Pyridostigmine enhances, but does not normalise, the GH response to GH-releasing hormone in obese subjects

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Abstract Obese patients are characterised by several neuroendocrine abnormalities, including characteristically a decrease in growth hormone responsiveness to GH-releasing hormone. In normal subjects, the GH response to GHRH is enhanced by the acetylcholinesterase inhibitor, pyridostigmine. We have studied the effect of this drug on GH secretion in gross obesity. Twelve obese patients were studied (mean weight 156% of ideal) and compared with a group of 8 normal volunteers. Each subject was initially studied on two occasions, in random order, with GHRH (1-29) NH₂ 100 µg iv alone and following pretreatment with pyridostigmine 120 mg orally one hour prior to GHRH. In obese patients, the GH response to GHRH was significantly blunted when compared to controls (GH peak: 20 ± 4 vs 44 ± 16 µg/l; mean ± SEM). After pyridostigmine, the response to GHRH was enhanced in the obese subjects, but remained significantly reduced compared to non-obese subjects treated with GHRH and pyridostigmine (GH peak: 50 ± 5 vs 77 ± 20 µg/l, respectively). In 6 subjects, higher doses of GHRH or pyridostigmine did not further increase GH responsiveness in obese patients. Our results suggest that obese patients have a disturbed cholinergic control of GH release, probably resulting from increased somatostatinergic tone. This disturbed regulation may be responsible, at least in part, for the blunted GH responses to provocative stimuli.

Obese patients have blunted growth hormone responses to a wide variety of stimuli (1-8). It has also been shown that these patients have decreased GH responses to GH-releasing hormone (9-14). This could be due to reduced pituitary sensitivity to GHRH, excess of hypothalamic somatostatin, or a chronic state of GHRH deficiency. Several lines of evidence point to a role for somatostatin in the decreased GH release found in obesity. Obese patients have blunted thyroid stimulating hormone response to TSH-releasing hormone (15), which might also reflect an increase in somatostatinergic tone. Furthermore, genetically-obese rats have increased hypothalamic somatostatin content (16) and increased hypothalamic somatostatin release in vitro (17). However, there is little direct evidence in the human.

Pyridostigmine is a drug which activates cholinergic receptors by decreasing the activity of acetylcholinesterase, and enhances GH responsiveness to GHRH (18-21); this is considered to occur via the inhibition of hypothalamic somatostatin (22). Recent studies have suggested that pyridostigmine may increase the GH response to GHRH in obesity, but only single doses of the drug were used, which did not take into account the increased body weight of the obese group compared to the controls (23,24).

In order to investigate whether hypothalamic somatostatin activity is increased in obesity, we have therefore studied the effect of several doses of pyridostigmine on the GH response to GHRH in obese patients.
Subjects and Methods

The experimental protocol was approved by the Ethical Committee of Escola Paulista de Medicina, São Paulo, Brazil. Two groups of subjects were studied after providing informed consent. One included 12 obese patients (9 females, 5 males), who were >120% of ideal body weight (mean ± SEM = 156 ± 6%, range 126-196) as calculated from the Metropolitan Life Insurance Company Statistical Bulletin. Their mean age was 27 years (range 17-39), and they were on free caloric intake prior to the study. The other group included 8 normal volunteers (6 females, 2 males), mean age 26 years (range 18-52) who were <110% of ideal body weight (mean ± SEM = 102 ± 1%, range 98-107). All women had regular menstrual cycles and were studied during the follicular phase. None of the subjects had clinical evidence of endocrine or other disease, and all were free of any medication for at least four weeks before the study.

Experimental protocol
After an overnight fast, all subjects were kept in bed from 08.00 h until the end of the test. Thirty minutes before starting the test, an indwelling cannula was inserted into an antecubital vein and kept patent by a slow 0.9% saline infusion. The 12 obese patients and 8 normal controls were studied twice with either 120 mg pyridostigmine or placebo orally, double-blind and in random order with a 48-h interval between tests. Both groups received an iv bolus injection of 100 µg GHRH (1-29) NH₂ (KabiVitrum, Sweden) one hour after pyridostigmine or placebo administration (time 0). Blood was obtained at times -15 and 0 min, and then at 15-min intervals until 180 min.

In order to investigate whether any differences found were related to drug dosage, 6 obese subjects were also submitted to a further two tests, one with placebo tablets plus 300 µg GHRH (1-29) NH₂, and another with 180 mg pyridostigmine and 100 µg GHRH (1-29) NH₂, following the same protocol described above.

GH assay
Serum GH was measured in duplicate by a double-antibody radioimmunoassay as previously described (25). The sensitivity of the method is 1.4 µg/l with mean intra- and inter-assay coefficients of variation of 9 and 12%, respectively.

Statistical analysis
Data were analysed by the Wilcoxon matched-pairs signed-ranks test for comparisons within subjects. Analysis of variance was used to compare treatment effects between groups. Pearson’s coefficient of correlation (r) was calculated where appropriate. The GH response to GHRH was also analysed following calculation of the area under the curve, computed by trapezoidal integration.

Fig. 1.
Change in mean serum GH in 8 normal volunteers (upper panel) and 12 obese patients (lower panel) given GHRH 100 µg iv with (O) or without (●) pre-treatment with pyridostigmine 120 mg. Each point represents the mean ± SEM.
Undetectable GH levels (<1.4 µg/l) were mathematically treated as equal to 1.4 µg/l. Results are shown as mean ± SEM.

Results
In normal subjects, GH rose significantly after GHRH, and this response was significantly enhanced by pyridostigmine (Fig. 1). The mean peak plasma GH levels after GHRH were 44 ± 16 µg/l in placebo-treated controls, increasing to 77 ± 20 µg/l with pyridostigmine (p<0.01); there was a significant increment of 50% in the area under the curve (data not shown). In obese patients, the GH response to GHRH was blunted, with peak GH values significantly lower than the values seen in the controls (20 ± 4 vs 44 ± 16 µg/l, respectively; p<0.02; Fig. 1). Following pyridostigmine, the GH response to GHRH in obese subjects was enhanced significantly between 15 and 75 min after GHRH, and was not different from the placebo-treated controls (peak GH values: 30 ± 5 µg/l). However, in the obese subjects the GH response to GHRH after pyridostigmine (both peak response and the area under the curve) was reduced when compared to normals treated with GHRH and pyridostigmine (Fig. 1). A negative correlation between peak GH levels after GHRH and percentage ideal body weight was found in obese subjects treated with both placebo (r= -0.70, p<0.01) and pyridostigmine (r= -0.81; p<0.005). Higher doses of GHRH did not further increase GH responsiveness in obese subjects (Fig. 2). With the higher dose of pyridostigmine (180 mg), the peak serum GH was slightly but non-significantly higher than with 120 mg; however, it was still less than that seen in the normal subjects (Fig. 3).

Adverse effects were mild in all patients. All subjects had facial flushing after GHRH 100 µg, which was accentuated with the higher dose. The principal adverse reactions associated with pyridostigmine were colicky abdominal pain in 44% and dizziness in 27%. The higher dose of pyridostigmine did not cause any increase in frequency or intensity of adverse reactions.

Discussion
Our results confirm that obese patients show a significantly blunted GH response to GHRH which is inversely related to body weight, as previously reported by other workers (9,10). They also demonstrate that pyridostigmine increases the response of GH to GHRH in obesity, suggesting that cholinergic mechanisms may be involved in this process. Enhancement of GH responsiveness to GHRH also suggests that somatotropes are functionally normal in obese patients.

These results are essentially in agreement with recent data from two studies in adults (23,24), and one in obese children (26). However, these workers administered GHRH as a single dose, such that the changes may have been secondary to sub-maximal stimulation of the somatotrope in the obese. We found that in these patients higher doses of GHRH were unable further to increase GH release, as suggested previously by Kopelman & Noonan (11). This excludes the administration of inadequate amounts of GHRH to obese patients as the primary cause of their hyporesponsiveness.

Fig. 2.
Change in mean serum GH (± SEM) in 6 obese patients given GHRH 100 µg (○) or 300 µg (●) as an acute iv bolus.

Fig. 3.
Change in mean serum GH (± SEM) in 6 obese subjects given GHRH 100 µg iv following pre-treatment with pyridostigmine 120 mg (○) or 180 mg (●).
The increase of GHRH-induced GH release by pyridostigmine in obese patients could be explained by several mechanisms. Pyridostigmine does not cross the blood-brain barrier and thus may be presumed to act either at the median eminence or at the level of the pituitary. An effect of pyridostigmine to increase endogenous GHRH release is unlikely, as cholinergic antagonists abolish the GH response to GHRH (18,19,27-31), and pyridostigmine enhances the GH response to supramaximal doses of GHRH (21). The effect of cholinergic drugs on pituitary GH release is controversial. Cholinergic agonists have been reported directly to stimulate pituitary GH release in vitro (29,32,33), although this has not been uniformly found (34). However, studies in the rat in vivo found that cholinergic modulation of GHRH-induced GH release did not occur when the animals were treated with cysteamine or were submitted to deafferentation of the mediobasal hypothalamus, procedures which decrease hypothalamic somatostatin concentrations (22). Furthermore, in vitro studies have shown that acetylcholine inhibits somatostatin release from hypothalamic fragments (35). Therefore, our results suggest that cholinergic mechanisms are involved in the blunted GH response to GHRH in obesity, acting via an increase in hypothalamic somatostatinergic tone. In agreement with this hypothesis, it has been shown that hypothyliami from genetically-obese rats release increased amounts of somatostatin in vitro (17).

Our results are also in agreement with both Cordido et al. (23) and Ghigo et al. (24) since pyridostigmine was found to increase GH responses to GHRH in obese subjects, but not to the levels found in controls tested with pyridostigmine. However, we have also shown that the responses still remain abnormal even when the dose of pyridostigmine is increased by 50% in the obese subjects, compatible with their increased weight. It is possible that still higher doses may be effective in normalising the GH responses, although we have no reason to believe that obese patients require a relatively higher dose on a µg/kg basis. It seems likely that, in addition to the cholinergic modulation, another mechanism may participate in the blunted GH response to GHRH in obesity. A decrease in GH stores consequent on a decrease in GHRH secretion cannot be excluded, but this seems improbable, as it has been shown that pituitary GH content is similar in genetically-obese and in lean control rats (17). Chronic inhibition of somatotropes by an excess of somatostatin may be an alternative related explanation. Further studies using repetitive administration of GHRH plus pyridostigmine may help elucidate this problem.

In conclusion, our results suggest that inhibition of GHRH-induced GH release in obesity is, at least in part, due to an increase in hypothalamic somatostatin release. This may in turn reflect changes in cholinergic control, although other neurotransmitters might also be involved in this process.

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