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Cardiac effects of hexarelin in hypopituitary adults

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Abstract

Growth hormone (GH)-releasing peptides possess specific pituitary, hypothalamic, and myocardial receptors. Seven adult male patients with GH deficiency (GHD) (age, mean ± S.E.M.: 42.0 ± 4.0 year) were studied by equilibrium radionuclide angiocardiography after i.v. administration of hexarelin, a peptide GH secretagogue. Data for these patients were compared with those for nine adult male controls (37.0 ± 2.7 year). The GH response to hexarelin was negligible in patients with GHD compared to control subjects (CS) (peak: 1.9 ± 0.9 vs. 45.7 ± 3.6 μg/l, $P < 0.001$). Basal left ventricular ejection fraction (LVEF) in patients with GHD was lower than that in CS (50 ± 1% vs. 63 ± 2%, $P < 0.001$). Hexarelin administration increased LVEF both in patients with GHD and in CS (peak: 57 ± 2 vs. 70 ± 2, respectively, $P < 0.05$ vs. baseline) without changing catecholamine levels, mean blood pressure (MBP), or cardiac output in either group. In conclusion, the acute administration of hexarelin exerts a short-lasting positive inotropic effect in humans, probably GH-independent and mediated by specific myocardial receptors for GH secretagogues.

Keywords: Hexarelin; Growth hormone (GH) secretagogue; Growth hormone deficiency (GHD), adult; Haemodynamic; Ventricular function, left; Radionuclide imaging

1. Introduction

The close relationship between the activity of growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis and myocardial function has been recently reviewed (Saccà et al., 1994). GH and IGF-I bind to specific receptors in the myocardium where IGF-I synthesis and release devoted to auto-/paracrine actions have been recently demonstrated (Isgaard et al., 1989, 1997; Timsit et al., 1990; Ito et al., 1993; Donath et al., 1994; Donohue et al., 1994; Saccà et al., 1994; Duerr et al., 1995; Cittadini et al., 1996). GH has both direct and IGF-I-mediated effects on myocardial growth and function in animals (Isgaard et al., 1989; Timsit et al., 1990; Saccà et al., 1994; Cittadini et al., 1996). Indeed, GH and IGF-I are able to induce the expression of mRNA for specific contractile proteins as well as myocyte hypertrophy and contractility (Ito et al., 1993; Duerr et al., 1995; Yang et al., 1995; Cittadini et al., 1996).

These findings may explain the abnormalities of cardiac function demonstrated in GH hyper- and hypo-secretory states. In acromegaly, first GH and then IGF-I hypersecretion induces a cardiac hyperkinetic syndrome, with morphological abnormalities such as ventricular hypertrophy with interstitial fibrosis leading to progressive impairment of diastolic function over time (Saccà et al., 1994). Low GH and IGF-I levels in GH deficiency (GHD) are accompanied by a reduced left ventricular mass and impaired cardiac performance (hypokinetic syndrome) (Salomon et al., 1989; Shahi et al., 1992; Amato et al., 1993; Merola et al., 1993; Cittadini et al., 1994; Saccà et al., 1994; Cuocolo

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et al., 1996; Johannsson et al., 1996). These abnormalities as well as alterations in metabolism, body composition and function are reversed by recombinant human GH replacement (Amato et al., 1993; De Boer et al., 1995; Johannsson et al., 1996).

Benefit from recombinant human GH treatment in patients with dilated cardiomyopathy has also been reported by some authors (Fazio et al., 1996), but not by others (Osterziel et al., 1998). An acute positive inotropic effect of GH and IGF-I administration has recently been reported in humans as well as in animals (Russel Jones et al., 1995; Donath et al., 1996, 1998; Volterrani et al., 1997). We did not find any acute effect of intravenous recombinant human GH administration on cardiac contractility in normal adults; however, cardiac contractility was increased by the acute intravenous administration of hexarelin, a peptide GH secretagogue (Bisi et al., 1999).

Peptide and nonpeptide GH secretagogues are synthetic molecules which markedly stimulate GH secretion via actions on specific receptors at the hypothalamic and pituitary level (Bowers et al., 1993; Arvat et al., 1997; Ghigo et al., 1997; Smith et al., 1997). Binding sites for GH secretagogues are present also in peripheral tissues, particularly in the myocardium (Howard et al., 1996; Guan et al., 1997; Ong et al., 1997, 1998; Bodart et al., 1998; Muccioli et al., 1998a,b), and it has been reported that treatment with GH secretagogues protects myocardial tissue against ischemia in both aged and GH-deficient rats (De Gennaro Colonna et al., 1997; Isgaard et al., 1998; Rossoni et al., 1999). These findings suggested a direct, GH-independent, cardiotropic activity of GH secretagogues.

To confirm this hypothesis, which has already been verified in normal adult subjects (Bisi et al., 1999), we studied the acute effects of hexarelin on cardiac performance evaluated by radionuclide angiocardiography as well as on GH secretion in adult patients with severe GHD. The results for patients with GHD were compared with those for a group of normal adult control subjects (CS) that was larger than that in our previous study (Bisi et al., 1999).

2. Materials and methods

2.1. Study design and protocol

Seven adult male patients with severe GHD (age, mean ± S.E.M.: 42.0 ± 4.4 year, Body Max Index, BMI: 26.2 ± 0.9 kg/m^2) and nine normal male adult volunteers (37.0 ± 2.7 year, 23.2 ± 0.8 kg/m^2) were studied.

Among the patients with GHD, three had acquired, adulthood-onset GHD with panhypopituitarism, while the others had childhood-onset GHD (one isolated, three with panhypopituitarism). In all subjects, the diagnosis of severe adult GHD was based on the demonstration of a GH peak below 3 μg/l after an insulin tolerance test and below 9 μg/l after a GH-releasing hormone (GHRH) + arginine test (Growth Hormone Research Society, 1998). All patients with pituitary insufficiency other than GHD were on optimized replacement therapy with thyroid hormone, cortisone acetate, gonadal steroids, and desmopressin acetate (DDAVP), as appropriate. All patients with childhood-onset GHD had been treated in childhood with recombinant human GH until they reached the predicted mid-parental height, while all patients with adulthood-onset GHD started recombinant human GH replacement therapy at the onset of hypopituitarism. In all patients, recombinant human GH replacement was withdrawn 3 months before they entered the present study.

All subjects gave their informed consent to participate in the study, which had been approved by the local, independent Ethics Committee. No subject had a history of hypertension, cardiovascular, renal, respiratory or hepatic disease, or was taking any drug other than the appropriate replacement therapy. Physical examination, blood pressure, and electrocardiographic findings were normal.

On the day of the study, subjects had breakfast at 8:00 a.m. and were admitted to the study room 2 h before the beginning of the test session (4:00 p.m.). The study room was maintained under constant conditions of temperature and light, and an absence of noise. One antecubital vein was cannulated for intravenous administration and the other for blood sampling; the latter being kept patent by slow infusion of isotonic saline. Electrocardiogram was continuously monitored in lead II. Blood pressure was monitored by an automated apparatus (SpaceLabs, Redmond, WA, USA).

After 45 min in recumbent position, all subjects received an intravenous dose of hexarelin (His–D2–methyl–Trp–Ala–Trp–D–Phe–Lys–NH_2, Europeptides, Argenteuil, France, 2.0 μg/kg at 0 min). Haemodynamic and hormonal parameters were evaluated at baseline and then every 15 min from 0 min to 60 min after administration of hexarelin.

2.2. Radionuclide imaging methods

Equilibrium radionuclide angiocardiography was performed after in vitro labelling of red blood cells with 925 MBq (25 mCi) of ^{99m}Tc. Subjects were imaged supine in the best septal anterior oblique projection. Acquisition was performed with a 400 T GE scintillation camera, equipped with a low-energy, all-purpose parallel hole collimator, at 24 frames/cycle, 64 × 64 matrix, and a ×1.5 zoom factor. For data processing, a standard, highly reproducible, validated semiautomatic procedure was used, involving multiple regions of interest and background subtraction. Left ventricular ejection fraction (LVEF) was calculated from the left ventricular curve. Right ventricular ejection

fraction (RVEF) was estimated from two regions of interest and background subtraction. Absolute left ventricular end-diastolic volume (LVEDV) and end-systolic volume were calculated by a validated nongeometric method (Links et al., 1982). Systolic, diastolic, and mean blood pressure (MBP) and heart rate (HR) were measured at each time point. Stroke volume, cardiac output and systemic vascular resistance were derived from the other measured parameters. Eight-minute repeated acquisitions were made for the first 8 min of each 15-min period from −15 to +60 min. For each acquisition, a minimum of 8 million counts was acquired.

2.3. Analytical methods

Blood samples for the assay of GH, epinephrine (E), and norepinephrine (NE) were collected under basal conditions (at −15 and 0 min) and then every 15 min from 0 min to 60 min after hexarelin administration.

Serum GH levels (μg/l) were measured by immunoradiometric assay (hGH-CTK, Sorin Biomedica, Saluggia, Italy). The sensitivity of the assay was 0.15 μg/l. The inter- and the intra-assay coefficients of variation were 2.9%–4.5% and 2.4%–4.0%, respectively.

Serum IGF-I levels (μg/l) were measured by radioimmunometric assay (Nicholls Institute Diagnostics, San Juan Capistrano, USA) after acid–ethanol extraction to avoid interference from binding proteins. The sensitivity of the assay was 0.2 μg/l. The inter- and intra-coefficients of variation were 8.8%–10.8% and 5.0%–9.5%, respectively. In our laboratory, the 3rd and 97th centile limits of normal IGF-I in adults are 65 and 385 μg/l, respectively.

Plasma E and NE levels (ng/l) were assayed after extraction on alumina by high-performance liquid chromatography with electrochemical detection. The sensitivity of the assay was 5.0 ng/l for both E and NE. The inter- and the intra-assay coefficients of variation were 8.5% and 4% for E, and 7% and 3% for NE, respectively.

2.4. Statistical analysis

Results are expressed as means ± S.E.M. Haemodynamic parameters are expressed as absolute values, abso-

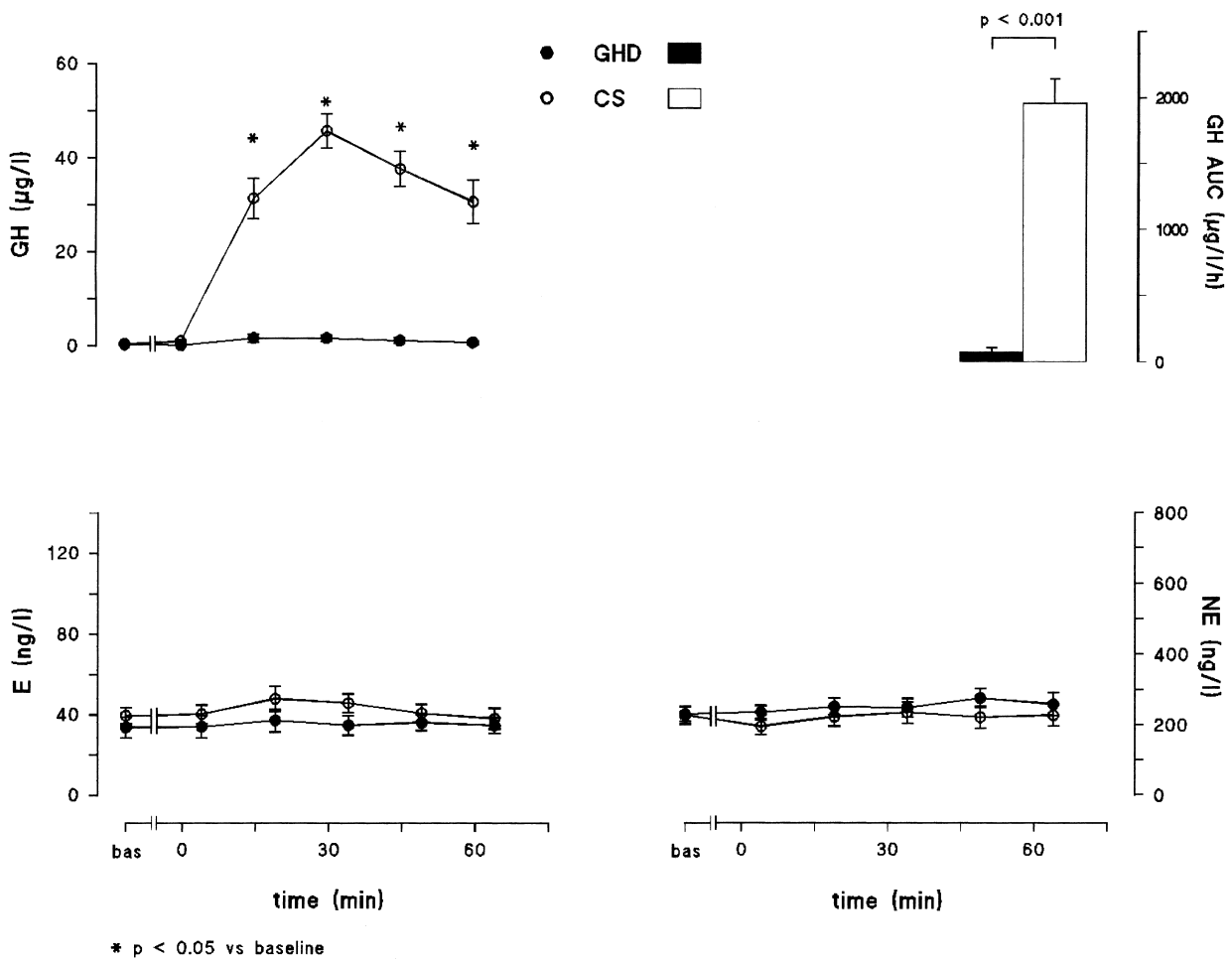

Fig. 1. Mean ± S.E.M. GH response, expressed as curves (upper left panel) or AUCs (upper right panel) and mean ± S.E.M. E (lower left panel) and NE response curves (lower right panel) after hexarelin in seven adult patients with severe GHD and in nine adult normal CS.

lute values corrected for body surface area, or 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 by analysis of variance (ANOVA) (Mann–Whitney *U*-test or Friedman two-way followed by Wilcoxon signed rank test, as appropriate).

3. Results

3.1. Hormonal parameters

Basal GH, IGF-I, and catecholamine levels were not different between patients with childhood-onset or adulthood-onset GHD. Basal GH levels were not significantly different in patients with GHD and CS (0.1 ± 0.1 μg/l vs. 0.9 ± 0.4 μg/l, respectively), while basal IGF-I levels in patients with GHD were significantly lower than those in CS (87.6 ± 21.1 vs. 210.2 ± 10.3 μg/l, $P < 0.001$). Patients with GHD showed a negligible increase of circulating GH levels after hexarelin administration (peak: 1.9 ± 0.9 μg/l, *P* NS vs. baseline), again with no differences between childhood-onset and adulthood-onset GHD. In contrast, hexarelin induced a marked increase in GH secretion in CS (45.7 ± 3.6 μg/l, $P < 0.01$ vs. baseline and $P < 0.001$ vs. peak in patients with GHD) (Fig. 1).

Basal E and NE levels in patients with GHD (34 ± 5 and 233 ± 21 ng/l) were similar to those in CS (40 ± 5 and 194 ± 22 ng/l). E and NE levels did not vary after administration of hexarelin in either group (patients with GHD: 35 ± 4 and 257 ± 33 ng/l, respectively; CS: 48 ± 6 and 234 ± 30 ng/l, respectively) (Fig. 1 and Table 1).

3.2. Haemodynamic parameters

Both at baseline and after hexarelin administration, haemodynamic parameters did not differ between patients with childhood- or adulthood-onset GHD. At baseline, LVEF and RVEF were lower in patients with GHD than in CS (50 ± 1 vs. 63 ± 2%, $P < 0.001$ and 35 ± 3 vs. 46 ± 2%, $P < 0.02$), while LVEDV corrected for body surface area was higher in patients with GHD than in CS (99 ± 9 vs. 66 ± 4 ml/m^2, $P < 0.01$) (Fig. 2 and Table 1). In patients with GHD and in CS, hexarelin administration did not induce a significant change in MBP and HR, though a trend towards a decreased HR was apparent in both groups (Fig. 3 and Table 1). Hexarelin administration increased

Table 1
Effects of hexarelin administration on haemodynamic and hormonal parameters in seven adult patients with severe GHD and in nine normal adult CS[a]

	GHD patients		Normal subjects (NS)	
	Basal	Peak of effect on LVEF (60 min)	Basal	Peak of effect on LVEF (30 min)
LVEF (%)	50 ± 2[b]	57 ± 2[c,d]	63 ± 2	70 ± 2[c]
RVEF (%)	35 ± 3[e]	36 ± 3[e]	46 ± 2	47 ± 2
LVEDV corrected for body surface area (ml/m^2)	99 ± 9[f]	95 ± 7[c,d]	66 ± 4	66 ± 4
MBP (mm Hg)	101 ± 6	105 ± 4	91 ± 3	90 ± 5
HR (beats/min)	64 ± 3	61 ± 2	66 ± 3	62 ± 2
Stroke volume corrected for body surface area (ml/m^2)	49 ± 3	54 ± 3[c]	42 ± 2	47 ± 4
Cardiac output corrected for body surface area (l/min/m^2)	3.1 ± 0.2	3.3 ± 0.2	2.7 ± 0.2	2.9 ± 0.2
Systemic vascular resistance corrected for body surface area (dyn s cm^{-5}/m^2)	829 ± 78	821 ± 111	832 ± 59	801 ± 81
E (ng/l)	34 ± 5	35 ± 4	40 ± 5	48 ± 6
NE (ng/l)	233 ± 21	257 ± 33	194 ± 22	234 ± 30

[a] In the present study, we present an enlarged and updated group of CS with respect to our previous data (Bisi et al., 1999).
[b] $P < 0.001$ vs. NS.
[c] $P < 0.05$ vs. baseline.
[d] $P < 0.005$ vs. NS.
[e] $P < 0.05$ vs. NS.
[f] $P < 0.01$ vs. NS.

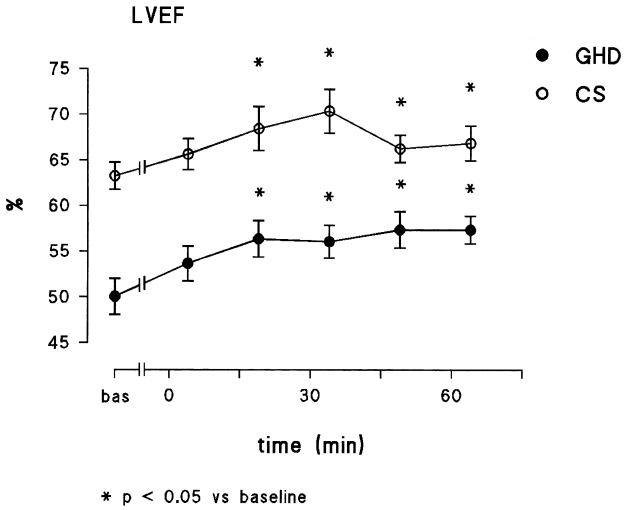

Fig. 2. Mean ± S.E.M. variations from baseline of LVEF after hexarelin in seven adult patients with severe GHD and in nine adult normal CS.

LVEF both in patients with GHD (peak: 57 ± 2%, $P < 0.03$ vs. baseline) and in CS (70 ± 2%, $P < 0.04$) (Figs. 2 and 3 and Table 1).

In both groups, LVEF increased 15 min after hexarelin administration, and this effect persisted until 60 min. However, the timing of the hexarelin-induced LVEF maximum in patients with GHD was delayed with respect to that of CS (60 vs. 30 min). The mean percentage increase in LVEF after hexarelin (15%) was higher than that in CS (11%), though this was not statistically significant. However, the peak of the LVEF in patients with GHD was lower than that in CS ($P < 0.002$) (Figs. 2 and 3).

In both groups, hexarelin administration enhanced stroke volume corrected for body surface area (peak vs. baseline, patients with GHD: 54 ± 3 vs. 49 ± 3 ml/m^2, CS: 47 ± 4 vs. 42 ± 3 ml/m^2), but this increase reached statistical significance ($P < 0.03$) only in patients with GHD. However, in both groups, cardiac output corrected for body surface area was not significantly modified (peak vs. baseline, patients with GHD: 3.3 ± 0.2 vs. 3.1 ± 0.2 l/min/m^2, CS: 2.9 ± 0.3 vs. 2.7 ± 0.2 l/min/m^2), probably due to the above mentioned trend towards a concomitant decrease in HR (Table 1). In agreement with these findings, a significant decrease in LVEDV was apparent in patients with GHD (LVEDV corrected for BSA, nadir: 95 ± 7

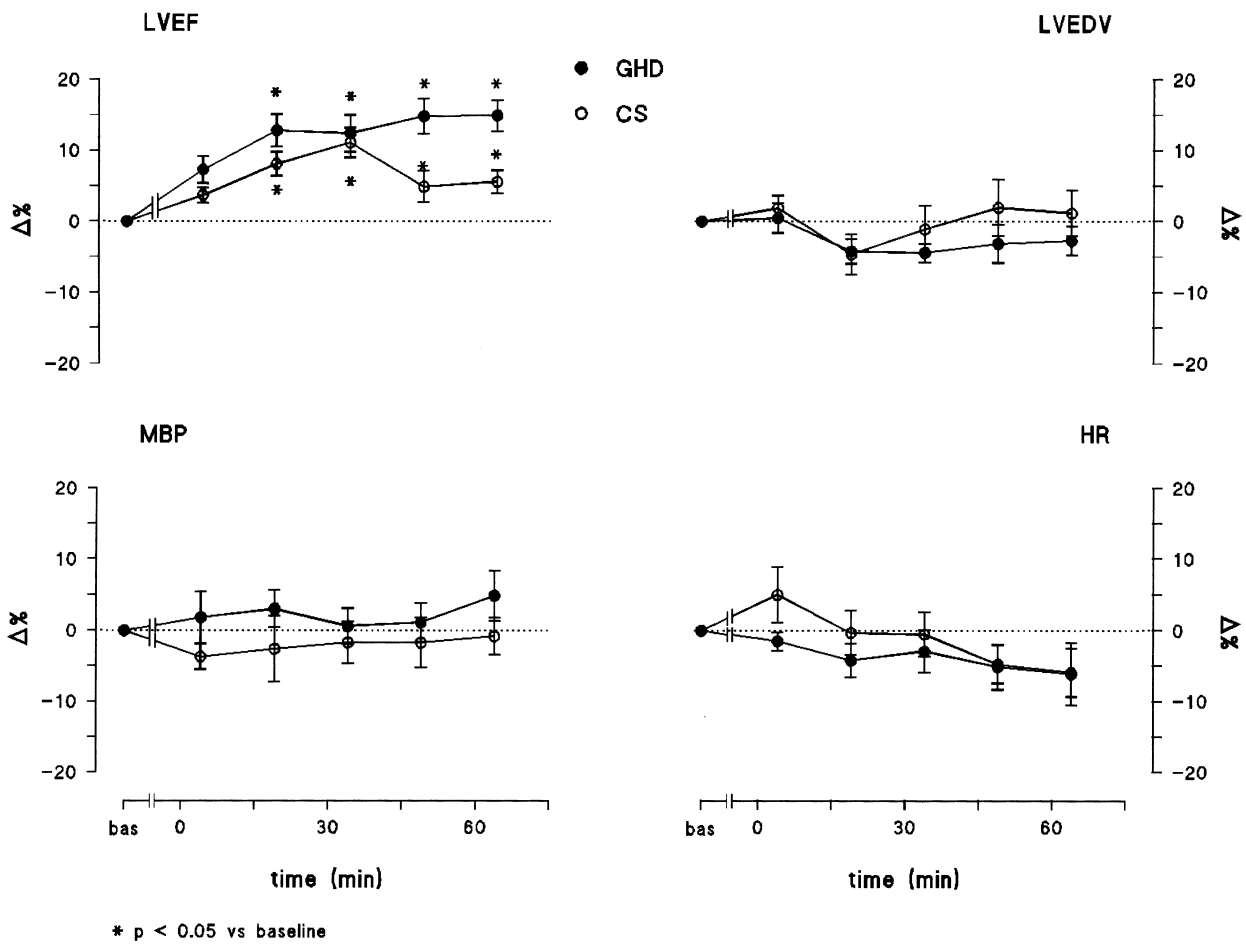

Fig. 3. Mean ± S.E.M. percentage variations from baseline of LVEF (upper left panel), LVEDV (upper right panel), MBP (lower left panel), and HR (lower right panel) after hexarelin in seven adult patients with severe GHD and in nine adult normal CS.

ml/m^2, $P < 0.05$ vs. baseline), but not in CS (66 ± 4 ml/m^2) (Table 1).

The administration of hexarelin did not modify systemic vascular resistance in either group (Table 1).

3.3. Side effects

Transient facial flushing, lasting less than 30 s, was recorded immediately after hexarelin administration in two patients with GHD and in five CS.

4. Discussion

The present study shows that the acute intravenous administration of hexarelin, a peptide GH secretagogue, increases LVEF in adult patients with severe GHD as well as in NS. The positive inotropic effect of hexarelin was not accompanied by any change in blood pressure, HR, or catecholamine levels in either group; similarly, GH levels did not change in adults with GHD.

Consistent with the close relationship between the GH/IGF-I axis and the heart (Saccà et al., 1994), the existence of abnormalities of cardiac function in adults with GHD has been reported by many authors (Salomon et al., 1989; Shahi et al., 1992; Amato et al., 1993; Merola et al., 1993; Cittadini et al., 1994; Saccà et al., 1994; Cuocolo et al., 1996; Johannsson et al., 1996). These alterations appear to be positively correlated with IGF-I levels (Mangieri et al., 1996) and are more evident during physical exercise (Longobardi et al., 1998). Our evaluation at baseline by equilibrium radionuclide angiocardiography agrees with previous data showing an impairment of ventricular function in patients with GHD (Salomon et al., 1989; Shahi et al., 1992; Amato et al., 1993; Merola et al., 1993; Cittadini et al., 1994; Cuocolo et al., 1996; Johannsson et al., 1996).

The endocrine effects of GH secretagogues, and in particular their strong, reproducible GH-releasing activity after intravenous, subcutaneous and even oral administration, have been extensively studied in humans as well as in animals (Bowers et al., 1993; Arvat et al., 1997; Ghigo et al., 1997; Smith et al., 1997). The present data confirm the potent GH-releasing activity of hexarelin under physiological conditions and, as expected, demonstrate that patients with severe GHD show a negligible response to the hexapeptide, in agreement with previous studies with peptide or non peptide GH secretagogues (Leal-Cerro et al., 1995; Chapman et al., 1997).

The possibility that GH secretagogues such as hexarelin might influence cardiac activity by indirect means was suggested by the cardiotropic actions of GH and IGF-I, and by the evidence that recombinant human GH replacement reverses abnormalities of cardiac function in adults with GHD (Amato et al., 1993; Cittadini et al., 1994; De Boer et al., 1995; Cuocolo et al., 1996; Johannsson et al., 1996). Acute inotropic effects of recombinant human GH or recombinant human IGF-I administration have recently been reported in humans as well as in animals (Russel Jones et al., 1995; Donath et al., 1996, 1998; Volterrani et al., 1997). In normal adults, however, we did not find any acute effect of intravenous recombinant human GH on cardiac contractility which was, however, increased by the acute intravenous administration of hexarelin (Bisi et al., 1999).

GH secretagogues act through specific receptors in the hypothalamus and the pituitary (Bowers et al., 1993; Arvat et al., 1997; Ghigo et al., 1997; Smith et al., 1997), but also in peripheral tissues, particularly in the myocardium (Howard et al., 1996; Guan et al., 1997; Ong et al., 1997, 1998; Bodart et al., 1998; Muccioli et al., 1998a,b). In GH-deficient and in aged rats subjected to low-flow ischaemia and reperfusion, hexarelin pretreatment protects against post-ischaemic left ventricular dysfunction (De Gennaro Colonna et al., 1997; Isgaard et al., 1998; Rossoni et al., 1999). In these experiments, hexarelin also counteracted the vasopressor response of the coronary vasculature to angiotensin-II, restored the release of 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin (PGI_2), to normal levels, and blunted the increase in creatine kinase in the post-ischaemic recovery phase (De Gennaro Colonna et al., 1997; Rossoni et al., 1999).

All these findings suggest a direct, GH-independent, cardiotropic activity of GH secretagogues, putatively mediated by specific myocardial receptors. The present study was planned to further verify this hypothesis, by determining the effects of hexarelin on cardiac performance by equilibrium radionuclide angiocardiography, which is highly reliable in comparison with other techniques (Burow et al., 1977; Okada et al., 1990). Our findings demonstrate that acute hexarelin administration induces a prompt and clear increase of LVEF in hypopituitary adult patients with severe GHD who show negligible somatotroph responsiveness to the hexapeptide. These results for patients with GHD provide further evidence that GH secretagogues possess acute, GH-independent cardiac activity. Moreover, the present study confirms, in a larger group of normal adults subjects, the acute inotropic effect of hexarelin reported in a previous study from our group (Bisi et al., 1999). All these data are in agreement with those for rats (De Gennaro Colonna et al., 1997; Isgaard et al., 1998; Rossoni et al., 1999). The hexarelin-induced increase in LVEF may be a direct reflection of the decrease in afterload following peripheral vasodilatation or an indirect response via an increase in coronary blood flow.

As no variation of MBP was recorded after hexarelin, a decrease of peripheral vascular resistance is unlikely to explain the increase in cardiac contractility. Moreover, hexarelin did not modify HR and circulating catecholamine levels in patients with GHD or in NS. In agreement with our findings, in dogs, intra-arterial infusion

of hexarelin into the left adrenal gland does not modify E output into the adrenal vein (Ong et al., unpublished data).

It is unlikely that the acute inotropic effect of GH secretagogues is mediated by an increase in circulating IGF-I levels. However, given that an acute inotropic effect of recombinant human IGF-I has been recently shown in humans, both in NS and in patients with dilated cardiomyopathy (Russel Jones et al., 1995; Donath et al., 1996, 1998), the possibility that GH secretagogues act via an increase in myocardial IGF-I levels (D'Ercole et al., 1984; Donohue et al., 1994) cannot be definitively ruled out.

5. Conclusion

The present human data indicate that hexarelin, a GH secretagogue, possesses acute positive inotropic activity which is independent of GH and which is putatively mediated by specific myocardial receptors for GH secretagogues.

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