Effect of Combined Administration of Growth Hormone (GH)-Releasing Hormone, GH-Releasing Peptide-6, and Pyridostigmine in Normal and Obese Subjects

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Growth hormone (GH) secretion in response to all provocative stimuli is decreased in patients with obesity. Recently, we found that the combined administration of GH-releasing hormone (GHRH) and the hexapeptide GH-releasing peptide-6 (GHRP-6) induced a large increase in plasma GH levels. To gain further insight into the disrupted mechanism of GH regulation in obesity, we investigated whether the inhibition of somatostatinergic tone with pyridostigmine could further increase the GH response to combined administration of GHRH and GHRP-6. In normal subjects, administration of GHRH plus GHRP-6 induced a marked increase in plasma GH with a peak at 30 minutes (mean ± SEM, 76.7 ± 9.7 μg/L), which was similar to that obtained after pretreatment with pyridostigmine (74.7 ± 9.4 μg/L). In obese patients, combined administration of GHRH plus GHRP-6 induced a clear increase in GH secretion with a peak at 15 minutes of 42.2 ± 10.0 μg/L, which was also unaffected after pretreatment with pyridostigmine (38.4 ± 5.8 μg/L). The GH response was lower in obese patients than in controls as assessed by the area under the curve after administration of both GHRH plus GHRP-6 (1,846 ± 396 *v* 4,773 ± 653, $P < .01$) and pyridostigmine plus GHRH plus GHRP-6 (1,989 ± 372 *v* 5,098 ± 679, $P < .005$). In conclusion, these data suggest that GHRP-6 can behave as a functional somatostatin antagonist, and that somatotrope responsiveness to the combined administration of GHRH plus GHRP-6 is largely independent of somatostatinergic tone. Therefore, our findings in obese subjects of a relatively high GH response to GHRH plus GHRP-6, albeit low in comparison to that in normal subjects, with or without pyridostigmine suggest that the somatotrope cell in obesity has a considerable GH secretory capacity.

A SYNTHETIC HEXAPEPTIDE His-DTrp-Ala-Trp-DPhe-Lys-NH_2, growth hormone (GH)-releasing peptide-6 (GHRP-6) has been developed via a combination of conformational energy calculations, synthesis, and biological activity testing.[1,2] Previous studies in normal prepubertal children and young adult subjects have shown that acutely intravenously administered GHRP-6 is a potent GH-releasing substance.[3-6] This hexapeptide has been shown to stimulate GH secretion in a dose-dependent and specific manner in all species tested so far.[3-12] The synergistic action exerted by maximal doses of GH-releasing hormone (GHRH) and GHRP-6 indicates that this hexapeptide acts through a non–GHRH-dependent mechanism.[4,5] Interest in this peptide has been strengthened by the fact that GHRP-6 is partially protected against enzymatic cleavage by two D–amino acid substitutions, allowing it to markedly increase plasma GH levels even when administered via the oral route.[10,13]

Obesity is associated with an impairment of GH secretion elicited by all stimuli known to date,[14-21] but the basic mechanisms of this alteration are not yet clear. Although obese subjects exhibited an increase in GH clearance rate, it is widely accepted that the main alteration is a decrease in both spontaneous and stimulated pituitary GH secretion. The finding that the cholinergic agonist pyridostigmine—which acts by inhibiting hypothalamic somatostatin release—partially restores stimulated GH secretion in obese subjects[22] suggested the existence of an increased somatostatinergic tone in obesity. More recently, we found that the combined administration of GHRH and GHRP-6 in obese subjects induced a large increase in plasma GH levels, supporting the existence of a considerable but not necessarily full complement of pituitary GH content in releasable-granule storage form.[23] To gain further insight into the disrupted mechanism of GH regulation in obesity, we investigated whether the inhibition of somatostatinergic tone with pyridostigmine could further increase the GH response to combined administration of GHRH plus GHRP-6. Thus, in the present study, we assess the effect of pyridostigmine administration on GHRH plus GHRP-6–induced GH secretion in normal and obese subjects.

SUBJECTS AND METHODS

The study involved 12 obese women who weighed more than 130% of their ideal body weight—as determined by the Fogarty Center Conference on Obesity.[24] A group of six obese patients (aged 29.5 ± 2.5 years), with a body mass index of 33.4 ± 2.0 kg/m^2 were administered GHRH 100 μg intravenously (IV) (GHRH-1-29, Geref; Serono, Madrid, Spain) plus GHRP-6 100 μg IV (GHRP-6, Peninsula Laboratories, Merseyside, UK) at 0 minutes. The second group of six obese patients (aged 29.8 ± 4.1 years with body mass index of 36.2 ± 2.4 kg/m^2) were also administered GHRH plus GHRP-6 at the same dose of 100 μg IV each at 0 minutes, preceded by either placebo or 120 mg pyridostigmine (Mestinon; Roche, Madrid, Spain) orally 1 hour earlier.

Eight normal volunteers (four women and four men) aged 20 ± 1.4 years and within their ideal body weight were also studied. They were administered GHRH plus GHRP-6 at the dose of 1 μg/kg IV preceded by placebo or pyridostigmine 120 mg orally at −60 minutes. The doses of GHRH and GHRP-6 used in this study result from previous data showing that pyridostigmine increased GH responses to either 100 μg GHRH or GHRP-6 administered independently,[5,24,25] and that combined administration of these two peptides elicited a synergistic interaction in terms of GH release.[5,23]

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Approval for this study was obtained from the Hospital Committee, and all subjects provided informed consent to participate in the study. Obese patients had normal menstrual cycles, and none had diabetes mellitus or other medical problems. All normal and obese women were studied in the follicular phase of the menstrual cycle.

The tests were initiated at 9 AM, with the subjects recumbent after an overnight fast. An indwelling catheter was placed in a forearm vein and kept patent with a slow infusion of 0.9% NaCl. Thirty minutes later, the tests were started and blood samples were obtained at different time intervals. Human plasma GH level was measured by radioimmunoassay with commercial kits (Nichols Institute, San Juan Capistrano, CA). Intraassay coefficients of variation were 4.2%, 2.9%, and 2.8% for low, medium, and high plasma GH levels, respectively, and the sensitivity of the assay was 0.2 μg/L. All samples from each subject were analyzed in the same assay.

Results were compared by nonparametric tests, with the Wilcoxon-paired test examining mean hormone levels at time intervals or areas under the secretory curves. The area under the curve was calculated by a trapezoidal method. The level of significance was set at $P < .05$.

RESULTS

In normal subjects (Fig 1A), administration of GHRH plus GHRP-6 induced a marked increase in plasma GH, with a peak at 30 minutes (mean ± SEM, 76.7 ± 9.7 μg/L), which was similar to that obtained after pretreatment with pyridostigmine (74.7 ± 9.4 μg/L).

In obese patients (Fig 1B), combined administration of GHRH plus GHRP-6 induced a clear increase in GH secretion, with a peak at 15 minutes of 42.2 ± 10.0 μg/L, which was not affected by pretreatment with pyridostigmine (38.4 ± 5.8 μg/L). The GH response was lower in obese patients than in controls as assessed by the area under the curve after administering both GHRH plus GHRP-6 (1,846 ± 396 *v* 4,773 ± 653, $P < .01$) and pyridostigmine plus GHRH plus GHRP-6 (1,989 ± 372 *v* 5,098 ± 679, $P < .005$).

DISCUSSION

In agreement with previous data, we found that combined administration of GHRH and GHRP-6 markedly increased plasma GH levels in normal and obese subjects.[5,23] However, the GH response in obese subjects was lower than in normal subjects. This decrease in responsiveness could be due to an increase in somatostatinergic tone and/or a decrease in the pituitary GH-releasable pool in obesity. Thus, we decided to compare the GH response to this combined stimulus after pretreatment with the cholinergic agonist pyridostigmine.

It is now widely accepted that cholinergic muscarinic pathways play a major role in GH secretion. Muscarinic cholinergic agonist drugs such as pyridostigmine stimulate basal GH release and the GH response to GHRH in normal subjects[5,25,26] and markedly potentiate GH responses to GHRH in obesity.[22] Conversely, antagonism of endogenous cholinergic pathways with muscarinic receptor-blocking drugs causes a striking reduction in basal and stimulated GH release.[14-21] Since it has been shown that the inhibitory effect of atropine on the GH response to GHRH can be abolished by antisomatostatin antibodies, it is widely accepted that acetylcholine regulates GH secretion by inhibiting somatostatin release from the hypothalamus.[27] In agreement with this hypothesis, it has been shown that pyridostigmine increases the GH response to either GHRH or GHRP-6 administered alone in normal subjects.[5,26] In contrast, in the present study, we have found that pyridostigmine failed to further increase the GH response to combined administration of these peptides in both normal and obese subjects, which is in agreement with recent data reported by others.[28] This lack of effect of pyridostigmine could be due to the fact that the massive GH discharge induced by this stimulus represents the full secretory capacity of the somatotrope. Alternatively, it is possible that GH responses to GHRH plus GHRP-6 are due to the action of this stimulus at both the pituitary and hypothalamic levels. A direct pituitary interaction of both peptides

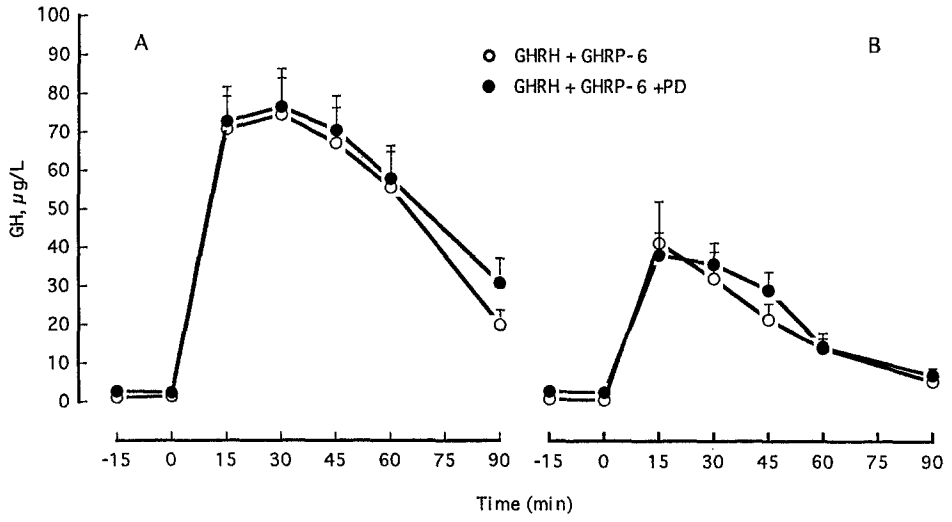

Fig 1. Plasma GH levels (mean ± SEM) in normal (A) and obese (B) subjects after combined administration of GHRH (100 μg) plus GHRP-6 (100 μg) either alone or after pretreatment with pyridostigmine ([PD] 120 mg) orally 1 hour before.

is supported by in vitro data showing an additive effect of maximal doses of GHRH and GHRP-6 on GH secretion. However, the finding that both compounds administered in combination exhibited a greater synergistic interaction in vivo than in vitro led some investigators to suggest that GHRP-6 could be acting by inhibiting hypothalamic somatostatin release.[8,9] In addition, by acting at the pituitary level, GHRP-6 is able to antagonize somatostatin-induced hyperpolarization of the somatotrope cell membrane.[29,30] Finally, it should be noted that while somatostatin completely abolished in vitro GH responses to GHRH and GHRP-6 when administered alone, it was unable to exert a similar effect when both compounds were administered in combination in vitro. Together, these data suggest that GHRP-6 can behave as a functional somatostatin antagonist, and in any event, somatotrope responsiveness to the combined administration of GHRH and GHRP-6 is largely independent of somatostatinergic tone.

Therefore, our findings in obese subjects of a relatively high GH response to GHRH plus GHRP-6, albeit low in comparison to that in normal subjects, with or without pyridostigmine suggest that the somatotrope cell in obesity has a considerable GH secretory capacity, although lower than in normal subjects. Whether this decrease in the GH-releasable pool present in obesity could be accounted for by an increase in free fatty acids or by other hormonal and metabolic alterations remains to be established.

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