Growth hormone-releasing peptides and the cardiovascular system

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Les sécrétines peptidiques de l’hormone de croissance (GH-RP) et le système cardio-vasculaire


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Growth Hormone (GH)-releasing peptides (GHRPs) and their non-peptidyl analogues are synthetic molecules which exhibit strong, dose-dependent and reproducible GH-releasing activity but also significant PRL- and ACTH/cortisol-releasing effects. An influence of these compounds on food intake and sleep pattern has been also shown. The neuroendocrine activities of GHRPs are mediated by specific receptors subtypes that have been identified in the pituitary gland, hypothalamus and GH secretagogues (GHS) are synthetic peptidyl (GH-releasing peptides, GHRPs) and non-peptidyl molecules which possess strong, dose-dependent and reproducible GH-releasing activity in vivo in several species and in man after intravenous, subcutaneous, intranasal and even oral administration [6, 10, 17, 32]. Among members of GHS family, those mostly studied in humans include peptidyl molecules (GHRPs) such as GHRP-6 and its superanalogs GHRP-1, a heptapeptide, GHRP-2 and hexarelin, two hexapeptides, as well as non-peptidyl GHRP mimetics, such as MK-677, a spiroindoline which shows marked bioavailability and long lasting effect after oral administration [6, 10, 17, 32].

The activity of GHS, however, is not fully specific for GH. In fact, both peptidyl and non peptidyl GHS also possess significant PRL- and remarkable ACTH/cortisol-releasing effects [16, 17]. Other central actions of GHS include stimulation of food intake and influence sleep pattern [15, 21].

The activities of GHS are mediated by specific receptors subtypes which are mainly present at the pituitary and hypothalamic level but also in other CNS areas and even at the peripheral level in both endocrine and non endocrine human tissues [19,
and various extra-hypothalamic brain regions with 125I-Tyr-Ala-hexarelin, an octapeptide of the GHRP family. In addition, GHRP receptors were also present in different peripheral tissues such as heart, adrenal, ovary, testis, lung and skeletal muscle, with a density significantly higher than that found in the hypothalamo-pituitary system. A remarkable specific 125I-Tyr-Ala-hexarelin binding was observed in the human cardiovascular system where the highest binding levels were detected in ventricles, followed by atria, aorta, coronaries, carotid, endocardium and vena cava. The binding of the radioligand to cardiac membranes 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 non peptidyl GHRP analog. In other experiments on H9c2 myocytes, a fetal cardiomyocytes-derived cell line, specific GHRP binding was found and hexarelin showed an anti-apoptotic activity. On the other hand, in vivo studies in animals and in humans showed that GHRPs possess direct cardiotrophic actions, in fact, hexarelin protects from ischemia-induced myocardial damage in aged and GH deficient rats while hexarelin shows a positive inotropic effect in normal subjects as well as in patients with GH deficiency. In conclusion, GHRPs possess extra-neuroendocrine biological activity and, particularly, show direct GH-independent cardiotropic effects.

**Key words:** Growth hormone-releasing peptides (GHRP), cardiac receptors, cardiotropic actions.

We studied the effects of sex and age on specific 125I-Tyr-Ala-hexarelin binding sites in human pituitary gland, hypothalamus and other CNS areas from subjects of both sexes (age ranging from 18 to 93 yr). GHS receptor density did not vary as a function of sex in pituitary, hypothalamus and other human brain areas in agreement with evidence that the GH response to GHS in men and women is similar [6, 17, 18, 27, 32]. Age did not affect the binding of 125I-Tyr-Ala-hexarelin to membranes from pituitary gland of middle-aged and elderly subjects. However, an age-related decrease of GHS receptor density was observed in the hypothalamus of both middle aged and elderly subjects [2] in agreement with evidence showing that the GHRP releasing effect of GHS undergoes an age-related reduction from adulthood to aging [1, 6, 7, 17, 32].

Our recent studies demonstrate that 125I-Tyr-Ala-hexarelin binding sites are also present in peripheral tissues [24]. In fact, a specific binding for this radioligand was found in the heart, adrenal, 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. Appreciable binding was also found in kidney, epiphysis and thyroid gland but not in intestinal smooth muscle, pancreas, parotid gland and spleen. Focusing on GHRP binding in the human cardiovascular system, considerable specific 125I-Tyr-Ala-hexarelin binding was detected in the ventricular myocardium, atrial myocardium, aorta, coronaries, carotid, endocardium and vena cava with values that were higher (p < 0.05) than those found in the pituitary gland, the exception being the endocardium and vena cava (fig. 1). GHRP binding in the cardiovascular tissues was independent of gender. Scatchard analysis of the binding to ventricular membranes revealed the presence of a single class of sites with a Kd of 2.2 ± 0.3 nM and a Bmax of 2786 ± 225 fmol/mg protein. 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 non peptidyl GHRP analog. Also various cardioactive substances such as angiotensin II, endothelin-1, IGF-I, epinephrine and acetylcholine did not inhibit cardiac GHRP binding.

**CARDIOVASCULAR ACTIVITIES**

**Studies in vitro and in animals in vivo**

The cellular effects of hexarelin on H9c2 myocytes, a fetal cardiomyocyte-derived cell line have been investigated. In fact, hexarelin binds to specific high affinity binding sites on H9c2 cells. The preliminary results of our studies indicate that hexarelin promotes the survival of H9c2 cells after treatment with cytotoxic agents such as tumor necrosis factor and doxorubicin. We also found that hexarelin activates Akt serine kinase, an enzyme which, by phosphorylating Bcl-2 and Bad, mediates the anti-apoptotic signaling induced by several survival factors. Thus, our preliminary results indicate that GHRPs exert an anti-apoptotic activity through a specific, receptor-mediated mechanism which involves the activation of a well known anti-apoptotic signaling pathway (personal unpublished results).

On the other hand, both in animals and in humans there is already evidence showing that GHRPs possess direct cardiotropic actions. In fact, in young rats with selective GH deficiency induced by passive immunization against GHRH, hexarelin pretreatment is able to protect against myocardial ischemic damage induced by low-flow ischemia and reperfusion [8, 9]. Such a protective activity was associated with a recovery of prostacyclin release and a normalization of the vasoressor activity of angiotensin II [8, 9]. In fact, it is well known that GH deficiency induces a clear exacerbation of ischemic tissue damage during low flow ischemia and reperfusion in rats. This worsening could be, at least partially, due to the reduced release of prostacyclin during the preischemic phase (particularly during reperfusion) and to the enhanced responsivity of coronary smooth muscles to angiotensin II which increases the coronary artery resistance during reperfusion [8, 9].

Similar results were observed in aged rats in which hexarelin pretreatment achieved a strong protection against myocardial stunning [29]. Complete recovery of the cardiac function was present on reperfusion and the simultaneous reduction of creatine-kinase levels testifies to the integrity of myocardial cell membranes and the preservation from the contractile impairment that follows oxygen readmission [29].

Noteworthy, in aged rats the cardioprotective activity of hexarelin was observed in absence of significant stimulation of somatotroph secretion. More recently, definitive evidence of a GH-independent, direct cardiotropic activity of GHRPs which could be mediated by the activations of specific myocardial receptors [5, 25, 29] has been provided by showing the same cardioprotective effects of hexarelin in hypophysectomized rats [20]. It has to be emphasized, however, that all these effects of hexarelin have been recorded after prolonged but not after acute or short-term hexarelin treatment.

**Studies in humans**

Recent data suggest that in humans as well as in animals hexarelin exerts cardiotropic activities. In fact, the acute intravenous administration of hexarelin is able to induce clear and prompt increase in left ventricular ejection fraction evaluated by radionuclide angiocardiography in healthy volunteers [4]. Indeed, hexarelin administration induced clear-cut increase in circulating GH
levels but it has to be emphasized that similar increase in circulating GH levels induced by the acute administration of rhGH did not modify the left ventricular ejection fraction in the same subjects [4].

In further agreement with the hypothesis that the acute cardiotropic activity of hexarelin is direct and GH-independent, like in normal subjects, a prompt and clear increase of left ventricular ejection fraction after acute hexarelin administration has been observed even in hypopituitary adult patients with severe GHD who show negligible somatotroph responsiveness to the hexapeptide [34].

The hexarelin-induced increase in left ventricular ejection fraction could directly reflect the decrease of the afterload charge following peripheral vasodilatation or, indirectly, the increase of the coronary blood flow. As no variation of the mean blood pressure was recorded after hexarelin, it is unlikely a decrease of the peripheral vascular resistance explains the increase in cardiac contractility [4, 34]. Moreover, hexarelin did not modify heart rate and circulating catecholamines levels in GHD as well as in normal subjects [4, 34]; in agreement with our findings, in dogs in vivo, the intra-arterial infusion of hexarelin into the left adrenal gland did not modify the epinephrine output in the adrenal vein (Ong et al., unpublished data).

It is also unlikely that the acute inotropic effect of hexarelin is mediated by an increase of circulating IGF-I levels which are not increased by the acute hexarelin administration in humans [18, 28]. However, taking into account that acute inotropic effect of rhIGF-I has been recently shown in humans, both in normal subjects and in patients with chronic heart failure [12, 13, 30], the possibility that GHS act via an increase of myocardial IGF-I [11, 14] cannot definitively be ruled out.

Interestingly, consideration has to be given to the evidence that the increase of cardiac ejection fraction after hexarelin in humans takes place after acute administration while the protective effect of hexarelin in rats needs prolonged treatment; this evidence suggests that different mechanisms underlie these different cardiotropic activities of GHRPs.

**CONCLUSIONS**

GH secretagogues (GHS) are synthetic peptidyl and non-peptidyl molecules which possess strong, dose-dependent and reproducible GH-releasing activity but also significant PRL- and ACTH-releasing effect. The neuroendocrine activities of GHS are mediated by specific receptors mainly present at the pituitary and hypothalamic level but also in other CNS areas. GHS receptors are also present at the peripheral level in both endocrine and non endocrine human tissues. The functional significance of peripheral GHS receptors is generally unknown, except for the notable evidence that GHRPs very likely have GH-independent cardiotropic activities both in animals and in humans.

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