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The Growth Hormone Response to Hexarelin in Patients with Different Hypothalamic-Pituitary Abnormalities

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ABSTRACT

We evaluated the GH-releasing effect of hexarelin (Hex; 2 μg/kg, iv) and GHRH (1 μg/kg, iv) in 18 patients (11 males and 7 females, aged 2.5–20.4 yr) with GH deficiency (GHD) whose hypothalamic pituitary abnormalities had been previously characterized by dynamic magnetic resonance imaging (MRI). Ten patients had isolated GHD, and 8 had multiple pituitary hormone deficiency. All patients were receiving appropriate hormone replacement therapy. Twenty-four prepubertal short normal children (11 boys and 13 girls, aged 5.9–13 yr, body weight within ±10% of ideal weight) served as controls. MRI studies revealed an ectopic posterior pituitary at the infundibular recess in all patients. A residual vascular component of the pituitary stalk was visualized in 8 patients with isolated GHD (group 1), whereas MRI showed the absence of the pituitary stalk (vascular and neural components) in the remaining 10 patients (group 2), of whom 8 had multiple pituitary hormone deficiency and 2 had isolated GHD. In the short normal children, the mean peak GH response to GHRH (24.8 ± 4.4 μg/L) was significantly lower than that observed after Hex treatment (48.1 ± 4.9 μg/L; $P < 0.0001$). In the GHD patients of group 2, the mean peak GH responses to GHRH (1.4 ± 0.3 μg/L) and Hex (0.9 ± 0.3 μg/L) were similar and markedly low. In the patients of group 1, the GH responses to GHRH (8.7 ± 1.3 μg/L) and Hex (7.0 ± 1.3 μg/L) were also similar, but were significantly higher that those observed in group 2 ($P < 0.0001$). In the whole group of patients, a significant correlation was found between the GH peaks after Hex and those after GHRH ($r = 0.746$; $P < 0.0001$). In this study we have confirmed that the integrity of the hypothalamic pituitary connections is essential for Hex to express its full GH-releasing activity and that Hex is able to stimulate GH secretion in patients with GHD but with a residual vascular component of the pituitary stalk. (*J Clin Endocrinol Metab* **83:** 3886–3889, 1998)

GH-RELEASING peptides (GHRPs) and their nonpeptidyl analogs are a class of compounds with potent GH-releasing activity in both animals and humans (1). Their action is mediated through specific receptors on the hypothalamus and the pituitary (2) and use signaling mechanisms distinct from those of GHRH and somatostatin (3, 4). The presence of specific receptors for GHRPs suggests that these compounds may represent synthetic analogs of an endogenous ligand. Although their precise mechanism of action is not completely understood, several data indicate that they may act as functional somatostatin antagonists as well as amplifiers of GHRH action (1).

Previous studies have shown that the GH responses to GHRP-6 and hexarelin (Hex), a synthetic hexapeptide analog to GHRP-6, are absent or severely blunted in patients with hypothalamic-pituitary disconnection (5, 6), indicating that the hypothalamus is their principal site of action, and that for GHRPs to express their full GH-releasing activity it is essential that intact hypothalamic-pituitary connections be present.

Magnetic resonance imaging (MRI) has been of great aid in the diagnosis of disorders of the hypothalamic pituitary area. More recently, dynamic MRI has allowed identification of discrete abnormalities of the hypothalamic-pituitary stalk in patients with GH deficiency (GHD), making it possible to visualize residual components of the pituitary stalk with significantly more accuracy than conventional MRI (7). Interestingly, the absence of the vascular pituitary stalk was strongly associated with the presence of multiple pituitary hormone deficiencies (MPHD), whereas the presence of a residual vascular component was predictive of a less severe impairment of pituitary function (7). It has also been shown that the GH response to GHRH was correlated with the hypothalamic-pituitary abnormalities (8).

The aim of this study was to further investigate the site and mechanism of action of Hex. To this end, we evaluated the GH-releasing effect of Hex in a group of patients with GHD and MPHD whose hypothalamic pituitary abnormalities had been previously characterized by dynamic MRI.

Subjects and methods

Eighteen patients with GHD (11 males and 7 females, aged 2.5–20.4 yr) were studied. At the time of diagnosis all patients underwent complete studies of pituitary function. In particular, GH secretion was evaluated by arginine (0.5 g/kg, iv, given over 30 min), insulin (0.1 U/kg, iv), and GHRH-(1–29) (1.0 μg/kg, iv) tests. GHD was diagnosed when GH levels failed to rise above 10 μg/L after arginine and insulin administration. The blood samples for GH determinations were obtained at 0, 30, 60, 90, and 120 min. Thyroid function was evaluated by deter-

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mination of serum T_4, free T_4 (FT_4), and basal and TSH response to iv 200 $\mu g/m^2$ TRH (Reflact, Hoechst, Frankfurt, Germany). Low T_4 and FT_4 with low normal basal TSH and delayed TSH response to TRH were documented in 8 patients. Pituitary-adrenal axis function, evaluated by determination of morning ACTH and cortisol levels at 0800 h and the cortisol response during insulin-induced hypoglycemia, were compatible with subclinical ACTH deficiency in 8 patients. In these patients, the cortisol response to im 0.25 mg ACTH-(1–24) (Synacthen, Ciba-Geigy, Basel, Switzerland) was less than 350 nmol/L (normal, >550 nmol/L). Hypogonadotropic hypogonadism was diagnosed in 2 patients on the basis of their serum FSH and LH responses to 100 $\mu g/m^2$ iv GnRH (Relisorm L, Serono, Milan, Italy). Ten patients had isolated GHD, and 8 had MPHD. All patients were receiving appropriate hormone replacement therapy. Twenty-four prepubertal short normal children (11 boys and 13 girls, aged 5.9–13 yr, body weight within ±10% of ideal weight) served as controls. All control children were referred to our institutions for short stature and were found to have constitutional growth delay or familial short stature. Data for these children have been previously published (10). The main clinical and hormonal findings of the patients at the time of diagnosis are summarized in Table 1.

MRI studies were performed with a spin-echo technique and the use of a 1.5-T superconductive unit (Magnetom, Siemens, Iselin, NJ) before and after gadopentetate-dimeglumine. Sagittal and coronal T1-weighted images [400/15 (repetition time, milliseconds per echo), three excitations, 3-mm thick sections, 256 × 256 pixels, and 20-cm field of view] were obtained for all patients (7). MRI revealed an ectopic posterior pituitary at the infundibular recess in all patients. A residual vascular component of the pituitary stalk was visualized in 8 patients with isolated GHD (group 1), whereas MRI showed the absence of the pituitary stalk (vascular and neural components) in the remaining 10 patients (group 2), of whom 8 had MPHD and 2 had isolated GHD. Mean serum insulin-like growth factor I concentrations was 48.5 ± 10.3 and 66.8 ± 9.0 $\mu g/L$ in groups 1 and 2, respectively (normal age- related values in our laboratory: 1–3 yr, 30–120 $\mu g/L$; 3–6 yr, 35–145 $\mu g/L$; 6–9 yr, 75–195 $\mu g/L$; 9–12 yr, 95–320 $\mu g/L$; 12–15 yr, 160–340 $\mu g/L$; 15–21 yr, 210–520 $\mu g/L$).

All subjects were tested with Hex (prepared and supplied by Europeptides, Argenteuil, France) at a dose of 2 $\mu g/kg$, iv. Blood samples were drawn from an indwelling catheter inserted in an antecubital vein 15 min and immediately before injection of the peptide and 15, 30, 45, 60, 90, and 120 min after injection. All experiments started between 0800–0900 h after the patients fasted overnight. In the patients, GH treatment was discontinued for 3 weeks before the test sessions. The time elapsed between the diagnosis and the time of these studies was 2.8 ± 1.0 yr (range, 0–11.2 yr).

GH was measured by fluoroimmunoassay using a commercial kit (AutoDELFIA hGH, EG&G, Wallac, Oy, Finland). The intra- and interassay coefficients of variations were 5.1% and 2.5%, respectively at 0.430 mU/L, 2.7% and 2.1% at 5.08 mU/L, and 2.2% and 1.4% at 21.1 mU/L. Cross-reactivity was less than 0.001% for PRL and human placental lactogen. TSH, T_3, T_4, FT_4, PRL, ACTH, cortisol, and FSH/LH were measured using commercially available RIAs. Statistical analysis of the results was carried out using the Wilcoxon test for paired data and the Mann-Whitney U test to compare groups; correlations between GH peaks after GHRH and Hex administration were performed by regression analysis. All values are given as the mean ± SEM.

Results

None of the subjects experienced adverse side-effects after Hex administration. In the short normal children, the mean peak GH response to GHRH (24.8 ± 4.4 $\mu g/L$) was significantly lower than that observed after Hex administration (48.1 ± 4.9 $\mu g/L$; $P < 0.0001$). In the GHD patients of group 2 the mean peak GH responses to GHRH (1.4 ± 0.3 $\mu g/L$) and Hex (0.9 ± 0.3 $\mu g/L$) were similar and markedly low. In the GHD patients of group 1, the GH responses to GHRH (8.7 ± 1.3 $\mu g/L$) and Hex (7.0 ± 1.3 $\mu g/L$) were also similar, but significantly higher that those observed in group 2 ($P < 0.0001$). A comparison between the GH responses to GHRH and Hex in the three groups of subjects is shown in Fig. 1. A clear-cut difference in the GH responses to GHRH and Hex was evident between both groups of patients and the control group. In the whole group of patients, a significant correlation was found between the GH peaks after Hex and those after GHRH treatment ($r = 0.746$; $P < 0.0001$). The peak GH responses to Hex in the individual patients are shown in Table 1.

In the patients with isolated GHD, Hex caused significant increases in serum ACTH (from 20.6 ± 2.1 to 32.9 ± 4.1 pg/mL; $P < 0.02$) and PRL (from 7.6 ± 1.3 to 8.9 ± 1.44 ng/mL; $P < 0.05$) concentrations.

Discussion

MRI has greatly improved the diagnosis of disorders affecting the hypothalamic-pituitary area in patients with idiopathic hypopituitarism. More recently, fast framing MRI

TABLE 1. Clinical, hormonal, and MRI findings and peak GH response to Hex in the patients studied

Case no./sex	Age (yr)	Ht (SD score)	IGF-I ($\mu g/L$)	GH peak ($\mu g/L$)				Associated hormone deficiencies	MRI findings
				Arginine	Insulin	GHRH	Hex		
1/M	2.5	−2.4	22	1.8	1.5	8.9	13.2		VPS
2/M	2.8	−2.7	18	1.9	1.6	7.8	7.5		VPS
3/F	5	−3.1	27	1.8	1.8	9.7	11		VPS
4/F	7	−3.9	47	1.6	2.2	11.4	6.8		VPS
5/F	2.9	−2.7	32	2.1	1.9	1.7	1.3	TSH/ACTH	PSA
6/M	5	−3.2	44	1.6	1.9	1.1	1.5	TSH/ACTH	PSA
7/F	6.30	−4.2	63	2.3	1.5	2.1	1.8	TSH/ACTH	PSA
8/F	11.3	−4.5	119	1.8	1.7	1	1.7	TSH/ACTH	PSA
9/F	7.7	−2.8	79	3.1	1.8	11.7	10		VPS
10/M	9.3	−3.5	65	2.4	1.8	1.2	0.1	TSH/ACTH/FSH-LH	PSA
11/M	8.3	−2.7	102	1.7	1.1	6.4	10.2		VPS
12/M	8.9	−3.6	103	1.5	1.6	5.8	2.8		PSA
13/M	9.8	−4.4	43	1.7	2.1	3	0.4	TSH/ACTH	PSA
14/M	13.20	−5.3	89	0.9	1.4	0.3	0.1	TSH/ACTH/FSH-LH	PSA
15/M	3.8	−2.6	47	1.5	1.5	1.9	0.2		PSA
16/M	3.7	−4	63	0.5	1	0.7	0.4	TSH/ACTH	PSA
17/M	2.40	−1.6	51	0.9	0.5	6.4	4.2		VPS
18/F	6.9	−4.4	42	1.7	0.5	5.4	4.7		VPS

VPS, Vascular pituitary stalk; PSA, pituitary stalk agenesis.

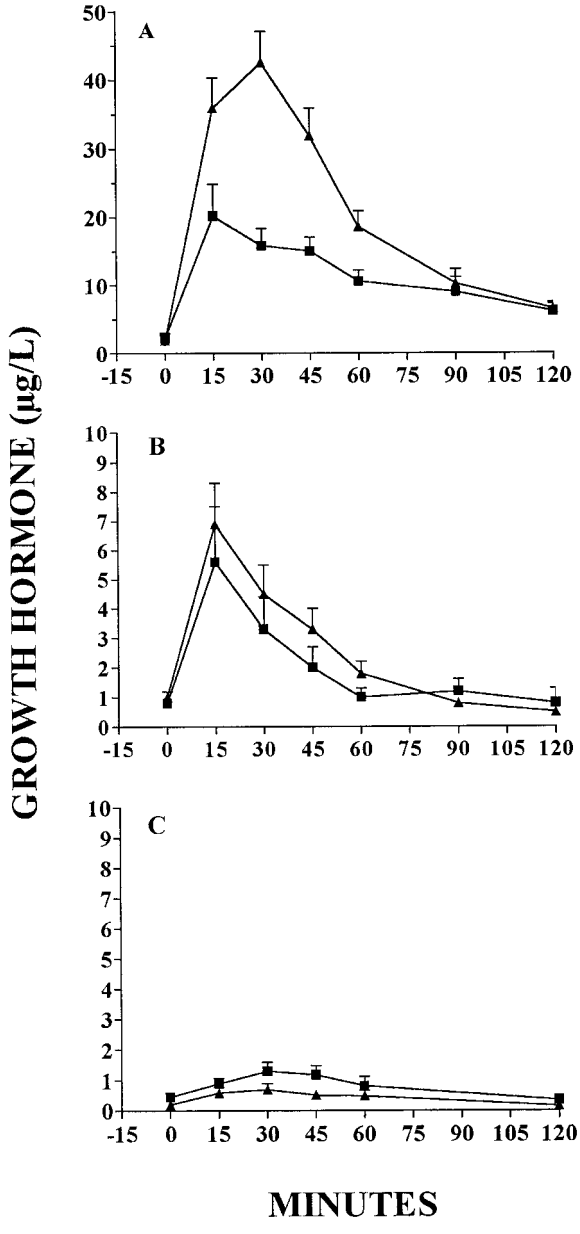

FIG. 1. Mean (±SEM) serum GH responses to GHRH (■; 1 μg/kg, iv) and Hex (▲; 2 μg/kg, iv) in 24 short normal children (A), the patients with GHD in group 1 (B), and the patients with GHD in group 2 (C).

has provided new information on the blood supply of the hypothalamus and the pituitary in normal subjects and patients with pituitary diseases (10). The use of this imaging technique has allowed identification in patients with GHD of residual vascular components of the pituitary stalk not recognized by conventional MRI. Interestingly, a GH response to GHRH has been demonstrated in the great majority of GHD patients with a residual vascular connection, as opposed to a lack of response in all patients with unidentifiable pituitary stalk (8). In this study, we have confirmed that Hex is unable to stimulate GH secretion in patients with hypothalamic-pituitary disconnection, *i.e.* those in whom the pituitary stalk was not visible after contrast enhancement. In addition, we have also shown that Hex can stimulate GH secretion in patients with residual vascular component of the pituitary stalk, although to a lesser extent than in normal subjects. We have previously observed a clear-cut, albeit low, GH response to Hex in some patients in whom the pituitary stalk was not visualized by conventional MRI and concluded that these low responses could be due to a direct somatotroph stimulation by the peptide (5). In light of the present findings it might be argued that the ability of Hex to elicit a GH response in those patients could have been related to the presence of a residual vascular connection.

A specific receptor for GHRPs has been cloned (2). Although these receptors are found in both the hypothalamus and the pituitary, several data suggest that the action of GHRPs is exerted mainly on the hypothalamus. In fact, GHRPs can stimulate GH secretion by pituitary cells *in vitro* (11), but their effectiveness is far greater when the experiments are carried out on hypothalamic-pituitary incubates (11). The effects of Hex on GH synthesis (12) and of GHRP-6 on GH release (13) are markedly reduced in rats and sheep after hypothalamic-pituitary disconnection. Moreover, the GH responses to Hex and GHRP-6 are absent or severely blunted in patients with hypothalamic-pituitary disconnection of different etiology (Refs. 5, 6, and 14 and this study). In normal subjects, the GH responses to Hex (9) and GHRP-6 (6, 14) are consistently higher than that elicited by a maximal dose of GHRH, whereas in adults with intracranial lesions (9, 14), the GH response to GHRP is lower than or similar to that observed after GHRH treatment. Also, in this study in the patients with GHD and residual vascular connection, the mean GH response to Hex was similar to that after GHRH, suggesting that Hex normally may act by potentiating the action of GHRH. The enhancing effect of GHRPs on GHRH-induced GH secretion *in vivo* has been well documented (1). Interestingly, this synergistic effect is absent in the patients with hypothalamic-pituitary disconnection (14). In rats, passive immunization against GHRH significantly reduces Hex stimulation of GH secretion (15). Furthermore, in animals with transection of the pituitary stalk, the GH-releasing activity of a nonpeptydil GH secretagogue is reduced, and it is restored to normal by administration of GHRH (16). A recent study has also shown that previous treatment with a GHRH antagonist eliminates most of the GH response to GHRP-6 in man (17). Taken together, these observations indicate that the presence of endogenous GHRH is essential for GHRPs to express their full GH-releasing activity. Thus, although the absent GH response to Hex observed in the patients not responsive to GHRH may reflect either the absence of functioning somatotrophs and/or the absence of endogenous GHRH, the observation of a sizable GH response to both GHRH and Hex in the patients of group 1 might indicate that the action of the latter is mediated by modulation of endogenous GHRH action.

In the patients of this study, Hex was able to stimulate GH secretion only in patients responsive to GHRH. Recent studies have shown that pretreatment of neonatal rats with an anti-GHRH serum induces permanent impairment of the somatotroph function (18), suggesting a crucial role for GHRH in somatotroph development and function. Thus, the absence of GH responses to either GHRH or Hex in our patients may reflect the absence of functioning pituitary so-

matotrophs, a hypothesis put forward by other investigators (14). Alternatively, even a normal number of pituitary somatotrophs chronically deprived by endogenous GHRH may need to be primed to normally synthesize and release GH (19).

In conclusion, we have confirmed that the integrity of the hypothalamic pituitary connections is essential for Hex to express its full GH-releasing activity and have shown that Hex is able to stimulate GH secretion in patients with GHD but with a residual vascular component of the pituitary stalk.

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