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Growth Hormone–Independent Cardiotropic Activities of Growth Hormone-Releasing Peptides in Normal Subjects, in Patients with Growth Hormone Deficiency, and in Patients with Idiopathic or Ischemic Dilated Cardiomyopathy

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Growth hormone releasing peptides (GHRPs) are synthetic molecules endowed with potent neuroendocrine activities mediated by specific receptors in the pituitary and in the central nervous system. GHRPs receptors have been reported even in perpheral tissues, particularly in the myocardium, where they probably mediate growth hormone (GH)–independent activities. We studied in humans the cardiac effects of hexarelin administration in 7 normal adults, in 7 severe GH-deficient patients, and in 12 patients with severe dilated cardiomyopathy. Left ventricular ejection fraction (LVEF), mean blood pressure (MBP), heart rate (HR), and GH levels were evaluated at baseline and every 15 min up to 60 min after acute 2.0 μg/kg iv hexarelin administration. Basal LVEF in dilated cardiomyopathy was impaired and lower ($p < 0.001$) than in GH deficiency, in turn lower ($p < 0.001$) than in normal subjects. Hexarelin signficantly ($p < 0.05$) increased LVEF in normal and in GH-deficient subjects, but not in dilated cardiomyopathy, without significant variations in MBP and HR. Hexeralin significantly ($p < 0.05$) increased GH levels in normal subjects and in dilated cardiomyopathy but not in GH deficiency. These findings suggest that, in humans, the acute administration of hexarelin exerts a GH-independent positive inotropic effect likely mediated by specific GHRPs myocardial receptors.

Key Words: Hexarelin; GH-secretagogues; dilated cardiomyopathy, GH deficiency; left ventricular ejection fraction.

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Introduction

Growth hormone secretagogues (GHSs) are synthetic peptidyl (growth hormone–releasing peptides, [GHRPs]) and non-peptidyl molecules that possess dose-dependent and reproducible GH-, prolactin-, adrenocorticotropic hormone/cortisol-releasing effect in vivo in several species and in humans after iv and even oral administration *(1–3)*.

The activities of GHSs are mediated by specific receptor subtypes that are mainly present at the pituitary and hypothalamic levels but also in other central nervous system areas and even at the peripheral level in both endocrine and non endocrine human tissues *(4,5)*, particularly in cardiovascular tissues, where specific GHS receptor subtypes have been recently isolated *(6)*. In fact, in human tissues, considerable specific GHRP binding has been detected in the ventricular and atrial myocardium, aorta, coronary arteries, carotid artery, endocardium, and vena cava with values that were higher than those found in the pituitary gland, with the exception of the endocardium and vena cava *(5)*.

The presence of specific human receptors suggested the existence of a natural GHS-like ligand that could be represented by a recently discovered gastric peptide named ghrelin *(7)*.

It has already been reported that cardiovascular GHRP receptors mediate GH-independent biologic activities. In fact, in vitro, hexarelin, a synthetic GHRP, exerts an antiapoptotic activity in H9c2 myocytes, a fetal cardiomyocyte-derived cell line that possesses high-affinity binding sites for GHRPs, through a specific, receptor-mediated mechanism involving well-known antiapoptotic signaling pathways *(5)*.

On the other hand, in vivo, in aged as well as in GH-deficient and even in hypophysectomized rats, prolonged hexarelin pretreatment is able to protect against myocardial

ischemic damage induced by low-flow ischemia and reperfusion *(8–10)*, as shown by a reduction in creatine kinase levels, testifying to the integrity of myocardial cell membranes and preservation from the contractile impairment that follows oxygen readmission *(8–10)*.

All these findings suggest a direct, GH-independent, cardiovascular activity of GHRPs that could be mediated by the activation of specific myocardial GHRP receptor subtypes *(5,6)*.

To verify this hypothesis, we studied the acute effects of hexarelin on cardiac performance evaluated by radionuclide angiocardiography as well as on GH secretion in normal subjects, patients with severe GH deficiency, and patients with severe impairment of cardiac function owing to idiopathic or postischemic dilated cardiomyopathy.

Results

At baseline, left ventricular ejection fraction (LVEF) in dilated cardiomyopathy (23.0±2.9%) was clearly impaired and lower ($p < 0.001$) than that in GH deficiency (50.0 ± 2.0%), which in turn was lower ($p < 0.001$) than that in normal subjects (64.0 ± 1.6%). On the other hand, left ventricular end-diastolic volume (LVEDV) was higher in dilated cardiomyopathy than in normal subjects (351.4 ± 34.1 vs 122.9 ± 6.1 mL; $p < 0.001$), in which, in turn, was similar to that in GH deficiency (98.9 ± 9.0 mL).

At baseline, mean blood pressure (MBP) and heart rate (HR) were not significantly different in the three groups (normal subjects: 96.9 ± 2.0 mmHg and 66.7 ± 3.5 bpm, respectively; GH deficiency: 92.4±3.6 mmHg and 63.9±3.2 bpm, respectively; dilated cardiomyopathy: 94.7±3.9 mmHg and 75.4±2.6 bpm, respectively).

In both normal subjects and GH deficiency, hexarelin administration induced a clear and prompt increase in LVEF (peak: 70.7 ± 3.0%, $p < 0.05$ vs baseline and 57.3 ± 1.5%, $p < 0.05$ vs baseline, respectively). In both groups, LVEF increased 15 min after hexarelin administration, and this effect persisted up to the end of the test. After hexarelin, the percentage of increase of LVEF in GH-deficient patients (15%) was higher than that in normal subjects (10.2%), although this difference did not attain statistical significance. However, the peak of LVEF in GH deficiency was still lower than that in normal subjects ($p < 0.01$).

On the other hand, in dilated cardiomyopathy, hexarelin administration was not able to modify LVEF (peak: 24.7 ± 3.3%).

In all groups, hexarelin administration did not induce any significant change in LVEDV, MBP, and HR.

In normal subjects as well as in dilated cardiomyopathy, hexarelin induced a similar significant increase in GH levels (peak: 19.4 ± 2.1 μg/L $p < 0.05$ vs baseline and 21.4 ± 3.0 μg/L, $p < 0.05$ vs baseline). On the other hand, as expected, in GH-deficiency, hexarelin did not elicit any significant GH-releasing activity (peak: 1.9 ± 0.9 μg/L).

Basal epinephrine and norepinephrine levels in GH deficiency (33.9 ± 5.4 and 233.0 ± 20.5 ng/L) were similar to those in normal subjects (40.9 ± 5.8 and 183.7 ± 20.7 ng/L) and both were lower than those in dilated cardiomyopathy (90.7 ± 21.4 and 587.5 ± 36.2 ng/L). Both epinephrine and norepinephrine did not vary after hexarelin administration in all groups (GH deficiency: 34.6 ± 3.8 and 257.4 ± 33.2 ng/L, respectively; normal subjects: 47.1 ± 5.7 and 211.4 ± 33.6 ng/L, respectively; dilated cardiomyopathy: 131.2 ± 48.6 and 623.5 ± 83.5 ng/L, respectively).

Side Effects

Transient facial flushing was recorded immediately after hexarelin administration in two GH-deficient, three dilated cardiomyopathy, and in four normal subjects.

Discussion

The present study shows that the acute iv administration of hexarelin is able to induce a clear and prompt increase in LVEF evaluated by radionuclide angiocardiography in healthy volunteers and even in hypopituitary patients with severe GH deficiency but not in patients with dilated cardiomyopathy. This cardiovascular effect is independent of the GH-releasing activity of hexarelin; in fact, the administration of the hexapeptide induced a similar GH increase in normal subjects and patients with dilated cardiomyopathy but not in patients with severe GH deficiency. The hexarelin-induced increase in LVEF seems to be also independent of changes in MBP, HR, and catecholamine levels.

Peripheral specific binding sites for peptidyl GHSs have already been shown *(5)*, and it has been emphasized that cardiovascular binding sites for GHRPs seem more abundant than in the hypothalamus-pituitary unit *(5)*. In both animals and humans, cardiovascular activities of GHRPs have been pointed out *(5,8–10)*. Depending on the dose and experimental conditions, in rats it has been shown that GHRPs influence cardiac contractility *(11)*. Moreover, GHRPs have been shown able to protect rats from myocardial damage induced by low-flow ischemia and reperfusion *(8–10)*. Again, recent data indicate that GHRPs exert an antiapoptotic activity protecting rat cardiomyoblasts and cardiomyocytes from doxorubicin-, FAS ligand-, and tumor necrosis factor–induced apoptosis *(5)*.

In humans, it has been previously shown that the acute administration of hexarelin but not that of rhGH increases the LVEF in the absence of any variation in blood pressure, HR, and catecholamine levels *(12)*. Moreover, it has been shown that hexarelin also has the same effect on LVEF as in hypopituitary patients with severe GH deficiency *(13)*. The present study confirms these findings in a larger group of normal and hypopituitary patients.

The evidence that hexarelin increases LVEF in GH-deficient patients, who show negligible GH response to the hexapeptide, as well as in normal subjects indicates that

GHRPs exert a positive influence on cardiac contractility independently of their GH-releasing activity. In agreement with data in animals, the cardiotropic effect of GHRPs could be mediated by their specific myocardial receptors *(6)*. The hexarelin-induced increase in LVEF could directly reflect the decrease in the afterload charge following the peripheral vasodilatation or, indirectly, the increase in the coronary blood flow. Because no variation in the MBP was recorded after hexarelin, a decrease in the peripheral vascular resistance unlikely explains the increase in cardiac contractility *(12,13)*. Moreover, hexarelin did not modify heart rate and circulating catecholamine levels in GH-deficient and in normal subjects *(12,13)*. 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 (unpublished data).

It is also unlikely that the acute inotropic effect of hexarelin is mediated by an increase in circulating insulin-like growth factor-1 levels, which are not increased by the acute hexarelin administration in humans as well as in animals administered with GHRP doses that do not induce any significant GH response *(14,15)*.

The evidence that the acute administration of hexarelin does not modify LVEF in dilated cardiomyopathy patients who show normal GH response to the hexapeptide points toward two considerations. On the one hand, these findings further indicate that the cardiotropic activity of GHRPs is independent of their GH-releasing effect. On the other hand, the lack of any effect of these molecules on cardiac contractility of dilated hearts must be explained. More likely, this could be owing to refractoriness of the dilated myocardium to inotropic stimuli *(16)*. However, theoretically, the GHRP receptor status could be impaired in dilated cardiomyopathy and this could explain the lack of effect on the cardiac contractility. Moreover, the effects of GHRP administration on cardiac performances in dilated cardiomyopathy should also be studied to distinguish between patients with ischemic and idiopathic causes of dilated cardiomyopathy because important differences could underlie the different pathogenesis *(17)*. Moreover, the study of the effects of the prolonged treatment with GHRPs on cardiac performances in patients with dilated cardiomyopathy is another key point to clarify the cardiovascular effects of these molecules in this condition.

Materials and Methods

Seven healthy male adult volunteers; age [mean ± SEM]: 37.4 ± 3.4 yr; BMI: 23.3 ± 1.0 kg/m^2), 7 patients with severe GH deficiency (age: 42.0 ± 4.4 yr, BMI: 26.2 ± 0.9 kg/m^2), and 12 patients with severe impairment of cardiac function owing to idiopathic or postischemic dilated cardiomyopathy (age: 53.3 ± 2.1 yr.; BMI: 26.5±1.6 kg/m^2; New York Heart Association functional classes: I, $n = 0$; II, $n = 7$; III, $n = 4$; IV, $n = 1$) participated in the study. LVEF, LVEDV, and left ventricular end-systolic volume, evaluated by equilibrium radionuclide angiocardiography (*see* ref. *13* for methodologic details); systolic, diastolic, and mean blood pressure and HR-monitored by an automated apparatus (*see* ref. *13* for methodologic details); as well as GH, epinephrine, and norepinephrine levels were studied in basal conditions (at –15 and 0 min) and then every 15 min from 0 up to 60 min after acute hexarelin (2.0 µg/kg intravenously at 0 min) administration (*see* ref. *13* for methodologic details).

In the GH-deficient group, the diagnosis of severe GH deficiency was based on a GH peak <3 µg/L after insulin tolerance test and/or <9 µg/L after GHRH+arginine test *(18)*. All patients with other pituitary insufficiencies were in optimized replacement therapy. In all the patients, rhGH replacement was withdrawn 3 mo before entering the present study.

All dilated cardiomyopathy patients had a history of congestive heart failure and were on a waiting list for heart transplantation. All the patients had cardiomegaly demonstrated by chest X-ray and echocardiographic examination. The diagnosis of postischemic cardiomyopathy ($n = 5$) was based on history of prior myocardial infarction and/or coronary artery disease diagnosed by coronarography. All the patients were under standard therapy with diuretics, angiotensin-converting enzyme inhibitors, and digoxin.

All the subjects gave informed consent to participate in the study, which had been approved by the local, independent ethical committee.

Statistical Analyses

Results are expressed as mean±SEM. Hemodynamic parameters are expressed as absolute values and as percentage changes from baseline (Δ%). Hormonal parameters are expressed as absolute values or as areas under the curve (AUCs), calculated by trapezoidal integration. The statistical analysis of the data was performed using analysis of variance (Mann-Whitney U test or Friedman two way followed by Wilcoxon signed rank test, as appropriate).

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