265.
78. Locatelli, V., Rossoni, G., Schweiger, F., et al. (1999). *Endocrinology* **140**, 4024–4031.
79. Perreault, A., Bouchard, J., Bodart, V., Deghenghi, R., Lamontagne, D., and Ong, H. (1999). 81st Annual Meeting of The Endocrine Society, San. Diego.
80. Bisi, G., Podio, V., Valetto, M. R., et al. (1999). *J. Endocrinol. Invest.* **22**, 266–272.
81. Bisi, G., Podio, V., Valetto, M.R., et al. (1999). *Eur. J. Pharmacol.* **381**, 31–38.
82. Broglio, F., Valetto, M. R., Benso, A., et al. (1999). 9th Meeting of the European Neuroendocrine Association, Odense, Denmark.