Reduced disorderliness of growth hormone release in biochemically inactive acromegaly after pituitary surgery

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Abstract

The episodicity of 24 h GH release was studied in 18 patients with active acromegaly, 12 patients 7–10 days after pituitary surgery, 14 patients long after operation (3–17 years), and 21 healthy gender- and age-matched control subjects, using a recently introduced scale- and model-independent regularity statistic, approximate entropy (ApEn). Blood samples were taken at 10-min intervals for 24 h, and plasma GH concentrations were measured by immunofluorometric assay (detection limit 11.5 ng/l). For this study we selected operated patients who were biochemically in remission, defined by normal circulating IGF-I and insulin-like growth factor-binding protein-3 (IGFBP-3) concentrations, normal glucose-suppressed plasma GH concentration ($<0.38 \mu\text{g/l}$), and the normalization of the paradoxical rise of GH to TRH or GnRH. In patients with active acromegaly ApEn was 1.23 ± 0.04 , with no overlap with the control subjects ($P = 1.2 \times 10^{-16}$), who had an ApEn of 0.40 ± 0.04 . ApEn in patients shortly after surgery was 0.71 ± 0.09 ($P < 0.001$ vs controls), and long after surgery 0.56 ± 0.05 ($P < 0.011$ vs controls). ApEn values in treated and untreated patients correlated significantly with the plasma concentration of IGF-I ($r = 0.531$) and IGFBP-3 ($r = 0.598$), and the log-transformed 24 h GH secretion rate ($r = 0.749$). Shortly after surgery only one-third of the patients had a normal ApEn value, whereas long after surgery about 70% of the patients had a normal ApEn value. Although ApEn eventually normalized in about 70% of the operated patients, the cause of the persistence of abnormal GH release in the remainder of the subjects is not known, and might reflect permanent hypothalamic–pituitary dysfunction or a very early recurrence of the somatotroph adenoma.

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Introduction

Growth hormone (GH) secretion in clinically active acromegaly is characterized by increased pulse frequency, and raised basal (nonpulsatile) secretion (1, 2, 3). Recent studies, applying a new regularity statistic, approximate entropy (ApEn), have revealed that the GH secretion pattern is also highly irregular (4, 5, 6). This inference has been supported further by other independent studies showing reduced predictability of GH release (by an adaptive neural network analysis) in acromegalic patients (7). Irregular and disorganized secretion might be a fundamental feature of endocrine tumors, since it has also been described for aldosterone-secreting adenoma (8), and for adrenocorticotropin (ACTH) and cortisol secretion in patients with Cushing's disease (9). Notably, eight surgically treated acromegalic patients studied by Hartman *et al.* (4) showed intermediate irregularity, i.e. somewhat increased ApEn values and hence greater disorderliness compared

with control subjects, yet with lower values than untreated patients (4). The purpose of the present study was to investigate in a considerably larger group of surgically treated acromegalic patients whether preoperatively disorganized GH secretion could normalize eventually after treatment. Therefore, we investigated 24 h GH profiles 7–10 days after surgery in patients who ultimately remained in clinical and biochemical remission 0.5–5 years after the operation. In addition, another group of patients, some of whom were studied shortly after surgery, underwent this investigation 3–17 years after the operation. By using this strategy, we could test the potential reversibility of quantifiably increased disorderliness of GH release in transsphenoidally operated acromegalic patients and concomitantly could evaluate the utility of ApEn as a potential marker of clinical outcome. This work was presented in part at the 4th International Congress of Endocrinology, San Francisco, 1996.

Patients and methods

Ten healthy men and 11 healthy women of normal height and body mass index (BMI) served as matched controls for the acromegalic patients. Controls and patients originated from the same community, and were evaluated in an identical sampling paradigm and GH assay (below).

Informed consent was obtained from all subjects and the study was approved by the ethics committee of the Leiden University Medical Center. The age of the male controls was 45 ± 2.6 years (mean $\pm$ S.E.M.) and of the female controls 51 ± 3.7 years. The three groups of patients investigated included 18 untreated subjects with active acromegaly, 12 patients shortly after successful pituitary microsurgery (i.e. 7–10 days), and 14 patients long after surgery (3–17 years, mean 6.9 years). The control subjects, the patients with untreated acromegaly, and the patients shortly after pituitary surgery had participated in a previous study, in which GH secretion was measured with a multiparameter deconvolution analysis (3). The follow-up period of the patients who were studied shortly after surgery was 0.5–5.0 years, with a mean of 4.2 years. One patient who had the shortest follow-up period unfortunately died during gastroscopy in a nearby general hospital. All the patients had a normal suppressed GH concentration ($<0.38 \mu\text{g/l}$ or $<1 \text{ mU/l}$) during glucose loading in yearly studies. In addition, in all operated patients paradoxical reactions of GH to thyrotropin-releasing hormone (TRH) and/or gonadotropin-releasing hormone (GnRH) normalized. The ages of the various patient groups and their BMIs are listed in Table 1. Not unexpectedly, BMI was increased in the acromegalic patients, irrespective of their GH secretory status, since body composition in patients with active acromegaly is characterized by increases in total body weight, extracellular water, and lean body mass, and decreased fat mass, compared with healthy height-adjusted controls (10, 11). In patients after surgical treatment, and in clinical and biochemical remission, body weight and BMI remained unchanged. This was accounted for by an increase of body fat to normal values, and a decrease of extracellular water and decrease of lean body mass.

None of the treated or untreated acromegalic patients used hormone substitution therapy with the exception of

a female patient, who was treated surgically for papillary thyroid cancer 10 years before the acromegaly was discovered and received thyroid hormone treatment. The postmenopausal women (five controls, four patients shortly after surgery, and four long after surgery) studied here did not use estrogen therapy. Patients and volunteers were hospitalized the evening before the sampling studies. On the following morning, an indwelling i.v. cannula was inserted in a large vein of the forearm, and blood samples were withdrawn at 10-min intervals starting at 0900 h and for the next 24 h. A slow i.v. infusion of 0.9% NaCl and heparin (1 U/ml) was used to keep the line open. The subjects were free to move around, but not to sleep, during the daytime. Meals were served at 0800, 1230 and 1730 h. Lights were turned off between 2200–2400 h, depending on the sleeping habits of the patient. No sleep monitoring was carried out. Premenopausal female control subjects were studied in the early follicular phase of the menstrual cycle.

The endocrine tests performed in the patients before and after surgery, and during follow-up studies, included an oral 75 g glucose tolerance test, and bolus corticotropin-releasing hormone (CRH), TRH and GnRH tests on separate days. In the surgically treated patients the mean nadir plasma GH concentration during the glucose tolerance test was $0.13 \pm 0.02 \mu\text{g/l}$ (range 0.015–0.37 $\mu\text{g/l}$). Paradoxical GH increases to TRH and/or GnRH, defined as a doubling of the basal GH concentration and an incremental increase above 1.9 $\mu\text{g/l}$, were absent after surgery in all patients. Patients with active acromegaly had an elevated circulating GH concentration during oral glucose loading (mean $31.0 \pm 6.9 \mu\text{g/l}$, range 7.3–113 $\mu\text{g/l}$). The plasma IGF-I concentration was increased in these patients (mean 59 nmol/l, range 35–118 nmol/l).

Assays

Plasma GH was measured with a sensitive time-resolved fluoro-immunoassay (Wallac, Turku, Finland). The assay is specific for the 22 kDa GH. The standard was biosynthetic recombinant human GH (Genotropin, Pharmacia Upjohn, Uppsala, Sweden), and was calibrated against the WHO First International Reference Preparation 80/505 (to convert $\mu\text{g/l}$ to mU/l, multiply

Table 1 Clinical and biochemical characteristics (mean $\pm$ S.E.M.) of the control subjects and patients.

	Number of subjects	Age (years)	BMI (kg/m^2)	IGF-I (nmol/l)	IGFBP-3 (nmol/l)	GH secretion ($\mu\text{g/l/day}$)
Controls	21	48.5 ± 2.4	23.9 ± 0.7	18.1 ± 1.5	82 ± 5.6	30 ± 4.6
Patients shortly after surgery	12	49.0 ± 3.6	27.7 ± 0.9^a	20.7 ± 1.3	95 ± 4.2	35 ± 5.4
Patients long after surgery	14	55.0 ± 2.6	28.1 ± 1.1^a	14.9 ± 1.3	79 ± 4.6	35 ± 6.9
Untreated patients	18	50.6 ± 3.1	27.1 ± 1.0^a	59.8 ± 6.4^b	164 ± 8.4^c	1620 ± 410^d

Statistical differences between patients and control subjects, ^a $P < 0.001$, ^b $P < 0.0001$, ^c $P < 0.0005$ (Student's *t*-test);

^d $P < 0.01$ (Wilcoxon test).

by 2.6). The limit of detection (defined as the value 2 s.d. above the mean value of the zero standard) was 11.5 ng/l. The intraassay coefficient of variation varied from 1.6–8.4% in the assay range from 0.1–18 μ g/l (0.26–47 mU/l), and the interassay coefficient of variation was 2.0–9.9% in the same range. The total plasma IGF-I concentration was measured by RIA (Incstar, Stillwater, MN, USA), after extraction and purification (12). The interassay coefficient of variation was less than 11%. The limit of detection was 1.5 nmol/l. Normal values are 9.0–34.0 nmol/l for subjects aged 30–50 years, 8.0–27.0 nmol/l for those aged 50–70 years, and 8.0–22.0 nmol/l for those aged 70 years or more. Plasma IGFBP-3 concentration was measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The interassay coefficient of variation was below 6.8% at different concentrations. The limit of detection was 2.8 nmol/l. Normal values are 45.6–122 nmol/l for subjects aged 30–50 years, and 49.0–112 nmol/l for subjects between 50–70 years.

Deconvolution analysis

Multiple parameter deconvolution was used to estimate various specific measures of hormone secretion and half-life from all plasma hormone concentrations and their dose-dependent intrasample variances considered simultaneously (13). Total production is the sum of basal production and pulsatile production, which were estimated as described (14, 15).

Approximate entropy, quantification of episodicity

The regularity or orderliness of GH release over 24 h was quantified by an approximate entropy statistic, ApEn (5, 6, 16, 17). ApEn provides a relative measure of pattern repetition within the hormone profile by assigning a single (positive) number whose value increases with greater disorder or more irregularity. This statistic exhibits high sensitivity (>90%) and specificity (>90%) in distinguishing the relative orderliness of GH, aldosterone, and ACTH release in normal vs tumoral secretory profiles and in the case of GH, in healthy men compared with women (4, 8, 9, 18). For the GH time series each comprising 145 observations, we used ApEn(1,20%) and ApEn(2,20%) as a scale- and model-independent statistic calculated for window lengths (m) of 1 and 2 respectively, and a tolerance (r) of 20% of the overall s.d. of the individual subject's 24 h serum GH concentration profile. Adjusting the tolerance to each subject's series s.d. normalizes ApEn to otherwise unequal mean hormone levels.

Statistical analysis

Data are given as the mean $\pm$ s.e.m., unless otherwise mentioned. Statistical analyses were done by using

Figure 1 ApEn(1,20%) values in controls, surgically treated patients and active untreated acromegalic patients. Open symbols are female subjects and closed symbols males. CTR = controls; PSAS = patients shortly after surgery; PLAS = patients long after surgery; UP = untreated active acromegalics.

ANOVA, regression techniques, and Student's *t*-test for paired and non-paired data. Statistical results were corroborated by non-parametric tests. Calculations were made with SPSS Windows version 7.0 and with Systat, version 6 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

In 10 healthy male controls, ApEn(1,20%) was 0.27 ± 0.04 , and in 11 female controls 0.52 ± 0.04 . In untreated patients with active acromegaly ApEn(1,20%) was highly significantly increased, with complete separation of the active acromegalic group and the sex- and age-matched controls (see Fig. 1 and Table 2). In 11 untreated male patients ApEn(1,20%) was 1.22 ± 0.06 ($P = 2.12 \times 10^{-10}$), and in 7 untreated female patients 1.24 ± 0.08 ($P = 8.3 \times 10^{-8}$). Shortly after surgery, ApEn(1,20%) was 0.74 ± 0.18 in 5 male patients ($P = 0.004$) and 0.62 ± 0.06 in 7 female patients ($P = 0.12$). Long after surgery ApEn(1,20%) was 0.48 ± 0.05 in 6 male patients ($P = 0.004$) and 0.68 ± 0.11 in 8 female patients ($P = 0.19$). For

Table 2 ApEn(1,20%) values (mean $\pm$ s.e.m.) in controls and acromegalic patients.

	Males	Females
Controls	0.27 ± 0.04	0.52 ± 0.04
Patients shortly after surgery	0.74 ± 0.18^a	0.68 ± 0.11^b
Patients long after surgery	0.48 ± 0.05^a	0.62 ± 0.07^b
Untreated patients	1.22 ± 0.06^c	1.24 ± 0.08^c

Statistical comparisons were made between sex-matched controls and the patient groups with the two-tailed Student's *t*-test.

^a $P = 0.004$, ^b NS, ^c $P < 10^{-7}$.

ApEn(2,20%), comparable statistical results were obtained (data not shown). In the ANOVA of ApEn the only significant factor was the subject group, i.e. controls and the various groups of patients, as outlined in the Methods section, but not the sex of the individuals or the interaction between these two categorical variables and therefore the ApEn values for the combined groups are also calculated. The mean ApEn(1,20%) in control subjects was 0.40 ± 0.04 , in active acromegaly 1.23 ± 0.04 ($P = 1.2 \times 10^{-16}$ vs controls), shortly after surgery 0.71 ± 0.09 ($P = 0.001$ vs controls), and 0.56 ± 0.05 longer after surgery ($P = 0.011$ vs controls). Comparable statistical results were obtained for ApEn(2,20%) (data not shown). The circulating plasma concentrations of IGF-I and IGFBP-3 and the total 24 h GH secretion rate expressed per liter distribution volume (V_d) of the controls and patients are listed in Table 1. No significant differences were present in circulating concentrations of IGF-I and IGFBP-3 between controls and treated patients. This was also true for the GH secretion rate per 24 h. In active acromegaly GH production was increased 50-fold, plasma IGF-I concentration 3-fold, and plasma IGFBP-3 concentration 2-fold.

In Fig. 2 the ApEn(1,20%) values for the surgically treated patients are plotted against the glucose-suppressed GH concentration. In addition, the figure also shows the upper normal limits (mean + 2 s.d.) for ApEn(1,20%) in males and females (0.50 and 0.70 respectively). Only one of the five male subjects who were investigated a week after surgery had a normal ApEn(1,20%), but in females three out of seven had reached a normal ApEn(1,20%). In contrast, long (3–17 years) after surgery, most subjects had ApEn values

Figure 2 Relationship between the suppressed serum GH concentrations obtained during the oral glucose tolerance test and ApEn(1,20%). Normal suppressed GH concentration, in our healthy controls, is less than $0.38 \mu\text{g/l}$ (to convert this value to mU/l multiply by 2.6). Horizontal lines refer to the upper normal limits of ApEn(1,20%): 0.5 for males and 0.7 for females. $\blacktriangledown$, ∇ patients shortly after surgery; $\blacktriangle$, $\triangle$ patients long after surgery. Open symbols are female subjects and closed symbols males.

within a normal control range, and only two female and two male subjects retained elevated ApEn values. As well, three other (non-cured) patients (two male and one female), who also underwent the sampling study shortly after surgery, but who did not normalize in terms of glucose-suppressed GH (0.37 , 0.41 and $0.85 \mu\text{g/l}$) or still had a paradoxical rise to TRH, showed increased ApEn(1,20%) values of 1.17 , 0.78 and 0.79 respectively.

A subgroup of six patients underwent a 24 h sampling study both immediately and later after surgery (two male and four female patients). The mean ApEn(1,20%) for this subgroup was 0.79 ± 0.10 shortly after surgery, and 0.57 ± 0.10 long after surgery. Although the mean ApEn became lower, the difference was statistically not significant ($P = 0.17$ in the two-tailed Student's *t*-test). The daily GH production rates were similar at both times, namely $30.0 \pm 5.0 \mu\text{g/l}_v$ and $36.0 \pm 9.2 \mu\text{g/l}_v$ ($P = 0.509$), and this was also true for the circulating concentrations of IGF-I and IGFBP-3. Plasma IGF-I and IGFBP-3 concentrations shortly after surgery were $19.8 \pm 0.7 \text{ nmol/l}$ and $88.0 \pm 5.6 \text{ nmol/l}$ respectively, and during follow-up $17.5 \pm 1.9 \text{ nmol/l}$ and $85.2 \pm 5.9 \text{ nmol/l}$ (not statistically significant).

Finally, we also explored the relationship between ApEn and the circulating concentrations of IGF-I, IGFBP-3, and the 24 h secretion rate of GH for treated and untreated patients. The correlation coefficients between ApEn(1,20%) and IGF-I, IGFBP-3 and the log GH production rate were 0.531 , 0.598 , and 0.749 respectively. The statistical significance of these regressions was high, and the *P* values were less than 0.0001 . In Fig. 3 the relationship between ApEn(1,20%) and the GH production rate is shown for untreated and treated acromegalic patients, clearly depicting the increase of ApEn with higher GH production.

Figure 3 Relationship between the calculated daily GH secretion rate, determined by deconvolution analysis, and ApEn(1,20%). $\blacksquare$, $\square$ patients with active acromegaly; $\blacktriangledown$, ∇ patients shortly after surgery; $\blacktriangle$, $\triangle$ patients long after surgery. Open symbols are female subjects and closed symbols males. The regression line is shown; the correlation coefficient was 0.749 , $P < 0.0001$.

Discussion

The present study first confirms earlier data by Hartman *et al.* (4) that GH release in active acromegaly is more markedly disorderly or irregular compared with normals, as demonstrated by highly increased ApEn values. In the study of Hartman and colleagues, the number of treated patients was small, they had circulating IGF-I concentrations in the high normal range of young adults and no clinical follow-up data were provided. Furthermore, some of these patients also underwent radiation therapy. Therefore that group of patients might not have been completely in remission, in contrast to our selected group of patients in whom long-term GH data were used to define their GH status both shortly and long after surgery. In addition, none of our patients underwent postsurgical radiation therapy, and none had partial or complete pituitary failure before and after operation. Therefore, several potential confounding factors on ApEn were excluded. As described previously, ApEn was larger in females than in males (4, 18). After microsurgery, even in the early postoperative period, ApEn values in our patients in biochemical remission were significantly lower than in active acromegaly, indicating greater regularity of GH release. One-third of the operated patients had an ApEn value in the normal range for age and gender soon after surgery. Long after surgery, ApEn values were normal in 70% of the patients, all of whom were carefully selected in terms of potential permanent cure. Each showed normal glucose-suppressed GH concentrations below $0.38 \mu\text{g/l}$ (1 mU/l), not only at the time of the 24 h sampling study, but also during follow-up studies. In addition, paradoxical reactions of GH toward TRH and/or GnRH were normalized, and this was also true for the circulating concentrations of IGF-I and IGFBP-3. By using these strict criteria, we are convinced that the investigated patients were in biochemical remission and that therefore the slightly raised ApEn values in some patients do not point necessarily to a very early recurrence of the disease.

We observed a tendency for lower ApEn values in paired comparisons of patients studied twice (early and late) after surgery, but the difference was not statistically significant because of the limited number of patients. This observation might indicate, in the absence of pituitary deficiencies, that surgery as such, or other, non-specified, postoperative factors might influence the regularity of GH release. This is supported by the observation that the GH production rate and GH-dependent proteins such as IGF-I and IGFBP-3 were normalized after surgery, and remained stable in this subgroup. At present, however, we cannot prove or refute a non-specific influence of pituitary surgery, since neither we nor others have studied GH secretion profiles in patients with non-GH secreting adenomas after pituitary surgery.

The mechanism underlying the decreased regularity (i.e. increased disorderliness) of GH secretion in

acromegaly is not established, but probably reflects loss of within-axis network coherence, reduced feedback control, and/or greater complexity of biochemical input to the tumoral secretory pathway. The present data do not allow a distinction among these possibilities. Several clinical observations have suggested that GH pulses in this disease are generated probably within the tumor, rather than from physiological input signals such as growth hormone-releasing hormone (GHRH) and somatostatin (1, 3, 4, 19). Further evidence for the tumoral origin of pulses was obtained by others in *in vitro* data of adenoma tissue, which exhibits episodic GH release in the absence of hypothalamic GHRH and somatostatin (20, 21). The presence of a pituitary tumor *per se* does not decrease the regularity of GH secretion, since we have observed normal ApEn values for plasma GH profiles in five patients with untreated prolactinomas (unpublished data). By using a system of multiple neural networks, acting in parallel (adaptive mixtures of local experts), Prank and coworkers (7) could distinguish normal controls from active acromegals and octreotide-treated patients, and their results corroborate our present data.

The approximate entropy in acromegalic patients correlated highly significantly with the GH secretion rate, and somewhat less with the circulating concentrations of IGF-I and IGFBP-3. The regression analysis was significant when all acromegals, surgically treated and non-treated, were considered together, but not when analyzed separately. A clear dissociation between unchanging ApEn values (data not shown), GH secretion rates and circulating IGF-I concentrations was observed in a previously reported series of acromegalic patients treated with octreotide (22). Therefore, the relationship as discussed above depends more on the presence of an adenoma than on GH concentration *per se* or other potentially confounding factors such as gender and age. In our normal controls, ApEn did not correlate with the 24 h GH secretion rate or the mean plasma GH concentration, indicating that ApEn in healthy subjects does not depend on GH production rate. In fact, it has been demonstrated that with increasing age and adiposity as plasma GH concentrations fall, ApEn levels increase (23–25). Finally, although we had expected normal ApEn values in all our patients in biochemical remission long after surgery, since they all had a normal total daily GH secretion rate, normal basal GH release, low interpulse serum GH concentrations, suppressed GH concentrations following glucose loading, and loss of anomalous responses to hypothalamic peptides, this was not found in four patients. These patients did not differ from the other treated subjects in terms of tumor size, severity or duration of the disease, or gonadal status. This observation might therefore point to a permanent derangement of the hypothalamus by unknown factors, altered intrapituitary architecture with disrupted paracrine regulation or the possibility of remarkably delayed

tumor recurrence. A determination of whether the persistence of increased values of ApEn postoperatively in clinically inactive acromegalic patients reflects continued hypothalamic–pituitary dysregulation or presages delayed tumor recurrence will require extended biochemical and clinical follow-up in such patient populations.

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