Pyridostigmine counteracts the blunted growth hormone response to growth hormone-releasing hormone of obese children

S. Loche¹, C. Pintor¹, M. Cappa², E. Ghigo³, R. Puggioni¹, V. Locatelli⁴ and E. E. Müller⁴

First Department of Pediatrics¹, Chair of Pediatric Endocrinology, University of Cagliari,
Department of Pediatric Endocrinology², Bambin Gesù Hospital, Rome,
Department of Biomedicine³, University of Turin,
and Department of Pharmacology⁴, University of Milan, Italy

Abstract. We have evaluated the effect of acute administration of pyridostigmine bromide, a cholinesterase inhibitor, on the GHRH-induced GH rise in 11 obese children and in 8 age-matched controls. The GH response to GHRH (hpGRF 1–40, 1 µg/kg iv), evaluated both as maximum GH peak and as integrated area under the curve, was significantly lower in the obese children than in the controls. Pretreatment with pyridostigmine bromide (60 mg orally 60 min before the GHRH injection) significantly increased both baseline GH levels and the GH response to GHRH in all the obese subjects, so that their mean baseline GH, peak GH levels and integrated area under the curve after pyridostigmine bromide plus GHRH were similar to those of the control children after GHRH. Also in control children pyridostigmine bromide increased (though not significantly) baseline GH levels, and caused a significant augmentation of the GH response to GHRH. Mean peak GH levels and mean integrated area under the curve after pyridostigmine bromide plus GHRH were significantly higher in the controls than in the obese children given the same treatment. Mean baseline Sm-C levels were significantly higher in the obese than in control children. These data show that enhancement of cholinergic neurotransmission, likely in the hypothalamus, counteracts the blunted GH response to GHRH present in the obese children, and that in simple obesity the potential of the pituitary to make a secretory response to a direct GH secretagogue is preserved.

Reduced GH responses to a wide variety of stimuli (see Glass et al. 1981 for review), including GHRH (Williams et al. 1984; Kopelman et al. 1985; Pertzelan et al. 1986; Pintor et al. 1986) is a characteristic feature of obese subjects. We (Loche et al. 1987) and others (Rosskamp et al. 1987) have recently reported high immunoreactive Sm-C levels in obese children, and, in our study, they were negatively correlated with the degree of the plasma GH response to a single bolus injection of GHRH. We have suggested that the elevated Sm-C levels of these obese children might inhibit GH secretion via a feedback mechanism mediated by an increased release of hypothalamic SRIH. That the hypothalamic component of Sm-C negative feedback on GH secretion may be mediated by SRIH is supported by the ability of Sm-C to induce an increased SRIH release from rat hypothalami in vitro (Berelowitz et al. 1981). Consistent with this view is also the increased release of SRIH from the hypothalami of rats with spontaneous experimental obesity (Berelowitz et al. 1983).

Recently, data have been presented showing that cholinergic muscarinic agonists or antagonistic drugs modulate in an opposite way the GH rise elicited by GHRH (Massara et al. 1984, 1986a,b; Ca-
sanueva et al. 1986; Yagi et al. 1986), likely via a somatostatinergetic mechanism (Richardson et al. 1980; Locatelli et al. 1986).

In the present study we have studied whether enhancement of the cholinergic neurotransmission by pyridostigmine bromide, a cholinesterase inhibitor (Taylor 1985), counteracts the blunted somatotrope responsiveness to GHRH in a group of obese children.

**Subjects and Methods**

Eleven obese children (8 males and 3 females) aged 5.2–13.0 years, with excess body weight ranging from 45.8 to 98.2% for their stature were studied. Eight age-matched normal children (5 males and 3 females aged 6.8–11.3 years) served as controls. They were referred to our Department for short stature, but were ultimately found to have normal GH secretion (peak GH levels > 10 µg/l following insulin-hypoglycemia or oral clonidine administration). One obese girl aged 11.1 years and one normal boy aged 12.1 years were in Tanner stage 2 of pubertal maturation, whereas all the other children were prepubertal. The study was approved by the ad hoc Ethical Committee of the Department of Pediatrics of the University of Cagliari, and informed consent was obtained from each subject or from their legal guardians prior to the study.

The existence of other endocrine abnormalities was excluded in all obese children by means of careful clinical and laboratory investigations. In particular, thyroid function (assessed by means of serum concentrations of T₃, T₄ and TSH), adrenal function (assessed by serum cortisol determinations at time 8.00 and 16.00 h), and skull X-ray were normal.

After an overnight fast, two basal samples were taken 30 min apart, the first drawn 30 to 60 min after an indwelling catheter was inserted into a forearm vein. GHRH (hpGRF 1–40, Bachem, Bubendorf, Switzerland), dissolved as reported elsewhere (Pintor et al. 1983), was then injected at a dose of 1 µg/kg iv over 30 sec. Blood samples were obtained after 15, 30, 60, 90 and 120 min. On separate occasions, all subjects underwent a second GHRH test after previous treatment with 60 mg pyridostigmine bromide (Mestinon®, Hoffman La Roche, Italy) administered po 60 min before GHRH. Blood pressure, pulse and respiratory rate, body temperature and neurological status were monitored throughout the test. Symptoms and signs were recorded every 20 to 30 min during the tests and for 3 h after the tests were completed. GH was measured by double-antibody RIA using reagents provided by CEA-IRE Sorin (Saluggia, Italy). The limit of detection of the assay was 1.0 µg/l with an intra- and inter-assay coefficient of variation of 2.8 and 5.1%, respectively.

Baseline Sm-C levels were also measured by RIA using reagents provided by Nichols Institute Diagnostic (S Juan Capistrano, CA); the limit of detection of the Sm-C assay was 100.0 U/l with an intra- and inter-assay coefficient of variation of 5.7 and 10.0%, respectively.

The statistical significance of the differences was calculated using paired or unpaired t-test preceded by ANOVA. Non-parametric analysis of variance was also performed where appropriate (Kruskall Wallis test). Plasma GH integrated areas under the curve (AUC-GH) after GHRH were calculated by means of trapezoidal integration. A P-value less than 0.5 (two-tailed) was considered to indicate a significant difference. All data are given as the mean ± SEM.

**Results**

Baseline GH values were 0.8 ± 0.1 and 1.8 ± 0.5 µg/l (P < 0.05) in the obese children and controls, respectively (Fig. 1). Administration of GHRH evoked a prompt and clearcut rise of plasma GH in all control subjects, with an increase from basal to peak values of 21.1 ± 3.9 µg/l (Fig. 1). In the obese children, mean peak GH levels after GHRH (6.4 ± 1.5 µg/l) were significantly lower (P < 0.001) than in the normal subjects (Fig. 1). Evaluation of AUC-
GH also showed a significantly lower ($P < 0.001$) GH response to GHRH in the obese children (34.4 ± 8.5 µg·min$^{-1}$·l$^{-1}$) than in the control group (129.6 ± 26.1 µg·min$^{-1}$·l$^{-1}$) (Fig. 2). Pretreatment with pyridostigmine bromide caused a significant increase in both baseline (1.5 ± 0.3 µg/l, $P < 0.05$) and GHRH-stimulated (25.9 ± 4.4 µg/l, $P < 0.001$) GH secretion in the obese subjects, so that mean baseline and peak GH levels after pyridostigmine bromide + GHRH were not different from those of the control group after GHRH alone (Fig. 1). Mean AUC-GH of the obese children after pyridostigmine bromide + GHRH (159.1 ± 39.4 µg·min$^{-1}$·l$^{-1}$) too was not significantly different from mean AUC-GH observed in the control children after GHRH alone (Fig. 2). In normal children, pretreatment with pyridostigmine bromide increased (though not significantly) mean baseline GH levels, and caused a significant augmentation of the GHRH-induced GH rise (mean peak GH levels = 44.4 ± 4.0 µg/l, $P < 0.0001$ vs GHRH (Fig. 1); mean AUC-GH = 273.8 ± 37.2 µg·min$^{-1}$·l$^{-1}$, $P < 0.05$ vs GHRH (Fig. 2). Mean peak GH levels (Fig. 1) and mean AUC-GH (Fig. 2) after pyridostigmine bromide + GHRH were significantly higher in the controls than in the obese children. Mean baseline Sm-C levels were significantly higher ($P < 0.02$) in the obese children (3300 ± 600 U/l) than in the control group (1300 ± 200 U/l).

No adverse effects, changes in pulse and respiratory rate, body temperature, blood pressure, and neurological status were observed in any of the subjects after GHRH administration, either alone or after pretreatment with pyridostigmine bromide.

Discussion

The blunted GH response to a single bolus injection of GHRH, as shown in this study, confirms previous reports (Pertzelan et al. 1986; Pintor et al. 1986; Loche et al. 1987), and, as previously observed (Loche et al. 1987; Rosskamp et al. 1987), this blunted response was associated with high immunoreactive Sm-C levels. A tendency to high Sm-C levels in obese prepubertal children associated with reduced 24-hour GH secretion has also recently been reported by Minuto et al. (1988). The new finding was that pretreatment with pyridostigmine bromide greatly potentiated the GH response to GHRH in all the obese children, and increased their baseline GH levels, so that their baseline and peak GH levels after pyridostigmine bromide + GHRH were superimposable on those of the control group after GHRH. Pretreatment with pyridostigmine bromide increased mean baseline GH levels also in normal children, though not significantly. This is not surprising since after pyridostigmine bromide administration to normal children and adults, peak GH levels are commonly observed after 90-120 min (Massara et al. 1986b; Ghigo et al. 1987). Furthermore, variations in baseline GH levels are difficult to interpret owing to the episodic secretion of the hormone throughout the day. Pyridostigmine bromide greatly potentiated the GH response to GHRH also in normal children, and peak GH levels and AUC-GH observed after pyridostigmine bromide + GHRH were significantly higher than those of the obese children given the same treatment.

It has recently been shown that agonists or antagonists of cholinergic muscarinic receptors potentiate (Massara et al. 1986a, 1986b) or inhibit (Massara et al. 1984; Casanueva et al. 1986; Yagi et al. 1986), respectively, the GH response to GHRH in man. A series of in vivo and in vitro studies in the rat have documented that the mechanism by which these compounds exert their neuroendocrine effects is via blockade (cholinergic agonists) or in-
crease (cholinergic antagonists) of hypothalamic SRIH release (Locatelli et al. 1986). A direct action of cholinergic drugs at the pituitary level was excluded in that study.

The finding of increased baseline GH levels and of a striking augmentation of the GH response to GHRH by pyridostigmine bromide in our obese children, in view of the drug's ability to antagonize cholinesterases and hence augmenting cholinergic neurotransmission (Taylor 1985), suggests counteraction by the drug of the endogenous SRIH tone. The latter, on the other hand, might result from the increased Sm-C production (Berelowitz et al. 1981). An involvement of SRIH could also explain why the GH response to all GH secretagogues, regardless of their nature and site of action, are invariably blunted in obese subjects (Glass et al. 1981; Williams et al. 1984; Kopelman et al. 1985; Pertzelsen et al. 1986; Pintor et al. 1986; Loche et al. 1987; Rosskamp et al. 1987). The observation that after pyridostigmine bromide the GH response to GHRH in obese children almost overlapped that in normal children after GHRH is of particular interest. It could imply that in simple obesity the potential of the pituitary to make a secretory response to a direct GH secretagogue is preserved, which is in keeping with the reversibility of the neuroendocrine defect after weight reduction (Williams et al. 1984). There was however, a clear-cut rise of plasma GH, which reached values higher than those observed in obese children, in control children receiving combined administration of pyridostigmine bromide + GHRH. This would rule out a major role for SRIH, and hence SRIH disinhibition, in dictating the extent of the GH responsiveness to GHRH in obese children, and would indicate, instead, that there is some chronic background suppression of the somatotropes in the latter which does not involve SRIH. In this context it is worth noting that Sm-C negative feedback on GH secretion is exerted on both the hypothalamus and the pituitary (Berelowitz et al. 1981), and therefore, the possibility of a Sm-C-mediated inhibition of GH synthesis (Ceda et al. 1985; Yamashita et al. 1987) cannot be ruled out. In addition, other hormonal and/or metabolic factors such as insulin (Ceda et al. 1985) and FFA (Imaki et al. 1985) which are known to be increased in the obese (Opie & Wallish 1963; Bogardus et al. 1981), or other unknown factors might also contribute to the pathophysiology of this phenomenon. It must be recalled, however, that pyridostigmine bromide was administered at only one dose level; thus we are unable, in our study, really to titrate from the plasma GH response to pyridostigmine bromide + GHRH the magnitude of the hypothalamic SRIH tone in obese and lean children, and to ascertain how this latter might be related to the circulating levels of Sm-C (Berelowitz et al. 1981, 1983).

In conclusion, our findings show that a cholinergic agonist, whatever its mechanism of action may be, counteracts the blunted GH response to GHRH in obese children, and strengthen the view that the combination pyridostigmine bromide + GHRH is a highly suitable test to study the secretory integrity of the somatotropes (Ghigo et al. 1987).

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References


