Mechanisms Underlying the Negative Growth Hormone (GH) Autofeedback on the GH-Releasing Effect of Hexarelin in Man

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The growth hormone (GH) response to GH-releasing hormone (GHRH) is strongly inhibited by previous administration of recombinant human GH (rhGH), likely as a consequence of a somatostatin-mediated GH negative autofeedback. Hexarelin (HEX), a synthetic hexapeptide belonging to the GH-releasing peptide (GHRP) family, possesses a GH-releasing activity greater than that of GHRH both in animals and in man. The mechanism of action of GHRPs is yet to be completely clarified, although concomitant actions at the pituitary and hypothalamic level have been hypothesized. To further clarify the mechanisms of action underlying the GH-releasing activity of HEX, in six normal young volunteers we studied the effects of rhGH (2 U intravenously [IV]) on the GH response either to GHRH (2 μg/kg IV) or to HEX (2 μg/kg IV) alone or combined with GHRH and/or pyridostigmine ([PD], 120 mg orally). The GH-releasing effect of HEX was higher than that of GHRH (area under the curve [AUC], 2,200.8 ± 256.9 *v* 792.2 ± 117.6 μg/L/h, $P < .001$), whereas combined administration of the two substances induced a true synergistic effect, with GH release after HEX plus GHRH (4,259.2 ± 308.0 μg/L/h) being higher ($P < .02$) than the arithmetic sum of the GH increases induced by each compound separately administered. After rhGH administration, the GH-releasing effect of HEX was blunted (1,468.9 ± 193.7 μg/L/h, $P < .04$; inhibition of 32.1%), whereas that of GHRH was nearly abolished (102.0 ± 7.8 μg/L/h, $P < .02$; inhibition of 86.1%). The GH response to combined administration of HEX and GHRH was also blunted by the previous rhGH bolus (3,070.6 ± 481.8 μg/L/h, $P < .02$; inhibition of 26.7%). PD did not modify the GH-releasing effect of HEX either alone (2,456.8 ± 317.5 μg/L/h) or combined with GHRH (4,009.1 ± 360.8 μg/L/h). rhGH was again able to blunt the GH response to HEX combined with PD (1,619.3 ± 237.9 μg/L/h, $P < .02$), but failed to modify the GH response to HEX combined with GHRH and PD (4,548.4 ± 698.0 μg/L/h). In conclusion, these results demonstrate that rhGH administration only blunts the GH-releasing activity of HEX, but abolishes that of GHRH. The blunting effect of rhGH on the GH response to HEX is probably mediated by a concomitant reduction in the activity of GHRH-secreting neurons and an increase of somatostatinergic tone.

NEURAL CONTROL of growth hormone (GH) secretion is mainly exerted by the tight interplay between GH-releasing hormone (GHRH), which stimulates GH synthesis and release, and somatostatin, which inhibits GH secretion. Besides hypophysiotropic neurohormones, several neurotransmitters, as well as insulin-like growth factors (IGFs) and metabolic fuels, have important roles in modulating somatotrope secretion.[1,2] Also, GH itself is able to directly and indirectly modulate its own secretion, and this negative GH-autofeedback control involves many of the above-mentioned factors.[1,2] In fact, GH increases IGF-I and free fatty acid levels, which in turn inhibit GH secretion acting both at the pituitary and the hypothalamic level.[2] However, the influence of IGF-I and free fatty acids does not account for the inhibitory effect shown by the acute administration of GH or GHRH on the GH response to GHRH both in animals and in man.[3-6]

Somatostatin antiserum in the rat[6-8] and substances inhibiting hypothalamic somatostatin release in man[3,9-13] are able to completely restore the GH response to GHRH when inhibited by a previous recombinant human GH (rhGH) or GHRH bolus. Thus, a somatostatin-mediated mechanism accounts for this short-loop negative GH autofeedback.

A new class of small synthetic GH-releasing peptides (GHRPs) has been discovered.[14-17] These compounds have no primary sequence homology with GHRH,[18] and in vivo they release more GH than the same dose of GHRH.[15,17,19-22] The mechanisms underlying their potent GH-releasing effect is still unclear, although concomitant actions at the pituitary[20,23-27] and hypothalamic[20,28-32] levels have been hypothesized, also based on evidence that GHRPs have specific binding sites at these levels.[33-35] At present, there are data favoring the hypothesis that GHRPs could act by counteracting somatostatinergic activity both at the pituitary and the hypothalamic level and/or, at least partially, via a GHRH-mediated mechanism.[20,23-32] However, the possibility that GHRPs act via an unknown hypothalamic factor is still open.[20]

We demonstrated that Hexarelin ([HEX], His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2), a new synthetic hexapeptide,[16] has a strong GH-releasing effect in man after intravenous (IV), subcutaneous, intranasal, and oral administration.[17] Like all other GHRPs,[15,19,21] HEX releases more GH than GHRH, and their coadministration has a synergistic effect.[17,22] In contrast to GHRH, the GH-releasing effect of HEX is only blunted by exogenous somatostatin and by substances stimulating hypothalamic somatostatin release, such as pirenzepine and glucose, as well as by free fatty acids, which probably also act at the pituitary level.[22,36] Together, all these data suggest that HEX and other GHRPs are likely to act by a mechanism that is at least partly different from that of GHRH, and that the HEX activity is partially refractory to inhibition by somatostatin. On the other hand, it has recently been reported that exogenous rhGH blunts

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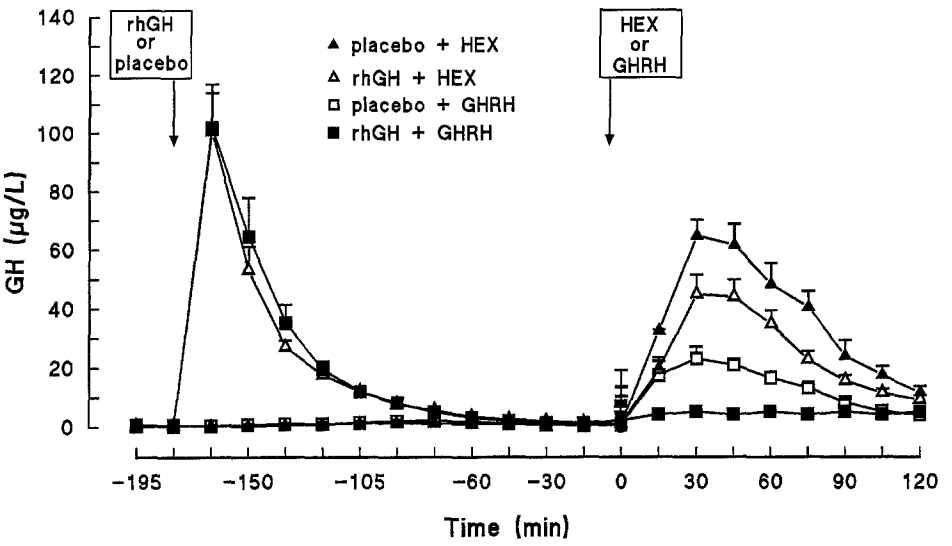

Fig 1. Mean ± SEM GH response curves after HEX or GHRH (both given at 0 minutes) preceded by placebo or rhGH (both given at −180 minutes) in 6 young volunteers.

the HEX-induced GH increase in man,[37] although this finding was not confirmed by others.[38]

To further clarify the mechanisms underlying the activity of GHRPs and their sensitivity to the negative GH autofeedback mechanism in man, we studied the effect of rhGH administration on the GH response to HEX alone or combined with GHRH and/or pyridostigmine (PD), a cholinergic agent that probably inhibits hypothalamic somatostatin release.[1,2]

SUBJECTS AND METHODS

Peptides and Drugs

HEX was synthesized by Bachem (Bubendorf, Switzerland). Vials containing 100 μg lyophilized HEX were prepared by Europeptides (Argenteuil, France). Vials containing 50 μg lyophilized GHRH-29 (Geref) were purchased from Serono (Milan, Italy). Vials containing 4 UI lyophilized rhGH (Genotropin) were purchased from Pharmacia (Stockholm, Sweden). Tablets containing 60 mg PD (Mestinon) were purchased from Hoffman-La Roche (Milan, Italy).

Study Design

Six healthy men aged 24 to 28 years volunteered to participate in the study. The subjects had a mean body weight of 72 ± 12 kg, and all were within 15% of their ideal body weight. Each subject provided written informed consent before taking part in the study. The study was approved by the Ethics Committee of our Department. The tests were performed in the morning after an overnight fast, starting at 8:30 to 9:00 AM, 30 minutes after forearm insertion of a venous catheter kept patent by slow infusion of isotonic saline. Blood samples were taken basally at −195 and −180 minutes, and then every 15 minutes for 5 hours. All subjects underwent the following tests in random order and at least 3 days apart: (1) placebo + HEX (saline 2 mL IV as a bolus at −180 minutes + HEX 2 μg/kg IV as a bolus at 0 minutes; (2) placebo + GHRH (GHRH-29 2 μg/kg IV as a bolus at 0 minutes); (3) placebo + HEX + GHRH; (4) placebo + HEX + PD (120 mg orally at −60 minutes); (5) placebo + HEX + GHRH + PD; (6) rhGH (2 UI IV as a bolus at −180 minutes) + HEX; (7) rhGH + GHRH; (8) rhGH + HEX + GHRH; (9) rhGH + HEX + PD; and (10) rhGH + HEX + GHRH + PD.

Serum GH levels were measured at each time point in duplicate by immunoradiometric assay (HGH-CTK IRMA; Sorin, Saluggia, Italy). All samples from an individual subject were analyzed together. The sensitivity of the assay was 0.15 μg/L. Interassay coefficients of variation were 4.9% and 6.5% for mean ± SD GH values of 40.8 ± 2.0 and 2.8 ± 0.18 μg/L, and the intraassay coefficients of variation were 1.5% and 2.9% for GH values of 3.0 ± 0.05 and 31.8 ± 0.95 μg/L.

GH secretory responses were expressed either as absolute values (micrograms per liter) or as areas under the curve ([AUC] micrograms per liter per hour) calculated by trapezoidal integration. Statistical analysis of the data was made using a nonparametric ANOVA (Kruskal-Wallis test) and the Wilcoxon test. Results are expressed as the mean ± SEM.

RESULTS

Mean basal GH levels (−195 and −180 minutes) ranged from 0.1 to 0.6 μg/L. Administration of rhGH elicited a marked GH increase overlapping in all five tests with a peak at −165 minutes (peak and AUC related to tests 6 to 10 ranged from 90.4 ± 19.0 to 103.5 ± 12.5 μg/L and from 1,040.4 ± 160.9 to 1,264.4 ± 227.7 μg/L/h, respectively). At 0 minutes, serum GH levels were again similar to the basal values (Table 1).

After placebo administration, the GH-releasing effect of HEX was higher than that of GHRH (AUC, 2,200.8 ± 256.9 *v* 792.2 ± 117.6 μg/L/h, $P < .001$). On the other hand, combined administration of HEX + GHRH induced a true synergistic effect on GH secretion, with GH release after HEX + GHRH (4,259.2 ± 308.0 μg/L/h) being higher ($P < .02$) than the arithmetic sum of GH increases induced by each compound separately administered (Figs 1 and 2).

After administration of rhGH, the GH-releasing effect of HEX was blunted (1,468.9 ± 193.7 μg/L/h, $P < .04$; inhibition of 32.1%), while that of GHRH was nearly abolished (102.0 ± 7.8 μg/L/h, $P < .02$; inhibition of 86.1%). The GH response to combined administration of HEX + GHRH was also blunted by the previous rhGH bolus (3,070.6 ± 481.8 μg/L/h, $P < .02$; inhibition of 26.7%), with a persistent synergistic effect on GH secretion ($P < .01$). The GH response to HEX + GHRH after rhGH was not different from the response to HEX alone after placebo (Figs 1 and 2).

After placebo administration, PD did not modify the GH-

Table 1. GH Levels (μg/L, mean ± SEM) During the Various Tests

Test	Time (min)																				
	−195.0	−180.0	−165.0	−150.0	−135.0	−120.0	−105.0	−90.0	−75.0	−60.0	−45.0	−30.0	−15.0	0.0	15.0	30.0	45.0	60.0	75.0	90.0	105.0	120.0
1. Placebo + HEX	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.6 ± 0.3	32.3 ± 3.3	64.5 ± 5.4	61.3 ± 7.1	48.1 ± 7.1	40.2 ± 5.5	23.8 ± 5.3	17.3 ± 3.0	11.4 ± 2.2
2. Placebo + GHRH	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.2	0.5 ± 0.3	17.2 ± 3.7	22.9 ± 4.8	20.8 ± 4.0	16.4 ± 1.6	13.0 ± 1.9	8.1 ± 1.5	5.1 ± 0.9	4.0 ± 0.6
3. Placebo + HEX + GHRH	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.8 ± 0.6	0.7 ± 0.6	83.2 ± 7.7	98.4 ± 9.2	96.8 ± 8.1	88.2 ± 7.2	78.3 ± 5.2	59.0 ± 7.0	45.6 ± 6.4	36.1 ± 5.0
4. Placebo + HEX + PD	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	1.4 ± 0.4	50.6 ± 8.0	66.8 ± 8.6	62.4 ± 8.7	52.7 ± 7.3	39.6 ± 5.5	27.8 ± 4.3	20.4 ± 2.2	13.3 ± 1.5
5. Placebo + HEX + GHRH + PD	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.3	1.7 ± 1.5	1.0 ± 0.8	0.3 ± 0.1	0.8 ± 0.3	4.9 ± 2.9	68.3 ± 17.8	93.9 ± 12.8	87.3 ± 8.6	83.8 ± 7.7	75.3 ± 7.0	62.3 ± 5.5	44.8 ± 7.6	32.9 ± 7.2
6. rhGH + HEX	0.3 ± 0.1	0.3 ± 0.1	100.4 ± 13.4	53.0 ± 7.9	26.9 ± 2.2	17.4 ± 1.5	11.8 ± 0.7	7.6 ± 0.9	5.5 ± 0.6	3.3 ± 0.4	2.3 ± 0.2	1.7 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	19.0 ± 4.2	44.7 ± 6.5	43.7 ± 5.9	34.5 ± 4.7	22.5 ± 2.8	15.4 ± 1.9	11.2 ± 1.4	9.0 ± 1.4
7. rhGH + GHRH	0.1 ± 0.0	0.2 ± 0.1	101.8 ± 20.3	64.4 ± 13.3	35.0 ± 6.1	19.3 ± 2.7	11.8 ± 1.7	8.1 ± 1.6	4.8 ± 1.1	2.9 ± 0.5	1.8 ± 0.3	1.3 ± 0.3	1.1 ± 0.2	0.9 ± 0.1	1.2 ± 0.1	1.7 ± 0.2	2.3 ± 0.3	2.4 ± 0.3	2.1 ± 0.2	1.6 ± 0.1	1.4 ± 0.2	1.0 ± 0.0
8. rhGH + HEX + GHRH	0.4 ± 0.2	0.6 ± 0.3	90.4 ± 19.0	49.1 ± 13.3	22.6 ± 2.4	17.4 ± 2.6	11.4 ± 2.4	7.0 ± 2.0	3.7 ± 1.1	2.3 ± 0.7	1.5 ± 0.4	1.2 ± 0.3	0.9 ± 0.1	0.6 ± 0.1	60.3 ± 10.1	68.3 ± 13.5	66.9 ± 7.9	72.5 ± 10.3	57.3 ± 8.8	42.2 ± 7.5	30.2 ± 6.7	22.9 ± 5.0
9. rhGH + HEX + PD	0.2 ± 0.1	0.3 ± 0.1	103.5 ± 12.5	51.7 ± 7.6	28.2 ± 3.4	18.9 ± 2.3	13.7 ± 1.8	8.8 ± 1.8	5.9 ± 1.1	3.6 ± 0.8	2.7 ± 0.5	2.1 ± 0.6	1.5 ± 0.4	1.1 ± 0.2	38.7 ± 13.8	44.2 ± 8.1	42.7 ± 6.2	30.9 ± 3.9	22.5 ± 1.8	17.7 ± 1.0	13.3 ± 0.7	10.8 ± 0.8
10. rhGH + HEX + GHRH + PD	0.2 ± 0.0	0.2 ± 0.1	96.3 ± 17.7	45.4 ± 12.2	24.5 ± 5.8	16.6 ± 5.5	11.3 ± 3.6	8.2 ± 2.5	5.5 ± 1.4	4.6 ± 1.4	3.0 ± 1.0	2.1 ± 0.8	1.4 ± 0.5	1.1 ± 0.4	88.5 ± 16.4	103.6 ± 17.9	105.0 ± 15.0	99.6 ± 13.1	78.8 ± 9.1	63.1 ± 10.3	48.5 ± 9.5	37.6 ± 9.0

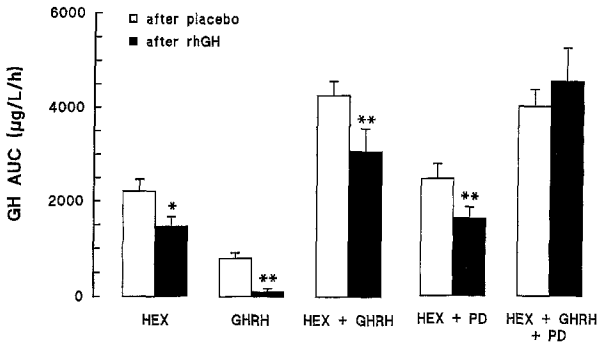

Fig 2. Mean ± SEM GH AUCs after HEX and/or GHRH alone and combined with PD, preceded by placebo or rhGH, in 6 young volunteers. *$P < .04$, **$P < .02$: *v* placebo.

releasing effect of HEX alone (2,456.8 ± 317.5 µg/L/h) or HEX + GHRH (4,009.1 ± 360.8 µg/L/h). On the other hand, rhGH was able to blunt the GH response to HEX + PD (1,619.3 ± 237.9 µg/L/h, $P < .02$), but failed to modify the GH response to HEX + GHRH + PD (4,548.4 ± 698.0 µg/L/h) (Fig 2).

Side Effects

Transient facial flushing was observed in all subjects after either GHRH or HEX administration. HEX also induced mild sleepiness. PD elicited mild abdominal pain and muscular fasciculations in five subjects, but no medication was required. Administration of rhGH produced no side effects.

DISCUSSION

Our results demonstrate that in man, in contrast to GHRH, the GH-releasing effect of HEX administered alone or in combination with GHRH is partially refractory to inhibition by rhGH. PD, which inhibits hypothalamic somatostatin release,[1,2] is able to counteract the blunting effect of rhGH on the GH response to HEX + GHRH, but not to the hexapeptide alone. On the other hand, PD is unable to enhance the somatotrope responsiveness to HEX either alone or combined with GHRH. There is evidence indicating that GHRPs, including HEX, act via a common action, but the precise mechanisms underlying their effects are still unclear. Animal and human data indicate that these peptides act concomitantly at the pituitary and the hypothalamic level,[14,36,39] where they have specific non-GHRH, non-somatostatin, non-opioid receptors.[33-35]

On the other hand, it has been accepted that both in animals and in man the negative GH autofeedback mechanism is mediated by the release of hypothalamic somatostatin. In fact, as with somatostatin antiserum in the rat,[6-8] in man neuroactive substances that probably inhibit hypothalamic somatostatin release are able to fully restore the GH response to GHRH abolished by a previous rhGH or GHRH bolus.[3,9-13] On the other hand, in the rat GH has been reported to exert its negative autofeedback mechanism also via reduction of the activity of GHRH-secreting neurons.[40-42]

Our present data showing that the GH response to HEX is only blunted by rhGH partially agree with the recent data from Massoud et al,[37] who showed a blunting effect of rhGH on the GH response to both HEX and GHRH. These findings all agree with others showing that the activity of HEX is partially refractory to other inhibitory influences known to abolish the GH-releasing effect of GHRH.[22,36] Based on the assumption that the negative GH autofeedback takes place via a somatostatin-mediated mechanism,[3,6-13] our results suggest that HEX partially counteracts the rhGH-induced somatostatinergic hyperactivity. Since GHRPs do not seem to modulate hypothalamic somatostatin release,[20,29-31] HEX could antagonize the inhibitory effect of somatostatin on somatotrope cells, in agreement with other data in animals.[25,27] In fact, in rats, GHRPs release GH via a depolarization of the somatotrope cell membrane and an increase of intracellular Ca^{2+}, while the opposite effects are induced by somatostatin.[25,27] It is noteworthy that in man the GH-releasing effect of HEX is partly refractory not only to inhibition by substances stimulating hypothalamic somatostatin release, but even to exogenous somatostatin.[22,36] On the other hand, an antagonizing effect of GHRPs even on the inhibitory activity of somatostatin on GHRH-secreting neurons has recently been reported.[32]

Our findings showing that GHRH has a true synergistic effect with HEX even after rhGH strengthen the hypothesis that HEX acts differently from GHRH.[19,22,25,27] On the other hand, our findings showing that GHRH partially counteracts the blunting effect of rhGH on the GH response to HEX agree with the data in animals indicating that the negative GH autofeedback also takes place via inhibition of the activity of GHRH-secreting neurons,[40-42] and that the GH-releasing activity of GHRPs is partially dependent on endogenous GHRH.[20,28-31] However, it remains that the GH response to HEX + GHRH is blunted by rhGH.

Interestingly, our data show that the GH response to HEX + GHRH, when inhibited by rhGH, is fully restored by PD. This is noteworthy, considering that PD is unable to modify the somatotrope responsiveness to HEX alone and preceded by rhGH, as well as that to HEX + GHRH after placebo. Based on the assumption that PD acts via inhibition of hypothalamic somatostatin release,[1] the evidence that the negative feedback effect of rhGH is fully antagonized only by coadministration of HEX, GHRH, and PD indicates that all these substances act by different mechanisms, and suggests that the increase of somatostatin and the decrease of GHRH secretion are induced by rhGH. In this context, it has also been hypothesized that the action of GHRPs could be mediated by an unknown factor[20] that may be involved in the negative GH autofeedback mechanism.

In conclusion, the present data indicate that HEX is partially refractory to the inhibitory effect of acute rhGH administration, which, on the contrary, abolishes the somatotrope responsiveness to GHRH. The mechanisms underlying inhibition of the GH-releasing activity of HEX by rhGH probably include the modulation of hypothalamic GHRH- and somatostatin-secreting neurons.

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