Growth Hormone (GH) Response to GH-Releasing Peptide-6 in Patients With Insulin-Dependent Diabetes Mellitus

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In insulin-dependent diabetes mellitus (IDDM), inappropriate growth hormone (GH) responses to several stimuli, including GH-releasing hormone (GHRH), have been described. A decreased hypothalamic somatostatinergic tone is one of the most likely explanations for these findings. His-DTrp-Ala-Trp-DPhe-Lys-NH_2 (GH-releasing peptide-6 [GHRP-6]) is a synthetic hexapeptide that stimulates GH release in vitro and in vivo. The mechanism of action of GHRP-6 is unknown, but it probably does not inhibit hypothalamic somatostatin secretion. Also, GHRH and GHRP-6 apparently activate different intracellular pathways to release GH. The aim of this study was to evaluate whether there is a differential effect of IDDM on GHRP-6– and GHRH-induced GH secretion. Six patients with IDDM and seven control subjects were studied. Each subject received GHRP-6 (1 μg/kg intravenously [IV]), GHRH (100 μg IV), and GHRP-6 + GHRH on 3 separate days. GH peak values (mean ± SE in micrograms per liter) were similar in controls and diabetics after GHRH (22.5 ± 7.8 *v* 24.0 ± 9.7) and after GHRP-6 (20.5 ± 5.3 *v* 24.4 ± 6.3). The association of GHRP-6 and GHRH induced a significantly higher GH release than administration of the isolated peptides in both groups. The synergistic GH response to combined administration of GHRP-6 and GHRH was not different in controls (70.5 ± 20.0) and diabetics (119.0 ± 22.2). In summary, the effectiveness of GHRP-6 in IDDM could reinforce the evidence that this peptide probably does not release GH through a decrease in hypothalamic somatostatin secretion. Moreover, our data suggest that both GHRH and GHRP-6 releasing mechanisms are unaltered in IDDM.
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IN PATIENTS WITH insulin-dependent diabetes mellitus (IDDM), basal growth hormone (GH) levels are either normal or elevated depending on glycemic control, and inappropriate or exaggerated GH responses to provocative stimuli are found.[1,2] In these patients both normal[2,3] and increased[4] GH-releasing hormone (GHRH)-stimulated GH release have been reported. Insulin-like growth factor-I (IGF-I) levels are either normal[5] or reduced,[1,6,7] and there is evidence of a reversible defect in IGF-I generation in these patients.[2,7] In addition, patients with IDDM have increased IGF binding protein-1 levels,[7] which are inversely related to insulin.[1] This could result in decreased IGF-I feedback on the hypothalamus and pituitary, leading to increased GH output.[1] There are several central mechanisms to explain the altered GH secretion in IDDM. A decrease in hypothalamic somatostatin secretion is, at least in part, the most likely explanation at present.[2,6,8-10] Reduced pituitary sensitivity to somatostatin, increased hypothalamic secretion of GHRH, or enhanced pituitary responsiveness to GHRH could also have a role.[1,3,6]

GH-releasing peptide-6 (GHRP-6) is a synthetic hexapeptide that specifically stimulates GH release in a dose-related fashion in several species, including man.[11-13] No gender- and age-related differences in GH response to GHRP-6 have been found.[14,15] This hexapeptide also has a potent GH-releasing activity in vitro.[16-18] The effects of the peptide are exerted through specific GHRP-6 receptors, which have been characterized in both the hypothalamus and the pituitary, and are different from those of GHRH, somatostatin, and opioids.[19] Several similar GH-releasing compounds have been recently described, including hexarelin and L-692,429.[13,20-22] When GHRP-6 is administered with GHRH, a synergistic effect on GH secretion is observed in normal men,[12,13,23] different from most results obtained in vitro.[16] This suggests that this peptide acts at both the pituitary and the hypothalamic level.[12,17,23-25] The mechanism of action of GHRP-6 is unknown. It may increase hypothalamic GHRH release and/or cause secretion of an unknown hypothalamic factor (U-factor), which would act synergistically with GHRH, stimulating GH secretion.[12,13,17] GHRP-6 probably does not decrease hypothalamic somatostatin secretion, although it may inhibit the effects of somatostatin on GH release.[26-29] At the pituitary level, it could act as a functional somatostatin antagonist.[27,28,30,31] At the intracellular level, GHRP-6 and GHRH release GH through different transduction pathways.[16,18]

Therefore, the aim of this study was to evaluate whether there is a differential effect of IDDM on GHRP-6– and GHRH-induced GH secretion.

SUBJECTS AND METHODS

Subjects

Six patients (three men and three women) with IDDM were studied. The mean age was 36.5 ± 1.9 years (mean ± SE), and the mean body mass index (BMI) was 21.3 ± 0.9 kg/m^2. The duration of diabetes was 13.7 ± 2.6 years. Mean levels of hemoglobin A_{1c} (HbA_{1c}) at the time of evaluation were 7.7% ± 0.2% (normal, <5.3%). The patients were receiving one or two injections of insulin daily. None had clinical or laboratory evidence of nephropathy. No medications other than insulin were being used by the patients before and during the study period.

A second group of seven normal subjects (six men and one woman) were also studied. The mean age was 29.0 ± 1.4 years and mean BMI 22.3 ± 0.6 kg/m^2. None of the control subjects were taking any medication.

Study Protocol

The experimental protocol was approved by the Ethics Committee of Universidade Federal de São Paulo, Escola Paulista de Medicina. All

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subjects provided written consent to participate in the study. Each subject underwent three tests in random order separated by at least 7 days. The tests were performed after an overnight fast, and subjects remained recumbent throughout each test. The patients received no insulin on the morning of the study. At 8 AM, an indwelling catheter was placed in a forearm vein and kept patent by a slow 0.9% saline infusion. Forty-five minutes later, the tests were started. After the first blood sample (time 0), each subject received GHRH(1-29)NH_2 (Geref; Serono, Geneva, Switzerland) at a dose of 100 μg intravenously (IV), GHRP-6 (Peninsula Laboratories, Merseyside, UK) at a dose of 1 μg/kg IV, or a combination of 100 μg GHRH plus 1 μg/kg GHRP-6 IV. Blood samples were taken every 15 minutes until 120 minutes for subsequent GH determination. Blood glucose level was measured every 30 minutes. Basal levels of IGF-I were also determined.

Assays

Serum GH levels were measured in duplicate by a two-site monoclonal antibody immunofluorimetric assay.[32] Monoclonal antibodies were developed as previously described.[33] The sensitivity of the assay is 0.05 μg/L. Intraassay and interassay coefficients of variation were 7% and 9%, respectively. IGF-I was determined by a radioimmunoassay after acid-ethanol extraction (Nichols Institute, San Juan Capistrano, CA). Glucose level was measured by the glucose-oxidase method using a glucose analyzer (Beckman, Palo Alto, CA). HbA_{1c} was determined by an affinity chromatography method (Isolab, Akron, OH). All samples from each subject were analyzed in the same assay.

Statistical Analysis

Friedman's ANOVA was performed to compare GH levels in each group. The Mann-Whitney test was used for comparisons between different groups. The GH response to each test was also analyzed by the area under the curve (AUC), which was calculated by trapezoidal integration. The Spearman correlation coefficient was calculated when appropriate. Undetectable GH levels (<0.05 μg/L) were considered equal to 0.05 μg/L for statistical purposes. Results are reported as the mean ± SE. *P* values less than .05 were considered significant.

RESULTS

In control subjects, mean GH peak values after GHRH administration were 22.5 ± 7.8 μg/L, and did not differ significantly from those observed after GHRP-6 injection (20.5 ± 5.3 μg/L). AUCs were 1,615.5 ± 567.5 and 1,101.5 ± 344.5 μg/L · 120 min, respectively (NS). GH release after combined administration of GHRH plus GHRP-6 was greater than after GHRH or GHRP-6 alone (peak, 70.5 ± 20.0 μg/L; AUC, 4,558.5 ± 1,193 μg/L · 120 min; $P < .05$) (Fig 1).

In diabetic patients, GHRH induced a mean GH peak of 24.0 ± 9.7 μg/L, and this value was not different from that observed after GHRP-6 injection (24.4 ± 6.3 μg/L). There were also no significant differences between AUCs (1,308.0 ± 597.5 *v* 1,213.5 ± 464 μg/L · 120 min). Administration of GHRH together with GHRP-6 induced a significantly higher GH release than the isolated peptides, with a mean GH peak of 119.0 ± 22.2 μg/L and AUC of 6,912.5 ± 1,754.0 μg/L · 120 min (Fig 2).

When diabetic subjects were compared with the normal control group, no significant differences were found between basal GH levels. GH responses to GHRH and to GHRP-6 were similar in both groups. Combined administration of both peptides also induced a similar GH response in diabetic patients and control subjects, although there was a trend for higher values in the former group ($P = .073$; Fig 2).

No significant changes in glucose levels were observed throughout and between the tests. Mean glucose levels at time 0 of GHRH, GHRP-6, and GHRH + GHRP-6 tests were 18.4 ± 3.0, 14.6 ± 1.9, and 15.8 ± 1.6 mmol/L, respectively, and did not differ significantly. Mean IGF-I levels were 204.4 ± 25.5 μg/L in the control group and 154.2 ± 33.2 μg/L in the diabetics, and did not reach statistical significance. There were no significant differences between the mean age in both groups.

Transient facial flushing was observed in seven subjects after administration of GHRH alone (four controls and three diabet-

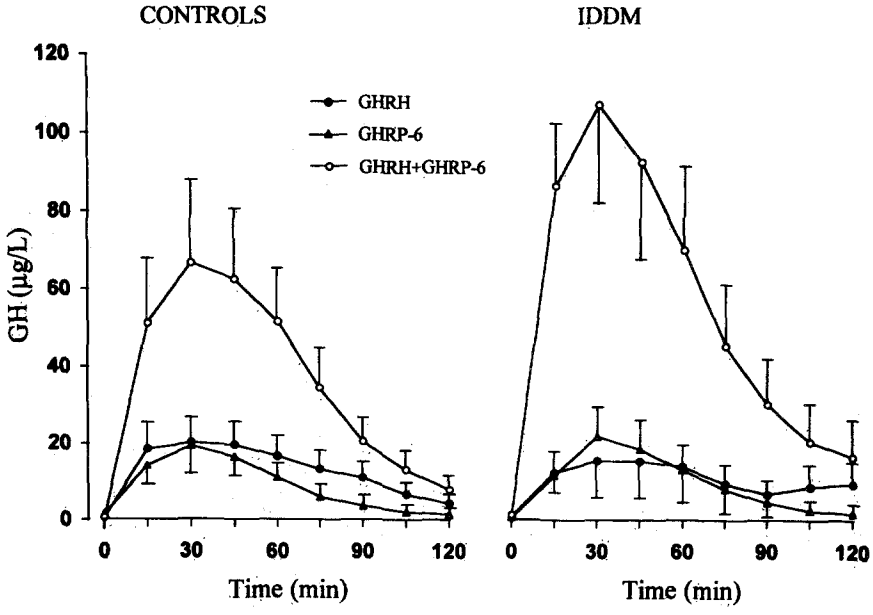

Fig 1. Mean plasma GH levels after administration of GHRH (100 μg IV), GHRP-6 (1 μg/kg IV), or GHRH + GHRP-6 in 7 control subjects and 6 patients with IDDM (mean ± SE).

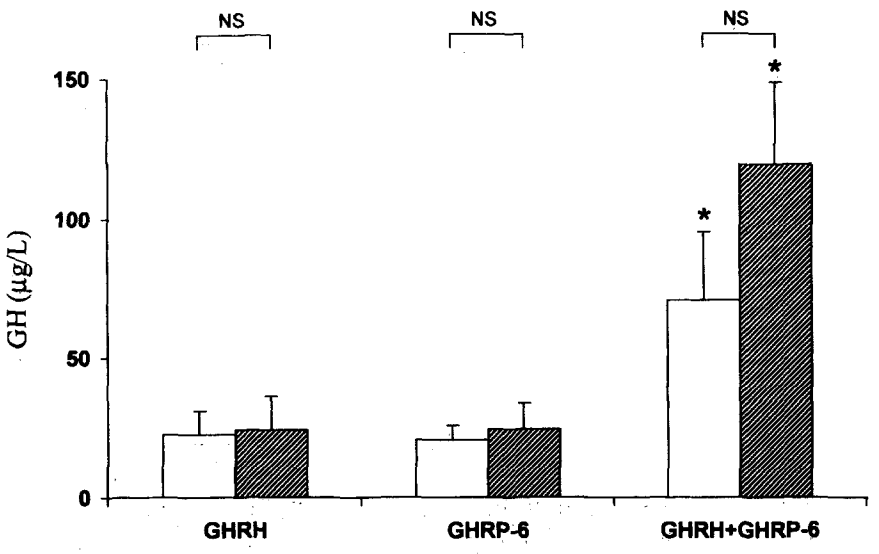

Fig 2. Mean GH peak levels after administration of GHRH, GHRP-6, or GHRH + GHRP-6 in (□) 7 control subjects and (■) 6 patients with IDDM (mean ± SE, *$P < .05$ v GHRH and GHRP-6).

ics) and in six subjects after GHRP-6 plus GHRH (four controls and two diabetics). Slight and transient nausea was noted in two control subjects after GHRP-6 injection.

DISCUSSION

Our results show that in normal subjects, similar GH release was observed after GHRH and GHRP-6 administration. Both equal and increased GH responses to GHRP-6 compared with GHRH have been previously described in normal subjects.[11,12,23]

In diabetic patients, basal GH levels were similar to those obtained in controls, which could reflect their glycemic control.[2] Although our sample sizes were small, in these patients GH release after GHRH was not different from that observed in normal subjects, confirming previous reports.[2] It has previously been shown that the same magnitude of GH responsiveness to GHRH is observed in both poorly controlled and well-controlled IDDM, suggesting that glycemic control does not cause major interferences with the pituitary responsiveness to GHRH.[2] However, a normal response in diabetes has been considered inappropriate by several groups, since the GH response to GHRH in normal subjects is blunted by hyperglycemia.[1,2,4,34] Interestingly, GH release after GHRP-6 administration to diabetic patients was not different from that observed in controls. Moreover, similar to normal subjects, GHRH- and GHRP-6–induced GH release did not differ significantly in patients with IDDM. This has been previously described in a preliminary report using GHRP-1 in diabetic patients.[35] The normal GH response to GHRP-6 in IDDM could eventually also be considered inappropriate, since it has been recently reported that oral glucose administration blunts the GH response to hexarelin, a GHRP-6 analog, in normal subjects.[36]

When GHRP-6 and GHRH were administered together, there was a synergistic GH release in control subjects, as demonstrated previously.[12,23] The GH response after combined administration of both peptides was higher than after the isolated peptides. In diabetic patients, the same pattern was observed. Moreover, there were no significant differences for the response to GHRH + GHRP-6 in diabetic and control subjects. Alster and Currie,[35] using GHRP-1 in association with GHRH also found a similar synergistic response in patients with diabetes and control subjects.

In patients with IDDM, there is an increase in the frequency of GH pulses and an elevation of interpulse GH concentrations.[6] These results and other data from the literature[2,8,9] have suggested that the deranged GH secretion observed in these patients is likely to be due, at least in part, to a reduced hypothalamic somatostatinergic tone. The effectiveness of GHRP-6 in this situation could reinforce the previous evidence that GHRP-6 probably does not release GH through a decrease in hypothalamic somatostatin secretion.[23,24,29,37,38]

Another hypothesis suggests an increase in hypothalamic GHRH secretion, which has been proposed as a possible mechanism of action of GHRP and related compounds.[24,26,39] Acute IV injection of GHRP stimulates neuronal activity and c-*fos* expression in the arcuate nucleus of rats, where GHRH is synthesized.[40] Moreover, hexarelin increases GHRH release into the hypophyseal portal blood of sheep.[24] However, an increase in hypothalamic GHRH secretion is unlikely to explain our results, since GHRH was administered at a maximal effective dose associated with GHRP-6. Other studies have also shown that the GH response to GHRP in humans and animals is probably not mediated by endogenous GHRH.[41-43]

In summary, we have shown that in IDDM, isolated or combined administration of GHRP-6 and GHRH induces GH release similar to that in normal subjects. If hypothalamic somatostatinergic tone is reduced in IDDM, the effectiveness of GHRP-6 in this situation could reinforce the evidence that GHRP-6 probably does not release GH through a decrease in hypothalamic somatostatin secretion. Moreover, our data suggest that both GHRH and GHRP-6 transduction mechanisms are preserved in IDDM. However, other studies are necessary to further clarify these hypotheses.

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