Neurosurgery restores late GH rise after glucose-induced suppression in cured acromegalics

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Abstract

Objective and design: A decrease of GH levels below 2 µg/l after an oral glucose tolerance test (OGTT) is still currently accepted as the gold standard for assessing cure in surgically treated acromegaly. Whether glucose-induced suppression of GH is accompanied by a restoration of normal GH late rebound has not yet been evaluated in this disease. In order to assess the restoration of normal GH regulation after removal of a pituitary adenoma, we have evaluated GH changes after an OGTT in a series of selected acromegalic patients (transsphenoidal surgery and lack of pituitary failure).

Methods: Twenty-nine patients (13 male, 16 female, age range 27–70 years) entered the study. Their neuroradiological imaging before neurosurgery showed microadenoma in 7, intrasellar macroadenoma in 8 and macroadenoma with extrasellar extension in 14. Plasma GH levels were assayed up to 300 min after glucose administration (75 g p.o.) and IGF-I on basal samples.

Results: Basal GH levels were below 5 µg/l in 20 patients and below 2 µg/l in 5 of these. Normal age-adjusted IGF-I levels were observed in 12 patients. GH values were suppressed below 2 µg/l during an OGTT in 13 patients, and below 1 µg/l in 7 of these. In 9 patients out of these 13, a marked rise in GH levels occurred after nadir. Baseline and nadir GH values of these 9 patients were not different from the corresponding values of the other 4 patients without OGTT-induced late GH peaks.

Conclusions: GH rebound after GH nadir occurs in acromegalic patients considered as cured on the basis of OGTT-induced GH suppression and/or IGF-I normalization. The restoration of this physiological response could be regarded as a marker of recovered/preserved integrity of the hypothalamic–pituitary axis. Even though the reason for this GH rebound has not yet been elucidated (GHRH discharge?/end of somatostatin inhibition?), the lack of late GH peak in the patients regarded as cured by the usual criteria could be due to injury to the pituitary stalk caused by the adenoma or by surgical manipulation.

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Introduction

The restoration of growth hormone (GH) suppression following an oral glucose (Glc) tolerance test (OGTT) is considered the gold standard for evaluating successful treatment of acromegaly after surgery (1) and the normalization of insulin-like growth factor-I (IGF-I) levels represents the aim of both surgical and medical treatment. Other criteria to evaluate cure of acromegalic patients include the disappearance of paradoxical stimuli for GH secretion, such as thyrotropin-releasing hormone (TRH) (2), and the restoration of normal GH dynamics (3).

It is known that in normal subjects a rebound of GH levels is frequently observed after the suppressive effect of an OGTT (4). This phenomenon has not yet been assessed in acromegalic patients. Therefore we felt it of interest to evaluate GH levels after an OGTT, by prolonging blood sampling up to 300 min, in a large series of operated acromegalic patients, with the aim of

disclosing the restoration of normal GH dynamics following removal of the adenoma.

Patients and methods

Patients

Acromegalic patients recently submitted to neurosurgical treatment entered the study after giving informed consent. All patients had been operated on by a single surgical team, following a clearly defined protocol.

Acromegaly had been diagnosed because of the clinical picture, GH levels not suppressible by oral Glc load to less than 2 µg/l, and high IGF-I levels for age. Exclusion criteria were neurosurgery via an approach different from the transsphenoidal, hypopituitarism (as demonstrated by levels of free thyroid hormones, plasma cortisol or urinary free cortisol, plasma testosterone or estradiol below the normal range) or previous radiotherapy. No patient was affected by diabetes mellitus, active hepatic or

renal disease. All subjects were within 20% of ideal body weight, even though it should be underlined that a larger value in acromegalic patients compared with normal subjects does not mean that the patients have an increased mass of fat tissue. It is well known that the lean body mass in this disease is increased (5).

Twenty-nine acromegalic patients (13 males, 16 females, age range 27–70 years) fulfilled these criteria and were evaluated. Individual demographic and clinical data are reported in Table 1. Hyperprolactinemia was present in eight patients (# 3, 5, 8, 13, 17, 20, 25, 28). At the preoperative neuroradiological imaging by high resolution CT scan or MRI, tumor sizes were classified into three groups: microadenomas ($n=7$); intrasellar macroadenomas ($n=8$); and tumors with extrasellar expansion ($n=14$).

Patients underwent an OGTT 3–6 months after neurosurgery, as outpatients. No patient was on any drug treatment aimed at lowering GH hypersecretion or potentially capable of interfering with GH secretion.

Blood samples were collected in the morning, after an overnight fast and rest, while the patients were supine

and awake, with an indwelling needle inserted in an antecubital vein and kept patent by slow saline infusion. Plasma GH levels were assayed basally every 15 min three times, thereafter patients were submitted to an OGTT (75 g Glc p.o.) with determination of GH and Glc levels every 30 min up to 180 min and then hourly up to 300 min after Glc administration. IGF-I was evaluated in the first basal sample.

Methods

GH and IGF-I were assayed in duplicate by an immunoenzymatic method and RIA after acid-ethanol extraction respectively. Plasma Glc was assessed by standard methods (Glc oxidase). All samples from a patient were run together.

Reagents were purchased from Sorin (Saluggia, Italy) for GH and Nichols (San Juan de Capistrano, CA, USA) for IGF-I.

Standards were calibrated against 1st IS 80/505 (1 ng = 2 μ IU) for GH and WHO 87/518 for IGF-I.

Table 1 Demographic and clinical data.

No.	Sex	Age (years)	TC/MRI ¹	IGF-I ²	IGF-I (%) ³	Basal GH ⁴	Nadir GH ⁵
1	F	63	μ	342	118	2.2	1.4
2	F	70	EX	220	76	1.6	1.1
3	M	65	μ	400	138	3.2	2.2
4	F	65	M	320	110	2.0	0.9
5	F	40	EX	492	137	15.1	9.9
6	F	54	EX	490	136	2.3	–
7	F	65	EX	400	138	24.3	2.6
8	F	48	EX	253	70	1.5	1.0
9	M	53	M	395	110	1.0	0.4
10	F	32	EX	1000	203	19.1	10.3
11	M	50	M	650	181	7.9	7.4
12	M	56	μ	275	95	2.4	–
13	M	52	EX	900	250	7.7	5.9
14	F	58	M	620	214	4.3	3.0
15	M	50	EX	359	100	2.1	0.1
16	M	55	EX	205	71	0.4	0.4
17	F	33	M	362	74	2.5	0.5
18	F	40	M	850	236	8.7	7.2
19	M	40	EX	388	108	2.5	–
20	F	27	EX	280	57	5.7	3.9
21	M	48	μ	206	57	2.0	0.1
22	F	38	μ	520	106	8.3	3.3
23	F	64	μ	383	132	4.1	2.7
24	F	42	M	283	79	2.2	1.9
25	M	51	EX	300	83	3.0	1.0
26	M	50	EX	626	174	2.4	–
27	F	42	μ	236	66	8.1	1.5
28	M	38	EX	343	70	0.2	0.1
29	M	44	M	500	139	3.2	3.0

¹ Presurgical neuroradiological imaging: μ = microadenoma, M = intrasellar macroadenoma, EX = macroadenoma with extrasellar extension.

² In μ g/l. Normal IGF-I values in our laboratory are 114–492 μ g/l in patients 25–39 years old, 90–360 μ g/l in patients 40–54 years old, 71–290 μ g/l in patients older than 55 years.

³ Expressed as percent of the upper limit of the normal age-adjusted range.

⁴ In μ g/l, mean of three samples.

⁵ GH nadir in μ g/l during OGTT, – = no decrease or paradoxical increase.

Intra-assay coefficients of variation were 3.5% for GH and 3.7% for IGF-I.

Normal values in our laboratory for IGF-I are: 114–492 $\mu\text{g/l}$ in patients 25–39 years old, 90–360 $\mu\text{g/l}$ in patients 40–54 years old, 71–290 $\mu\text{g/l}$ in patients older than 55 years.

For GH levels the detection limit was 0.1 $\mu\text{g/l}$.

Statistical analysis

Values are expressed as median and range, due to non-normal distribution. Basal GH levels are the mean of the three samples collected before Glc administration. Areas under curves (AUCs) were calculated by trapezoidal integration, subtracting basal values: AUC_{0-150} between 0 and 150 min, $\text{AUC}_{150-300}$ between 150 and 300 min. GH rebound was arbitrarily defined as a GH increase after Glc-induced suppression that was greater than 10 $\mu\text{g/l}$. IGF-I values are expressed both as absolute values and as percentages of the upper limit of the normal age-adjusted range.

Data were analyzed by Student's *t*-test, Mann–Whitney test, Wilcoxon test, Kruskal–Wallis ANOVA followed by Dunn's test, Pearson correlation test, or McNemar's test, as appropriate.

Values of *P* less than 0.05 were considered significant.

Results

Basal GH levels

GH was below 2 $\mu\text{g/l}$ in 5 patients (# 2, 8, 9, 16, 28) (two out of these, # 16 and 28, had levels <1 $\mu\text{g/l}$), ranged between 2 and 5 $\mu\text{g/l}$ in 15 (# 1, 3, 4, 6, 12, 14, 15, 17, 19, 21, 23–26, 29), and was ≥ 5 $\mu\text{g/l}$ in 9 (# 5, 7, 10, 11, 13, 18, 20, 22, 27).

IGF-I levels

In 12 patients (# 2, 8, 12, 15–17, 20, 21, 24, 25, 27, 28), normal age-adjusted values were reached. Among the other 17 patients, 11 (# 1, 3–7, 9, 19, 22, 23, 29) achieved IGF-I values ranging between 101 and 140% of the upper limit of the normal age-adjusted range.

OGTT

GH values were suppressed from 2.5 $\mu\text{g/l}$ (0.2–24.3) to 1.9 $\mu\text{g/l}$ (0.1–10.3) in the whole group ($P=0.006$). The analytical evaluation showed that in 16 out of 29 patients, GH did not decrease below 2 $\mu\text{g/l}$. In the remaining 13 patients (# 1, 2, 4, 8, 9, 15–17, 21, 24, 25, 27, 28), GH levels fell below 2 $\mu\text{g/l}$ and in 7 out of these (# 4, 9, 15–17, 21, 28), GH nadir was below 1 $\mu\text{g/l}$.

On considering as cut-off OGTT-induced GH levels <2 $\mu\text{g/l}$ and normal age-adjusted IGF-I values, the patients fell into two categories:

Group I – 13 patients with GH levels below 2 $\mu\text{g/l}$ after Glc load. Ten of them (# 2, 8, 15–17, 21, 24, 25, 27, 28) reached normal age-adjusted IGF-I levels. In the remaining three patients (# 1, 4, 9), IGF-I levels remained pathological but their individual IGF-I levels were near normal, i.e. 118, 110, and 110% of the upper limit of the normal age-adjusted range respectively.

Group II – 16 patients with GH levels greater than 2 $\mu\text{g/l}$ after Glc load. Fourteen of them (# 3, 5–7, 10, 11, 13, 14, 18, 19, 22, 23, 26, 29) had pathological IGF-I levels, whereas in two patients (# 12, 20) normal age-adjusted IGF-I levels were obtained.

If patients belonging to the two groups were distinguished according to the presurgical tumor size, no significant difference was observed in outcome.

Evaluation of the whole OGTT-induced GH curve

A rebound burst of GH release (≥ 10 $\mu\text{g/l}$) was observed in 9 (# 1, 4, 15–17, 21, 25, 27, 28) out of the 13 patients belonging to Group I (regarded as cured) (Fig. 1). It occurred between 180 and 240 min after Glc load and ranged between 8 and 200 times the nadir value. The basal GH level in these 9 patients was 2.1 $\mu\text{g/l}$ (0.2–8.1), OGTT-induced GH nadir was 0.5 (0.1–1.5) $\mu\text{g/l}$, late peak was 14.4 (10.7–19.1) $\mu\text{g/l}$; IGF-I was 320 (205–362) $\mu\text{g/l}$. Basal GH (1.6 $\mu\text{g/l}$ (1.0–2.2)), GH nadir after Glc load (1.1 $\mu\text{g/l}$ (0.4–1.9)), and IGF-I (268 $\mu\text{g/l}$ (220–395)) values of the other four patients (# 2, 8, 9, 24) without OGTT-induced late GH peaks were not different from the corresponding values of the nine patients reported above. No difference was observed in Glc nadir between the two groups. On the other hand, individual analysis of OGTT-induced GH nadir in these two groups showed GH below 1 $\mu\text{g/l}$ in six of nine of the rebound group vs one of four in the group without rebound.

On examining the AUCs of GH, a significant difference was observed only in the final portion of the OGTT curve, i.e. after 150 min (801 (468–1832) $\mu\text{g/l}$ per 150 min in patients with rebound vs 140.5 (–23 to +177) $\mu\text{g/l}$ per 150 min in patients without rebound, $P=0.006$), whereas the difference was not significant in the first part of the curve (–141 (–350 to +27) $\mu\text{g/l}$ per 150 min in patients with rebound vs –25 (–65 to +17) $\mu\text{g/l}$ per 150 min in patients without rebound).

Finally there was a significant inverse correlation ($r=-0.94$, $P=0.017$) between GH rebound and plasma Glc nadir. Once again, if these 13 patients were divided according to presurgical tumor size, no significant difference was observed in the occurrence of GH rebound. No GH rebound was observed in the 16 patients belonging to Group II.

Discussion

In the present study we report for the first time the restoration of GH rebound after OGTT-induced nadir in

Figure 1 Individual plasma levels of GH after an OGTT (75 g p.o. at time 0) obtained in the nine patients with late GH burst.

surgically cured acromegalics. This GH rebound (i.e. increase of GH levels $>10 \mu\text{g/l}$, 8–200 times the nadir value, and of AUC in the late portion of the sampling period) was observed in nine patients. In all these nine patients GH fell below $2 \mu\text{g/l}$ in the hyperglycemic phase of the OGTT (and below $1 \mu\text{g/l}$ in seven of them) and normal age-adjusted IGF-I values were obtained in seven (and near-normal in the other two patients). This phenomenon was observed in patients assumed to maintain a normally working hypothalamo-pituitary connection. These patients fulfilled the strict criteria reported in the Methods section, i.e. lack of pituitary failure, transsphenoidal surgery by the same neurosurgeon and no previous radiotherapy. The temporal relationship between OGTT-induced GH nadir and the subsequent burst of GH secretion (shaped as a curve in all the patients) seems to point to hormonal rebound, quite different from the occurrence of spontaneous fluctuations or secretory spikes. The inverse correlation between GH rebound and nadir Glc levels in the final phase of the OGTT could be regarded as further evidence that this GH burst is not a spontaneous peak.

We are not aware of previous results on this topic in acromegalic patients, whereas it has already been described in normal subjects. Glc administration has a biphasic effect on GH secretion in man. Following oral Glc, initial plasma GH suppression is followed by a marked rise (4). Only few data about the late GH peak in

normal subjects are available; indeed a wide variability of GH rebound after Glc administration was reported, as well as a poor or absent GH response in some individuals (6). Moreover, the reproducibility of this response is still to be demonstrated. The mechanism mediating the GH response to Glc is not yet fully elucidated. GH secretion is regulated mainly by the interplay between the hypothalamic hormones, growth hormone-releasing hormone (GHRH) and somatostatin (SRIH) (7), so that changes of these peptides are likely to be involved. Direct experimental evidence for this hypothesis is the lack of Glc effect on GH and thyrotropin release from rat pituitary cells *in vitro* (8) and the inverse relationship between Glc concentration and SRIH release from rat hypothalamic cells *in vitro* (9, 10). The clinical counterpart of this experimental evidence is Glc-induced block of GH response to stimuli acting at the suprapituitary level, such as arginine (11), exercise (12) and L-DOPA (13). All these data seem to imply that the action of Glc on GH release is exerted by a stimulation of hypothalamic SRIH.

As for the late GH rise after Glc administration, some hypotheses have been put forward. GHRH may be involved; when Glc is administered 3.5 h before GHRH, GH release is enhanced (14). Valcavi (6) hypothesized that oral Glc administration could enhance hypothalamic SRIH release, thus blunting plasma GH levels for 2–3 h. In this phase GH should be stored at the pituitary level; as SRIH tone fades and

endogenous GHRH resumes its activity, GH is released from pituitary stores, and a rebound in serum GH levels is observed.

The persistence of an SRIH tone in some patients with active acromegaly whose hypothalamus–pituitary network was intact has already been reported (15, 16). It was hypothesized that the high somatostatinergic tone induced by high circulating GH and IGF-I levels could not be further stimulated but could be suppressed in those patients.

Whatever the mechanism of this phenomenon (GHRH discharge?/end of SRIH inhibition?), GH rebound could be regarded as a marker of recovered integrity of the hypothalamic–pituitary axis and of normal GH secretion by pituitary cells. In contrast the lack of a late GH peak in the remaining patients regarded as cured by standard criteria could be due to injury to the pituitary stalk caused by the adenoma or by surgical manipulation. We hypothesize that, even though the injury is not great enough to cause pituitary failure, it might have provoked a subtle alteration in the delicate interplay of hypothalamic–pituitary hormones regulating GH secretion. In addition a lack of SRIH tone may be suggested.

As for the five patients of this series with conflicting GH and IGF-I values, it is disappointing to find a discrepancy. A discordance between GH and IGF-I levels has already reported in some acromegalic patients. Wass (17) reported normal GH values with elevated IGF-I levels in 8% of 73 acromegals off medical treatment and normal IGF-I values with GH levels still high in 19% of cases. Indeed GH suppression after the OGTT and IGF-I levels reflect different physiopathological mechanisms, i.e. the integrity of GH neuroregulation and the normalization of integrated daily concentration of GH respectively. As for the three patients with normal GH suppression after an OGTT and near normal IGF-I values, normally working hypothalamo–pituitary connections may be suggested, in the face of the persistence of a very low degree of GH hypersecretion due to the occurrence of abnormally frequent pulses of secretion (18). In contrast, for the two patients with normal IGF-I values but unsatisfactory suppression of GH after Glc load, a subtle disruption of the integrity of the hypothalamo–pituitary network can be hypothesized. Perhaps further evaluation in a larger series of patients with conflicting data between OGTT-induced GH suppression and IGF-I levels might show whether prolonged GH sampling after oral Glc administration could have a clinical usefulness in the evaluation of the surgical cure of acromegaly.

In summary, the present criteria of cure for acromegaly include the restoration of GH suppression after an OGTT and the normalization of IGF-I levels. Additional criteria of cure, i.e. the disappearance of paradoxical GH responses, such as to TRH, and the restoration of normal GH diurnal rhythm, are suggestive of the restoration of normal GH pathophysiology.

The occurrence of late GH rebound to Glc in cured acromegals, reported for the first time in this paper, could be regarded as a further tool in the evaluation of restoration of normal GH pathophysiology after neurosurgery. The follow-up of these patients will perhaps allow us to establish if the occurrence of GH rebound after Glc suppression may be predictive of ultimate cure.

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