CLINICAL STUDY

Somatostatin infusion withdrawal: studies in the acute and recovery phase of anorexia nervosa, and in obesity

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Abstract

Objective: Changes in GH/IGF-I axis activity occur in both anorexia nervosa (AN) and obesity (OB). A GH hypersecretory state with very low plasma IGF-I levels is present in AN, whereas in morbid OB, GH secretion is dull and plasma IGF-I levels are generally preserved. Endogenous GHRH activity in AN and OB has never been directly studied, although indirect evidence would indicate that GHRH function is altered in either condition, possibly enhanced and reduced respectively. Somatostatin (SS) infusion withdrawal (SSIW) is followed by a rebound rise of plasma GH in animals and humans, an event which, allegedly, is mediated by endogenous GHRH release.

Methods: In the present study, 28 young women, eight with active AN (A-AN), six with AN in the recovery phase (R-AN), eight with morbid OB, and six healthy age-matched normal weight subjects (NW), were studied. All subjects underwent, on different occasions, the following two tests: (i) acute GHRH injection (1 µg/kg, i.v.); (ii) infusion of SS (9 µg/kg per h i.v. over 60 min), with blood samples drawn prior to and at different intervals after drug injections. Plasma GH levels were measured at each time interval in all sessions, and, in addition, baseline plasma estradiol, free triiodothyronine, TSH, IGF-I and insulin were measured at –30 min.

Results: Baseline plasma GH concentrations were significantly higher in A-AN than in NW (4.7 ± 0.7 vs 2.1 ± 0.6 µg/l, $P < 0.01$). Baseline GH levels in R-AN were also higher than in NW, but the difference did not reach statistical significance (5.6 ± 1.7 µg/l, not significant (NS)). Baseline plasma GH concentrations were significantly lower in OB than in NW (0.3 ± 0.1 µg/l, $P < 0.01$). GHRH-stimulated GH release was significantly higher in A-AN than in NW (mean change in area under the curve (Δ AUC) 1904.9 ± 626.1 vs 613.9 ± 75.9 µg/l per min, $P < 0.01$), whereas no statistically significant difference was present between R-AN and NW (mean Δ AUC 638.2 ± 293.0 µg/l per min, NS); in OB, GHRH failed to evoke a plasma GH rise (mean Δ AUC 239.8 ± 89.9 µg/l per min vs A-AN, R-AN, and NW, $P < 0.01$). SS infusion markedly reduced plasma GH concentrations in both A-AN and R-AN and, to a lesser extent, in NW, but failed to do so in OB. In A-AN, SSIW was followed by a plasma GH rise markedly higher than that present in NW (mean Δ AUC 193.0 ± 42.3 vs 60.1 ± 11.4 µg/l per min, $P < 0.01$), whereas in R-AN the GH response after SSIW was nearly superimposable on that registered in NW (mean Δ AUC 72.9 ± 22.8 µg/l per min, NS). There were no changes in plasma GH levels after SSIW in OB (mean Δ AUC 22.8 ± 9.7 µg/l per min). In all groups, Δ AUCs of the GH response to GHRH and after SSIW were highly positively correlated ($r = 0.7$, $P < 0.01$).

Conclusions: These data support the view that a high endogenous GHRH tone, which subsides in the recovery phase of the disease, is present in AN, whereas GHRH hypofunction, possibly associated with pituitary impairment, might indicate OB.

European Journal of Endocrinology 148 237–243

Introduction

In anorexia nervosa (AN), as well as in malnutrition and catabolic states, low insulin-like growth factor I (IGF-I) levels are generally associated with growth

hormone (GH) hypersecretion (1–11), concordant with the existence of peripheral GH resistance (12, 13). In this disease, low circulating IGF-I levels could explain GH hypersecretion due to a reduction of the IGF-I-mediated negative feedback mechanism,

which is thought to take place directly at the pituitary level or, indirectly, via stimulation of hypothalamic somatostatin (SS) release (14–16).

Alternatively, in AN, a primary alteration in the neural control of somatotroph function involving GH-releasing hormone (GHRH) (5, 6) may underlie GH hypersecretion. In fact, so far, low or absent GH response to insulin-induced hypoglycemia (17) or dopaminergic agonists (18), exaggerated GH responses to GHRH (7, 8, 19, 20), paradoxical GH rises after glucose load (21, 22), thyrotropin-releasing hormone (23), or gonadotropin-releasing hormone (GnRH) (24) have been reported. GH secretion in AN has also been shown to be particularly refractory to the inhibitory effect of muscarinic cholinergic antagonists (7, 8), as well as to the stimulatory effect of cholinergic agonists or β -adrenergic antagonists (25, 26), or acute glucocorticoid administration (27). As central cholinergic and β -adrenergic functions and glucocorticoids would influence GH secretion via modulation of hypothalamic SS release (28), the existence of an impaired SS activity in AN has also been suggested (5, 6, 8, 25, 26). Along this line also are the low cerebrospinal fluid (CSF) concentrations of SS detected in anorectic patients (29).

In contrast, in morbid obesity (OB), circulating GH levels and the 24 h GH production rate are reduced (30). GH insufficiency in OB could denote neuroendocrine abnormalities, including GHRH hypoactivity (31), although alterations in peripheral hormones and metabolic factors could also play a role (32).

In OB, not only spontaneous GH secretion, but also the somatotroph response to provocative stimuli, including GHRH and GH-releasing peptide, is markedly blunted (33, 34), sometimes to levels as low as those recorded in patients with GH deficiency (35). Interestingly, in the obese subjects, the somatotroph responsiveness to either GHRH or arginine shows a peculiar refractoriness to the inhibitory effect of glucose load, whereas GH secretion is normally inhibited by pirenzepine, an antagonist of muscarinic receptors, thought to act via SS release, as well as by exogenous SS (36).

In recent years, studies in animals (37–39) and humans (40–42) have provided evidence that the rebound GH rise which follows SS infusion withdrawal (SSIW) is due, at least in part, to the functional activation of GHRH neurons of the recipient organism. Thus, SSIW may represent a test to indirectly probe

endogenous GHRH function, and a potential tool in the diagnosis of GH hyposecretory states (42) or to assess the declining GHRH function in aging (41). Very recently, a marked impairment of the GH response to combined SSIW and GHRH has been reported in a group of OB patients (43).

Based on the foregoing, we studied, in patients with AN in the acute and recovery phase of the disease, and in OB subjects the GH response which follows an SSIW. Our aim was that of gaining further insight into the GHRH (and/or SS) function in these clinical conditions.

Material and methods

Subjects and methods

Twenty-eight young women (eight patients with active AN (A-AN), aged 19–33 years; six with AN in the recovery phase (R-AN), aged 17–32 years; eight with morbid OB, aged 17–29 years; six healthy normal weight subjects (NW), aged 17–20 years) were studied. Clinical and hormonal characteristics of the study subjects are shown in Table 1. All AN patients met the diagnostic criteria for AN according to the *Diagnostic and Statistical Manual of Mental Disorders IV-TR* (44). All subjects gave informed consent to participate in the study, which had been approved by the Ethical Committee of our Institute. NW and OB subjects had no history or actual evidence of endocrine or psychiatric disorders, and had not been taking medications for the previous 6 months. Patients with R-AN, OB and NW were all eumenorrheic and were studied in the early follicular phase of the menstrual cycle. Patients with A-AN were amenorrheic.

After an overnight fast, an indwelling (i.v.) cannula was inserted in both forearms at 0800 h for separate blood sampling and drug administration. All subjects underwent the following tests at least 5 days apart: (i) GHRH (Geref; Serono, Milan, Italy), 1 μ g/kg, i.v. bolus at 0 min, with blood samples drawn at –30, 0, 30, 45, 60, 90 and 120 min; (ii) SS (Stilamin; Serono) infused i.v. in 50 ml normal physiological saline at a rate of 9 μ g/kg per h over 60 min (0–60 min of the study), with blood samples drawn at –30, 0, 30, 45, 60, 90, 105, 120, 135 and 150 min.

Table 1 Demographic and hormonal characteristics of the study subjects. Data are expressed as means $\pm$ S.E.M.

Group	Age (years)	BMI (kg/m ²)	Duration of disease (years)	E2 (pmol/l)	FT ₃ (pmol/l)	FT ₄ (pmol/l)	TSH (mU/l)	IRI (μ U/ml)	IGF-1 (μ g/l)
NW	17.8 $\pm$ 0.4	22.3 $\pm$ 0.6	–	145.0 $\pm$ 14.7	3.8 $\pm$ 0.4	18.5 $\pm$ 2.4	1.7 $\pm$ 0.2	9.3 $\pm$ 0.8	160.2 $\pm$ 12.50
A-AN	22.6 $\pm$ 1.7	13.8 $\pm$ 0.3 ^a	4.1 $\pm$ 1.6	39.4 $\pm$ 3.4 ^a	2.4 $\pm$ 0.4 ^a	12.4 $\pm$ 0.4 ^a	2.4 $\pm$ 0.4	2.9 $\pm$ 0.7 ^a	57.1 $\pm$ 7.67 ^a
R-AN	24.0 $\pm$ 2.1	19.0 $\pm$ 0.8	1.3 $\pm$ 0.3	123.7 $\pm$ 31.2	4.3 $\pm$ 0.5	12.8 $\pm$ 1.5 ^a	1.3 $\pm$ 0.2	8.6 $\pm$ 2.9	155.9 $\pm$ 12.98
OB	21.5 $\pm$ 1.8	37.4 $\pm$ 1.5 ^a	–	289.2 $\pm$ 85.2 ^a	4.7 $\pm$ 0.3	14.3 $\pm$ 0.6	2.2 $\pm$ 0.2	16.9 $\pm$ 3.3 ^a	122.4 $\pm$ 14.60

^a $P < 0.01$ vs NW.

Serum GH levels were measured at each time interval. Baseline plasma levels of estradiol (E2), free triiodothyronine (FT₃), free thyroxine (FT₄), thyrotropin (TSH), IGF-I and insulin (IRI) were also measured at -30 min before the GHRH challenge test.

Serum GH levels was estimated by chemiluminescence immunoassay (Nichols Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); sensitivity of the assay 0.01 ng/ml, intra- and inter-assay coefficients of variation 4.4 and 8.6% respectively. Serum IGF-I levels was estimated by chemiluminescence immunoassay (Nichols Advantage); sensitivity of the assay 6 µg/l, intra- and interassay coefficients of variation 4.2 and 6.3% respectively. Serum IRI was measured by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, Mannheim, Germany); sensitivity of the assay 0.2 µU/ml, intra- and interassay coefficients of variation 4.2 and 8.2% respectively, normal range in our laboratory 2.6–24.9 µU/ml. Serum TSH was measured by ECLIA (Roche Diagnostics); sensitivity of the assay 0.005 mU/l, intra- and interassay coefficients of variation 5.4 and 8.7% respectively, normal range 0.27–4.2 mU/l. Serum FT₄ was measured by ECLIA (Roche Diagnostics); sensitivity of the assay 0.30 pmol/l, intra- and interassay coefficients of variation 4.8 and 6.6% respectively, normal range 12–22 pmol/l. Serum FT₃ was measured by ECLIA (Roche Diagnostics); sensitivity of the assay 0.4 pmol/l, intra- and interassay coefficients of variation 5.1 and 9.0% respectively, normal range 2.8–7.1 pmol/l. Serum E2 was measured by ECLIA (Roche Diagnostics); sensitivity of the assay 18.4 pmol/l, intra- and interassay coefficients of variation 4.9 and 6.2% respectively, normal range for the follicular phase of the menstrual cycle 90.1–716 pmol/l.

No adverse effects were recorded during the challenge tests.

Statistical analysis

Results are expressed as means±s.e.m. To facilitate comparison of the GH secretory profiles after GHRH or SSIW, plasma GH responses were expressed either as absolute values or as GH ΔAUCs (area under the curve detected after GHRH bolus or SSIW minus the value of the plasma GH level at *t*₀ or *t*₆₀ multiplied by 120 min or 90 min respectively in each subject). The results were compared among all groups using an ANOVA. Student's paired and unpaired *t*-tests were used to evaluate individual differences between means. When applicable, a preliminary logarithmic transformation was used to satisfy the assumption of a normal distribution of variances. Correlations between GH ΔAUCs after GHRH and GH ΔAUCs after SSIW were performed by linear regression analysis.

Results

Table 1 shows the mean values±s.e.m. for age, body mass index (BMI), E2, FT₃, FT₄, TSH, IGF-I and IRI in A-AN, R-AN, OB and NW women. For A-AN and R-AN the duration of the condition is also reported.

Baseline plasma GH concentrations were significantly higher in A-AN than in NW (4.7 ± 0.7 vs 2.1 ± 0.6 µg/l, $P < 0.01$). Baseline GH levels in R-AN were also higher than in NW, but the difference did not reach statistical significance (5.6 ± 1.7 µg/l, not significant (NS)). Baseline plasma GH concentrations were significantly lower in OB than in NW (0.3 ± 0.1 µg/l, $P < 0.01$) (Fig. 1).

In A-AN the GH response to GHRH was enhanced, being markedly higher than that observed in NW

Fig. 1 Plasma GH concentration profiles (left panel) and GH ΔAUCs (right panel) (means±s.e.m.) after GHRH (1 µg/kg i.v. at 0 min) in normal weight women (NW), patients with anorexia nervosa in the acute (A-AN) and recovery (R-AN) phases and subjects with obesity (OB). The same general description applies to Fig. 2. * $P < 0.01$ vs NW. See text for further details.

(mean Δ AUCs 1904.9 ± 626.1 vs 613.9 ± 75.9 $\mu\text{g/l per min}$, $P < 0.01$; ranges 448.0–5158.1 $\mu\text{g/l per min}$ and 382.5–810.8 $\mu\text{g/l per min}$ respectively), while no difference with respect to NW was present in R-AN (mean Δ AUC 638.2 ± 293.0 $\mu\text{g/l per min}$, NS; range 133.8–1974.8 $\mu\text{g/l per min}$). The somatotroph response to GHRH in OB was significantly lower than that displayed by the other groups (mean Δ AUCs 239.8 ± 89.9 $\mu\text{g/l per min}$ vs A-AN, R-AN and NW, $P < 0.01$; range 43.8–809.5 $\mu\text{g/l per min}$) (Fig. 1).

Percent inhibition of plasma GH concentrations from t_{-30} to t_{60} during SS infusion was more pronounced in A-AN and R-AN than in NW, although the difference did not reach statistical significance (data not shown). The percent GH inhibition induced by SS in OB was similar to that recorded in NW (data not shown) (Fig. 2).

SSIW in A-AN resulted in a rise in plasma GH levels from 0.9 ± 0.4 $\mu\text{g/l}$, the value reached at the end of SS infusion, to a maximum of 4.7 ± 1.1 $\mu\text{g/l}$ at 150 min. Mean Δ AUC (193.0 ± 42.3 $\mu\text{g/l per min}$; range 25.8–359.3 $\mu\text{g/l per min}$) was significantly higher than that shown by NW (60.1 ± 11.4 $\mu\text{g/l per min}$; range 21.8–93.8 $\mu\text{g/l per min}$, $P < 0.01$). Termination of SS infusion in R-AN was followed by an increase in plasma GH levels from 0.4 ± 0.1 $\mu\text{g/l}$ to a maximum of 2.4 ± 0.7 $\mu\text{g/l}$ at 150 min. In this group, mean Δ AUC (72.9 ± 22.8 $\mu\text{g/l per min}$; range 22.5–164.0 $\mu\text{g/l per min}$) was similar to that present in NW subjects. Mean Δ AUC (22.8 ± 9.7 $\mu\text{g/l per min}$; range 0.3–74.1 $\mu\text{g/l per min}$) following SSIW was significantly lower in OB than in A-AN, R-AN and NW subjects respectively ($P < 0.01$) (Fig. 2).

There was a positive correlation between plasma GH Δ AUCs recorded after GHRH and SSIW ($r = 0.7$, $P < 0.01$), when all study subjects were considered.

Discussion

The existence of a robust somatotroph responsiveness to GHRH in A-AN is widely acknowledged (5, 7, 8, 26), although few exceptions have also been reported (45). In the present study, the GHRH-induced GH response in A-AN overrode the one recorded in NW, whereas it was within normal limits in R-AN.

The enhanced GH secretion of AN is, at least in part, linked to the malnourishment-dependent reduction of IGF-I synthesis with ensuing attenuation of the negative feedback exerted by IGF-I itself on GH secretion at the pituitary and/or via stimulation of hypothalamic SS release (9, 13–16). In AN, consistently low IGF-I levels in spite of elevated GH concentrations, indicate a condition of GH peripheral resistance, which has also been reported in other pathophysiological conditions, such as fasting (46), malnutrition (1), poorly controlled insulin-dependent diabetes mellitus (4), and liver cirrhosis (10, 47). Alternatively, or in addition, in AN, primary alterations in the neural control of somatotroph secretion for a state of GHRH hyperactivity and/or SS hypoactivity could be operative (5–8, 25, 26).

Reportedly, SSIW in either animals or humans elicits a rebound GH rise, which has been attributed to a hypothalamic component, i.e. disinhibition of GHRH neuronal function (37, 38, 48, 49). Our present finding of a highly positive correlation between the GH responses to SSIW and to exogenous GHRH in all the subjects studied reinforces this opinion. Along this line, the enhanced GH rebound observed in AN after SSIW might be related to an enhancement of the endogenous GHRH tone (6, 50). Indeed, the analysis of GH pulsatility in AN has documented an increased frequency of GH secretory pulses (6), which might be due to increased frequency of GHRH discharges (50).

Fig. 2 Plasma GH concentration profiles (left panel) and GH Δ AUCs (right panel) (means $\pm$ S.E.M.) of NW, A-AN, R-AN and OB subjects administered a 1 h infusion of SS ($9 \mu\text{g/kg per h i.v.}$). Bar indicates timing and duration of infusion. * $P < 0.01$ vs NW. See Fig. 1 legend for general description and text for further details.

In R-AN the GH response after SSIW was nearly superimposable on that observed in NW, which would denote subsiding of GHRH hyperfunction in this phase of the disease (8).

The view of a reduced hypothalamic somatostatin-ergic tone in AN rests on the presence of elevated inter-pulse GH levels (50) and of low CSF concentration of SS (29), as well as on the finding that substances acting via stimulation of hypothalamic SS release, while capable of abolishing the GH response to GHRH in normal subjects (28), only blunt it in anorectic patients (7, 8). Conversely, substances that inhibit hypothalamic SS release, such as pyridostigmine, an indirect cholin-ergic agonist, or atenolol, a β 1-adrenergic antagonist, while capable of potentiating the GH response to GHRH in normal subjects (28), fail to do so in AN (25, 26).

The above alterations in the hypothalamic control of somatotroph secretion in AN do not rule out *per se* the possibility that an altered sensitivity of somatotroph cells to endogenous SS may also be operative in this disease. Reportedly, the percent inhibitory effect of SS at physiological doses on the GHRH-stimulated GH secretion is similar in anorectic patients and in normal subjects (51). In our study SS induced a steep inhibition of GH release, greater but not significantly so in AN patients than in NW and comparable in the two groups of A-AN and R-AN patients, who, on the contrary, displayed significantly different GH responses to both SSIW and GHRH. These observations make it unlikely that an altered pituitary sensitivity to SS may be responsible for the robust somatotroph responsiveness to the above stimuli observed in A-AN.

It has to be noted that data presented here were derived from a one-point cross-sectional investigation of anorectic patients in a natural setting. Therefore, many confounding factors could be controlled only statistically and others (e.g. the individual recent history of weight changes) could not be controlled at all.

The interpretation of the results in OB patients only is apparently less complex. In these subjects GH secretion, either basal or evoked by GHRH or SSIW, was markedly blunted. A defect of GH secretion in OB is widely acknowledged. In fact, these patients, compared with NW subjects, display a reduced frequency of spontaneous secretory episodes and daily production rate of GH (30). Furthermore, in them GH secretion is impaired in response to all traditional pharmacological stimuli acting at the hypothalamus (31, 33, 52–55). The finding that compounds thought to inhibit hypothalamic SS release (pyridostigmine, arginine, galanin, atenolol) improve only partially, but do not normalize, the somatotroph response to GHRH (35, 53, 56) militates against the view of an increased SS tone as the main cause of the hyposomatotropism of OB. Although plasma and CSF levels of GHRH were reportedly similar in massively OB patients and in NW subjects (57), the frank impairment of GH release

after SSIW in OB might be related to a GHRH hyposecretory state (31). In this context, in genetically obese Zucker rats, decreased hypothalamic GHRH mRNA levels are thought to be critical for the attenuation of GH gene expression and the consequent reduction of circulating hormonal levels (58). However, the hypothesis of a reduced endogenous GHRH activity in OB is toned down by the clear-cut impairment of GH response to combined SSIW and GHRH recently reported in these patients (43). Also the observation that in OB repeated i.v. administration of GHRH boluses does not improve the somatotroph response to a subsequent GHRH bolus points to a defect of somatotroph function in this disease (59). This pituitary impairment might also contribute to the blunted GH response to SSIW displayed by OB patients.

Ghrelin, a recently isolated endogenous ligand for GH secretagogue receptors (60), is a stomach hormone sensitive to nutritional intake and capable of stimulating food intake in experimental animals (61). A high-fat diet decreases plasma ghrelin levels whereas a low-protein diet has an opposite effect (62). These mechanisms could also be operative or even amplified in OB and AN patients. A possible reduction or increase in ghrelin production may also account for functional failure or hyperactivity of the somatotrophic axis in OB and AN respectively (63, 64). In this context, recent studies have shown low circulating levels of ghrelin in OB (63) and, conversely, high plasma concentrations of the peptide in the A-AN, which subside when anorectic patients gain weight (64). It cannot be excluded, therefore, that SSIW may be a trigger that releases ghrelin from hypothalamic neurons and/or oxyntic glands (65), and that this event may contribute to the GH secretion evoked by SSIW (66).

On the whole, the present data support the view of a high endogenous GHRH tone in AN, which subsides in R-AN. GHRH hyposecretion, possibly associated with an enhanced SS function and pituitary impairment, would indicate the OB state. Further studies will allow a better understanding of the hypothalamic events triggered by SSIW and, possibly, the exploitation of this test in the diagnosis of GH hypo- and hypersecretory states (41, 42).

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Received 6 August 2002

Accepted 12 November 2002