Pituitary-hormone secretion by thyrotropinomas

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Abstract Hormone secretion by somatotropinomas, corticotropinomas and prolactinomas exhibits increased pulse frequency, basal and pulsatile secretion, accompanied by greater disorderliness. Increased concentrations of growth hormone (GH) or prolactin (PRL) are observed in about 30% of thyrotropinomas leading to acromegaly or disturbed sexual functions beyond thyrotropin (TSH)-induced hyperthyroidism. Regulation of non-TSH pituitary hormones in this context is not well understood. We there therefore evaluated TSH, GH and PRL secretion in 6 patients with up-to-date analytical and mathematical tools by 24-h blood sampling at 10-min intervals in a clinical research laboratory. The profiles were analyzed with a new deconvolution method, approximate entropy, cross-approximate entropy, cross-correlation and cosinor regression. TSH burst frequency and basal and pulsatile secretion were increased in patients compared with controls. TSH secretion patterns in patients were more irregular, but the diurnal rhythm was preserved at a higher mean with a 2.5 h phase delay. Although only one patient had clinical acromegaly, GH secretion and IGF-I levels were increased in two other patients and all three had a significant cross-correlation between the GH and TSH. PRL secretion was increased in one patient, but all patients had a significant cross-correlation with TSH and showed decreased PRL regularity. Cross-ApEn synchrony between TSH and GH did not differ between patients and controls, but TSH and PRL synchrony was reduced in patients. We conclude that TSH secretion by thyrotropinomas shares many characteristics of other pituitary hormone-secreting adenomas. In addition, abnormalities in GH and PRL secretion exist ranging from decreased (joint) regularity to overt hypersecretion, although not always clinically obvious, suggesting tumoral transformation of thyrotrope lineage cells.

Introduction

The major regulators of thyrotropin (TSH) secretion are thyrotropin-releasing hormone (TRH), the inhibitory neurotransmitters dopamine and somatostatin, and negative feed back by thyroid hormones, thyroxine and triiodothyronine [1]. The interplay among these regulators in time dictates the TSH secretion pattern, which is characterized by a diurnal variation of serum TSH concentrations with superimposed (small) bursts. The 24-h secretion profile of TSH has been well described in various pathophysiological conditions, including hyperthyroidism, hypothyroidism, obesity, fasting and in non-thyroidal illness, but not in detail in hyperthyroidism caused by a pituitary

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TSH-secreting adenomas [2–6]. TSH-secreting adenomas are relatively rare pituitary tumors (less than 1% of all pituitary adenomas), and are usually diagnosed when the TSH level is inappropriately elevated in a patient with raised serum thyroxine levels, combined with the presence of a pituitary (micro) adenoma [7]. In other hormone-secreting pituitary adenomas, e.g. Cushing's disease and prolactinomas, hormone secretion is marked by diminished or absent diurnal amplitude (one-half the difference between maximal and minimal concentrations), increased basal (i.e. non-pulsatile) secretion, elevated pulse frequency and reduced secretory regularity [8–10].

By analogy with results obtained in other hormone-secreting pituitary adenomas, we hypothesized that patients with thyrotropinomas would display a blunted diurnal secretion pattern, heightened basal and pulsatile TSH release, increased pulse frequency and attenuated regularity. Moreover, TSH adenomas may co-secrete other pituitary hormones, most frequently growth hormone and in decreasing order prolactin and gonadotropins, as well as uncombined α-subunit [7]. To examine the mechanistic secretory similarities between TSH and co-secreted hormones, we quantitated correlations between hormone concentration time series (TSH, GH, prolactin and α-subunit) using selected model-free tools. Finally, many patients with thyrotropinomas are currently treated with slow-release somatostatin analog formulations. Therefore we also explored the changes in fasting hormone levels in patients treated with long-acting somatostatin analogs. To this end serum hormone concentration profiles of six patients with thyrotropinomas were compared with those of 18 healthy controls that were investigated in a strictly comparable way.

Patients and methods

Six patients (4 m/2 f) with central hyperthyroidism caused by a TSH-secreting adenoma and 18 healthy non-obese (BMI 18–25 kg/m^2) control subjects of similar sex (12 m/6 f) and age distribution were enrolled in this study, after giving written acknowledgment of informed consent for participation. All premenopausal controls were required to have a regular menstrual cycle and not use oral contraceptives. Female subjects were studied in the early follicular phase of their menstrual cycle. Chronic disease, depression (present or in history), smoking, recent transmeridional flights, night shift work, weight change (>3 kg in 3 months), and use of medication (except for the patients) were exclusion criteria. All control subjects had an unremarkable medical history, and no abnormalities were found during physical examination, standard laboratory hematology, and blood chemistry and urine tests.

Clinical protocol

The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands). Subjects were admitted to the Clinical Research Unit of the Department of Internal Medicine. A cannula for blood sampling was inserted into an antecubital vein. The cannula was attached to a three-way stopcock and kept patent by a continuous saline infusion. Blood samples were taken at 10-min intervals for determination of plasma TSH, GH, prolactin and α-subunit concentrations. Subjects remained recumbent, except for bathroom visits. No daytime naps were allowed. Meals were served according to a fixed time schedule. Lights were switched off at 2300 h. Vital signs were recorded at regular time intervals, and great care was taken not to disturb patients while sampling blood during their sleep (no electroencephalography sleep recording was performed).

Assays

Samples were centrifuged at 4000 rotations/min at 4°C for 20 min, within 60 min of sampling. Subsequently, plasma was divided into separate aliquots and frozen at −80°C until assays were performed. Samples of each subject were determined in the same assay run. Plasma TSH concentrations were measured with a time-resolved immunofluorometric assay (Wallac, Turku, Finland), and its standard was calibrated against the World Health Organization second standard International Reference Preparation (80/558) hTSH for immunoassays. The limit of detection was 0.05 mU/liter, and the interassay coefficient of variation was less then 5%. Free T$_4$ (fT$_4$) was estimated using an automated system (Elecsys 2010, Roche Diagnostics Nederland BV, Almere, Netherlands). Triiodothyronine was measured with Abbott Axsym (Abbott Park, IL).

GH was measured with a sensitive time-resolved fluoro-immunoassay (Wallac Oy, 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 μg/l to mU/l multiply by 2.6). The limit of detection of this assay (defined as the value 2SD above the mean value of the zero standards) was 0.01 mU/l (0.0038 ng/ml). The intraassay coefficient of variation varied between 1.6 and 8.4% in the range from 0.01 to 18 μg/l and interassay coefficient of variation was 2.0–9.0% in the same range.

Total IGF-I was determined by RIA (Incstar, Stillwater, MN) after extraction and purification on ODS-silica columns. The intraassay coefficient of variation was less than 11%. The detection limit was 1.5 nmol/l. Age-related normal data were determined in the same laboratory.

Plasma PRL concentration was measured with a sensitive time-resolved fluoroimmunoassay with a detection limit of 0.04 μg/l (Delfia, Wallac Oy, Turku, Finland). The intra-assay coefficient of variation varied from 2.0 to 3.3% and inter-assay coefficient of variation is 3.4–6.2%, in the concentration range from 3.0 to 80 μg/l.

The α-subunits were measured by an immunoradiometric assay (Immunotech, Marseille, France). The assay is calibrated with WHO International Reference Preparation 75/569, and 1 mU is equivalent to 1 μg. The intra-assay coefficient of variation was 6.8% and the inter-assay coefficient of variation 9.1% in the concentration range observed here.

Calculations and statistics

Deconvolution analysis

Each hormone concentration time series was analyzed using a recently validated deconvolution method [11]. The automated Matlab program first detrends the data and normalizes concentrations to the unit interval [0, 1]. Second, successive potential pulse-time sets, each containing one fewer burst, are created by a smoothing process (a nonlinear adaptation of the heat-diffusion equation). Third, a maximum-likelihood expectation (MLE) deconvolution method estimates all secretion and elimination rates simultaneously for each candidate pulse-time set. The deconvolution model specifies basal secretion (β_0), two half-lives (α_1, α_2), an accumulation process and weak interpulse length dependency for secretory-burst mass (η_0, η_1), random effects on burst mass (σ_A), procedural and measurement error (σ_ε), and a 3-parameter secretory-burst waveform (β_1, β_2, β_3). Model selection is performed to distinguish among the candidate pulse-time sets using the Akaike information criterion [12]. Observed interpulse intervals are described by a 2-parameter Weibull process (more general form of a Poisson renewal process). The parameters (and units) are frequency (number of bursts per unit time, *lambda* of Weibull distribution), regularity of interpulse intervals (unitless *gamma* of Weibull), slow half-life (min), basal and pulsatile secretion rates (concentration per unit time), mass secreted per burst (concentration), and waveform mode (time delay to maximal secretion after burst onset, min) [11].

Approximate entropy (ApEn)

Approximate entropy, ApEn, is a scale-and model-independent univariate regularity statistic used to quantitate the orderliness (subpattern consistency) of serial stationary measurements [13, 14]. Mathematical models and feedback experiments establish that pattern orderliness monitors feedback and/or feedforward interactions within an interlinked axis with high sensitivity and specificity, both >90% [15]. Reduced pattern regularity typifies hormone secretion in puberty and aging, during diminished negative feedback or fixed exogenous stimulation, and by autonomous neuroendocrine tumors [16, 17]. Data are presented as normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1000 randomly shuffled versions of the same series. ApEn ratios close to 1.0 express high irregularity (mean randomness) of pulsatile hormone patterns.

Cross-Approximate Entropy (X-ApEn) is a scale- and model-independent two-variable regularity statistic used to quantitate the relative pattern synchrony of coupled time series [18, 19]. Clinical experiments establish that changes in two-hormone synchrony monitor feedback and/or feedforward adaptations within an interlinked axis with high sensitivity and specificity [15]. To normalize comparisons among subjects, X-ApEn is computed on the paired original time series (observed X-ApEn) and then recalculated 1000 times after each series in a pair is shuffled randomly [rearranged in order or sequence without replacement or loss (random X-ApEn). Repetition of the permutation procedure allows calculation of the maximum, mean, and SD of random X-ApEn for a given series length and assay pair. A normalized distributional measure is then the number of SDS (z scores) separating observed from the maximum or mean random X-ApEn. Higher absolute z scores denote more synchronous patterns. Lower X-ApEn ratios and higher absolute z scores denote greater pattern synchrony between (coordinate control of) the interlinked signals. Reduced regularity of hormone secretion typifies puberty, aging, diminished negative feedback due to target-gland failure, fixed exogenous stimulation, and autonomous neuroendocrine tumors [20, 21].

Diurnal rhythmicity

Nyctohemeral variation of TSH concentrations was determined by a nonlinear unweighted least-squares cosine regression, as reported earlier [22]. Ninety-five percent statistical confidence intervals were determined for the 24-h cosine amplitude (50% of the zenith-nadir difference), mesor (rhythmic mean) and acrophase (clock time of maximal value).

Statistics

Data are presented as mean ± SEM, unless otherwise specified. Means of TSH concentration and secretion parameters of both groups were compared using two-tailed independent Student's *t*-test after logarithmic transformation or with the Kolmogorov–Smirnov two-sample test.

Conventional cross-correlations between hormone concentration series were calculated after prewhitening of the series by first differencing in order to prevent spurious correlations. Statistical calculations were performed with Systat software, version 11 (Systat Inc, San Jose, CA). Significance level was set at 0.05.

Clinical presentation of the patients

Patient 1 was studied when he was 73 years old. His medical history revealed that he underwent thyroid surgery at the age of 53 yr because of presumed hyperthyroidism. After surgery he developed visual complaints and headaches and he was diagnosed with a large pituitary adenoma. He underwent pituitary surgery and because of incomplete resection pituitary irradiation as well. He was treated with thyroxine, testosterone supplements and cortisone acetate. For the pituitary tumor residue he received first bromocriptine, and later when immunostaining revealed β-TSH, short-acting octreotide, 100 µg t.i.d. The 24-h blood sampling study was performed after withholding octreotide for 4 weeks. He is currently treated with octreotide-LAR, hydrocortisone, thyroxine and testosterone replacement.

Patient 2, aged 44 years was referred of acromegaly and central hyperthyroidism. After detailed clinical studies and pretreatment with sc octreotide (100 µg t.i.d.) he underwent transsphenoidal adenomectomy leading to biochemical cure of the central hyperthyroidism, but insufficient control of GH secretion. He is currently treated with octreotide only.

Patient 3, aged 38 years was evaluated at a teaching hospital because of weight loss and nervous symptoms. She was hyperthyroid, but TSH levels were not suppressed and MRI of the pituitary gland revealed a macroadenoma. For detailed endocrine studies she was referred to the Leiden University Medical Center. Subsequently, she was treated with octreotide LAR, with a favorable clinical response. After one year of treatment the 24-h blood sampling study was repeated.

Patient 4, aged 26 years was referred because of long-standing nervousness, palpitations and excessive perspiration. He appeared to be hyperthyroid with non-suppressed TSH levels. Further studies revealed a pituitary adenoma and detailed biochemical studies, including a 24-h blood sampling were carried out. He was treated with octreotide LAR with good clinical response. After 3 years he underwent a repeat 24-h blood sampling study.

Patient 5, aged 61 years was referred because of central hyperthyroidism and a pituitary microadenoma. Detailed biochemical studies were carried out, including a 24-h blood sampling. The patient, however, refused treatment. During infrequent follow-up visits for the last 12 years his clinical condition and TSH and thyroid hormone levels remained unchanged.

Patient 6, aged 60 years was medically treated in 1998 for hyperthyroidism in another hospital for one year. In 2006 she was reevaluated because of persisting hyperthyroid complaints, which were confirmed by biochemical analyses. In addition, the TSH levels were non-suppressed and the MRI of the pituitary gland revealed a macroadenoma. After the endocrine studies in our facility, treatment with octreotide LAR 20 mg was started. However, although TSH concentration decreased, T4 levels remained elevated, so that after 3 months the octreotide dose was increased to 30 mg/4 weeks without a clinical satisfactory response. Subsequently, it was found that she had a toxic nodular hyperthyroidism as well, for which was recently treated with strumazol and radioactive iodine (613 Mbq). Because of atrial fibrillation she was put on anticoagulant therapy and octreotide LAR was withheld.

Clinical and biochemical details of the patients are displayed in Table 1. One patient had severe acromegaly, and two other patients had slightly elevated IGF-I concentrations.

Results

Results of the i.v. 200 µg TRH test are shown in Fig. 1. Without exception the TSH increase was subnormal,

Table 1 Clinical characteristics of the patients

Patient	Gender	Age	fT$_4$ (nmol/l)	T$_3$ (nmol/l)	TSH (mU/l)	α-Subunit/TSH molar ratio	Thyroid size	Pituitary MRI	Immunostaining adenoma
1	Male	73	31	n.d.	4.30	3.99	Enlarged	Macroadenoma	TSH +
2	Male	44	47	4.1	3.01	7.80	Enlarged	Macroadenoma	GH/TSH +/+
3	Female	38	36	3.2	1.40	1.30	Enlarged	Macroadenoma	n.a
4	Male	26	30	4.2	3.35	0.93	Enlarged	Macroadenoma	n.a
5	Male	61	34	3.3	5.84	0.62	Enlarged	Microadenoma	n.a
6	Female	60	38	3.2	1.01	14.1	Enlarged	Macroadenoma	n.a

Normal range (median) for fT4: 10–24 (16.0) nmol/l, normal range for T3 1.1–3.0 (2.1) nmol/l, normal range for TSH 0.72–2.99 (1.25) mU/l. *n.d.* not determined, *n.a.* not applicable
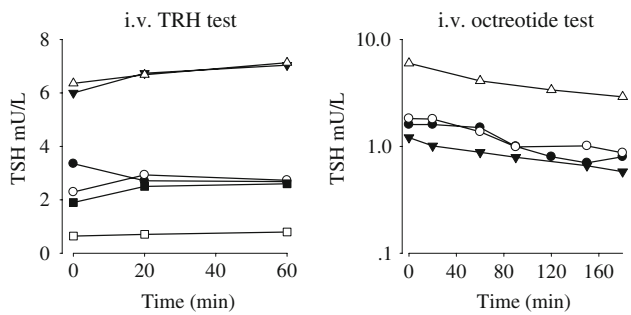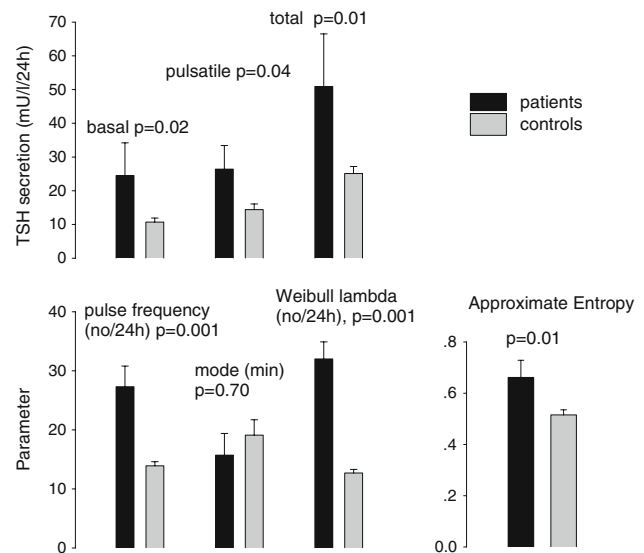
Fig. 1 Serum TSH response to 100 μg TRH. Note the subnormal response in all patients (*Left panel*). Effect of intravenous octreotide injection on TSH concentrations. Note the logarithmic scale of the abscissa (*Right panel*)
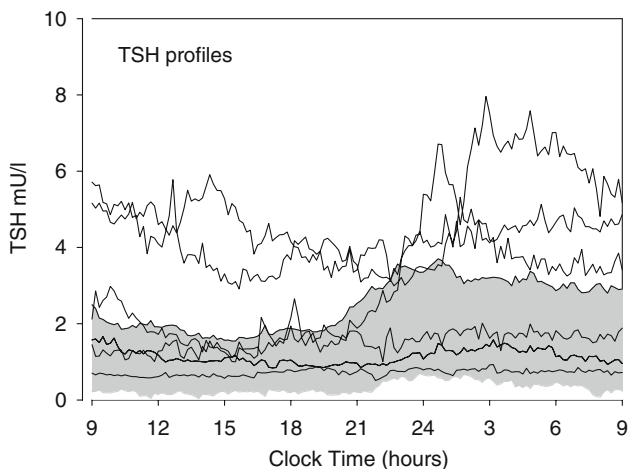
Fig. 2 Serum TSH concentration profiles of 6 patients with a thyrotropinoma. The shaded zone represents the 95% confidence interval of 18 healthy matched control subjects

Fig. 3 Results of the deconvolution analysis and approximate entropy calculation of the TSH profiles of 6 thyrotropinoma patients and 18 healthy controls. Data are mean ± SEM

ranging from 0 to 37%, with a mean of 15% (normal values exceeding 100%). An octreotide test (50 μg i.v.) was performed in 4 patients and the results are shown in Fig. 1. Acute inhibition of serum TSH concentration was remarkably similar among patients with a mean of 56%, ranging from 52 to 65%.

Serum TSH concentration profiles of the 6 investigated patients are shown in Fig. 2 with the 95% confidence interval for TSH profiles of non-obese controls. Results of the deconvolution analysis and of approximate entropy of the TSH concentration profiles are shown in Fig. 3. TSH secretion in thyrotropinoma patients was characterized by increased hormone burst frequency. Furthermore, basal and pulsatile TSH secretion was increased in patients, but the secretion-burst shape (waveform) was unchanged, as indicated by the mode. TSH secretory regularity of the subordinate patterns in patients was markedly decreased (increased ApEn ratio). Regularity of the interburst intervals however, was unchanged as demonstrated by the Weibull gamma results.

The estimated first component of the TSH half-life did not differ in patients and controls (24.5 ± 2.5 vs. 22.2 ± 2.2 min), but the slow component was shorter in patients (87.9 ± 10.7 vs. 108 ± 4.3 min, $P = 0.048$). This could denote reduced sialylation of tumoral TSH.

With the exception of one patient, GH secretion as also analyzed. The results are summarized in Table 2 and the GH concentration profiles are displayed in Fig. 4. Serum IGF-I concentrations and standard deviation scores were increased in patients 1–3, of whom only patient 2 had clinically active disease. However, patients 1 and 3 had (slightly) increased GH secretion, with respect to age and both had a definitely abnormal GH secretion profile, compared with the other two patients and examples of healthy controls (Fig. 4). Approximate entropy for GH secretion was only increased in the patient with active acromegaly.

Serum prolactin concentration profiles are displayed in Fig. 5. One patient (#3) had increased levels throughout the 24-h cycle, and two others had raised levels during the daytime. In contrast, the irradiated patient had subnormal levels. The regularity of PRL patterns in patients was diminished compared with controls, as defined by higher ApEn ratios in patients (0.788 ± 0.074, mean ± SD) than controls (0.461 ± 0.131, $P < 0.001$).

We also investigated cross-correlations between 24-h TSH concentrations and each of GH, α-subunit and PRL. Profiles of the serum concentrations of GH and TSH in two patients were rather similar, as illustrated in Fig. 6. The cross-correlation outcomes are shown in Table 3. Three patients had a highly significant correlation between serum

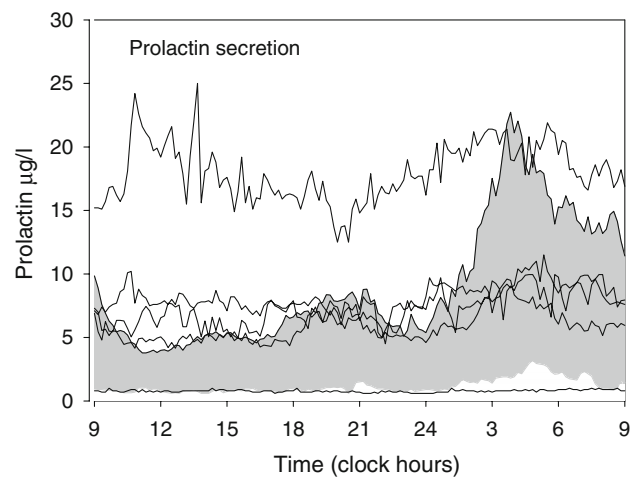**Table 2** Growth hormone secretion in thyrotropinoma

Patient	Age (years)	Sex	Fasting GH (mU/l)	GH secretion (mU/l/24 h)	IGF-I (µg/l)	IGF-I SDS	ApEn ratio
1	73	Male	2.0	169	228	3.00	0.483
2	44	Male	142	17070	550	10.4	0.787
3	38	Female	1.90	205	274	2.23	0.433
4	26	Male	0.11	174	144	0.02	0.310
5	61	Male	0.20	n.d.	182	1.80	n.d.
6	60	Female	2.50	81	135	1.16	0.480
Controls	30–68 (49)	Male	0.02–1.10 (0.18)	116–200 (45)	75–185 (107)	–	0.181–0.558 (0.330)
Controls	21–70 (37)	Female	0.06–2.80 (0.56)	57–247 (123)	87–234 (143)	–	0.260–0.514 (0.380)

GH secretion was calculated with deconvolution of the 24 h serum GH concentration profiles. Control values were obtained from 26 male and 22 female healthy subjects, who were investigated in a similar way. Data for controls are shown as the range and median. *n.d.* not determined, *SDS* standard deviation score
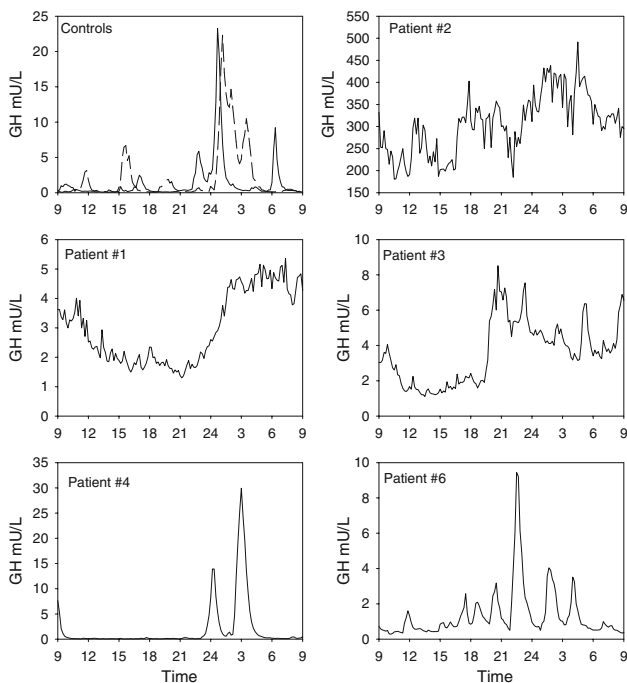

Fig. 4 Serum GH concentration profiles of 5 patients with a thyrotropinoma and 2 healthy representative controls (left upper panel, continuous line male subject, dashed line female subject). Note the difference in scales of the abscissa. The GH secretion patterns of patients #1–3 are clearly abnormal, while that of patient #6 only shows an increased basal (interpulse) level

Fig. 5 Serum prolactin concentration profiles of 5 patients with a thyrotropinoma. The shaded area represents the 95% confidence interval obtained from 18 matched healthy controls. The lowest profile was obtained in the irradiated patient
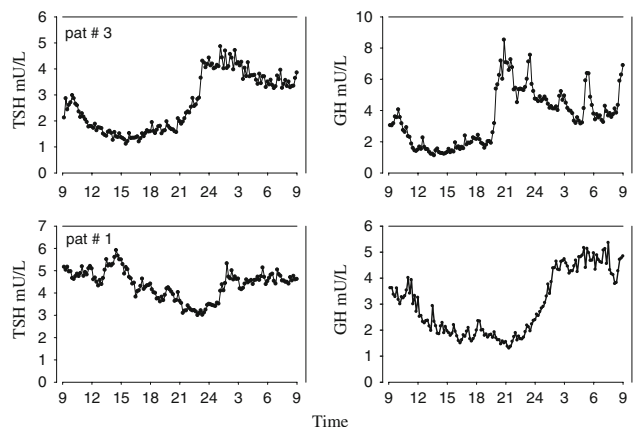
Fig. 6 TSH and GH profiles of 2 patients with a thyrotropinoma. Note the similarity of the patterns of both hormones

TSH and GH concentrations. In contrast, in healthy controls no correlation between these two hormones could be demonstrated. In two of 5 patients a significant correlation could be demonstrated between serum TSH and α-subunits concentrations, where an almost 100% concordance was expected when α-subunits are truly co-secreted with TSH. Unfortunately, we have no comparable data in controls subjects. The cross-correlation between TSH and prolactin was significant in all patients. However, two-thirds of

Table 3 Cross-correlations between TSH, GH and α-subunits in thyrotropinoma

Patient	TSH/GH	TSH/α-subunits	TSH/prolactin
1	0.337 ± 0.083	n.s.	0.350 ± 0.083
2	0.345 ± 0.084	0.427 ± 0.084	0.428 ± 0.083
3	0.279 ± 0.084	n.s.	0.190 ± 0.085
4	n.s.	0.320 ± 0.121	0.385 ± 0.083
6	n.s.	n.s.	0.405 ± 0.083

Cross-correlations were calculated after removing autocorrelations by first differencing. *n.s.* non-significant

Fig. 7 Effect of octreotide treatment on fasting morning hormone concentrations in 5 patients with a thyrotropinoma. fT4 denotes serum free thyroxine. Note the logarithmic scale for GH

healthy controls also showed a significant correlation between these two hormones, with a median correlation coefficient of 0.300, range 0.176–0.360.

Cross-ApEn ratios for TSH and GH were similar in patients and controls (0.716 ± 0.085 in patients vs. 0.678 ± 0.018 in controls), but cross-ApEn ratios for TSH and prolactin were increased in patients (0.774 ± 0.041 vs. 0.547 ± 0.022, $P = 0.001$), denoting significant loss of pattern synchrony.

All patients had significant diurnal TSH and PRL rhythms. For TSH the acrophase of the rhythm was shifted (time-delayed) from 2.83 to 5.58 h ($P = 0.02$) and for PRL from 4.36 to 6.20 h ($P = 0.07$). For TSH amplitudes were 0.83 ± 0.29 mU/l in patients and 0.54 ± 0.25 mU/l in controls (mean ± SD, $P = 0.09$), and for PRL 1.43 ± 0.44 µg/l and 0.97 ± 0.60 µg/l, respectively ($P = 0.09$).

Effect of octreotide treatment

Five of the six patients received octreotide as medical treatment, 4 as octreotide LAR. One patient (#5) refused medication. One other patient (#1) was treated preoperatively with octreotide (200 µg t.i.d.) for 3 months. Subsequently, he underwent transsphenoidal surgery, but had still (slightly) increased GH and IGF-I concentrations afterwards although TSH and thyroid hormone concentrations became and remained normal. This patient was treated with octreotide for the persisting GH excess. Finally, patient #6 had also a toxic nodular goiter, for which she was treated with radioactive iodine and suppressive thyroid medication after initial failure of normalization of thyroid hormone concentrations associated with (moderate) decrease of already low TSH levels. The effect of octreotide on fasting morning thyroxine, TSH, GH, IGF-I, prolactin and α-subunit concentrations is shown in Fig. 7. Free thyroxine concentrations normalized in all but one with a parallel reduction of TSH and also of IGF-I, GH and prolactin concentrations. The size of the adenoma on MRI decreased slightly in patients #3 and 4, but remained unchanged in patients #2 and 6.
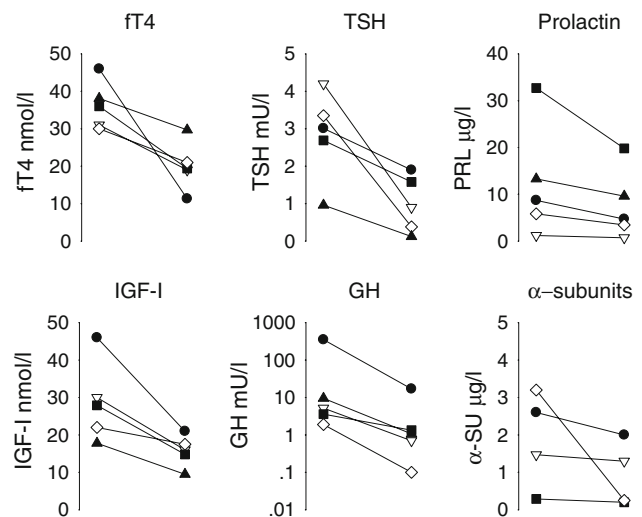
Discussion

This cohort of patients with a TSH-secreting pituitary adenoma maintained a high TSH pulse frequency with normal pulsing variability, increased basal and pulsatile TSH release and decreased secretory-pattern regularity. In addition, three of five patients also showed increased GH secretion, although clinically manifest in only one. Sustained increased PRL levels were present in one patient.

Previous investigations have demonstrated that frequent hormone pulses constitute feature of all endocrine adenomas, including somatotropinomas, prolactinomas, corticotropinomas and also hormone-secreting adrenal adenomas [8–10, 17]. This common finding among diverse tumors suggests that the cause of increased pulsatility is not primarily mediated via signaling of increased frequency of stimulatory peptides or neurotransmitters, but rather is an intrinsic feature of the adenoma per se.

Stability of the Weibull gamma parameter indicated that the increased TSH pulse frequency in patients is not associated with altered variability in length of interpulse intervals. Conversely, the elevated Weibull lambda parameter confirms increased TSH pulsing. The other remarkable finding was the decreased regularity of TSH secretory patterns as revealed by the increased ApEn. Approximate entropy has been investigated in a variety of altered feed-forward and feed-back states in healthy individuals, for instance in the somatotropic axis in healthy subjects during GHRH or GHRP-2 infusions and pegvisomant administration [23–25]. Increased stimulation of somatotropes is accompanied by decreased regularity (increased ApEn), and decreased IGF-I feedback by blocking the GH signal with the GH-receptor blocking

drug, pegvisomant, is also associated with decreased orderliness of GH secretion. Such studies indicate that higher ApEn reflects elevated feed-forward and reduced feed-back signaling of the studied system [15, 25]. For TSH comparable conditions of diminished feedback would be primary hypothyroidism and thyroid hormone resistance, which have not been studied. In contrast, endocrine tumors are generally not well regulated by exogenous stimuli (with the possible exceptions of some GHRH-secreting carcinoid tumors and ectopic CRH-producing tumors), although diminished feedback may be present, as observed in corticotropinomas (glucocorticoids), somatotropinoma (IGF-I) and parathyroid adenomas (calcium). Thus, increased TSH ApEn in thyrotropinomas likely reflects disorganized secretion from the tumor per se. Aberrant alternative splicing of thyroid hormone-receptor $\beta2$ mRNA encoding a thyroid hormone receptor variant lacking T_3 binding activity might in principle also contribute to irregular secretion [26, 27].

Co-secretion of GH and prolactin is found in about one-third of patients with a thyrotropinoma [7]. Up to 16% of patients co-secrete GH and 11% prolactin, while co-secretion of gonadotropins is rare (about 1%). Immunohistochemical studies report an even larger incidence of co-expression of GH and PRL mRNA in the thyrotrope tumors, suggesting that either these hormones are not secreted in quantities leading to clinical disease and furthermore that the tumor cell is derived from a common precursor cell [28–31].

Although all patients with a TSH-secreting adenoma secrete relatively excessive amounts of thyroid hormones, the TSH concentration in patients varies from normal to grossly elevated, in contrast with other hormone-secreting pituitary adenomas, e.g. somatotropinomas and corticotropinomas. This observation has been explained by increased biological activity of less acidic post-glycosylation isoforms of TSH [32]. Some preservation of feedback at the pituitary level cannot be ruled out, although it has not been studied in detail. Indeed, in our case series only two patients had an increased mean TSH concentration (Fig. 2), although the other patients should have had suppressed TSH levels due to elevated T_4 (T_3) concentrations. Terminal sialylation of TSH can reduce its metabolic clearance, and indeed we found a 25% increase of the second component of the TSH half-life in our patients.

The other major cause of central hyperthyroidism is thyroid hormone resistance. This syndrome is not associated with a pituitary tumor [33]. The TRH test in this condition is normal or hyperresponsive in contrast to the blunted TSH response in thyrotropinomas. In addition, patients with this disorder do not respond to somatostatin analog therapy [34]. The results of the TRH and octreotide tests are relevant and important in this context, because we have no histological proof for a thyrotropinoma in 4 of the 6 patients, although the presence of a (micro) adenoma would not favor a resistance syndrome. Other indirect methods to discriminate between thyroid hormone resistance and thyrotropinomas are the dissociated effects of TRH on TSH and α-subunits and the molar ratio of α-subunits and TSH. Taking into account that one of the patients was postmenopausal (although she had a normal α-subunit level) only two of our patients had an increased ratio. However, recent data suggest that high levels of α-subunit and an increased ratio are not invariably present and are actually normal in 20% of patients with a thyrotropinoma [1].

Treatment modalities of thyrotropinomas include transsphenoidal surgery and/or (primary) medical treatment. Generally, surgery may cure up to 50–80% and therapy with long-acting somatostatin analogues may be effective in about 80% of patients [35–41]. In line with the literature fasting hormone levels (TSH, fT4, GH, IGF-I and prolactin) decreased in all patients (except for T_4 in the patient with a toxic nodular goiter) during treatment with octreotide-LAR, thus demonstrating that the suppressive effect was not restricted to TSH secretion. Rare subjects have had both primary hyperthyroidism and central hyperthyroidism [36, 42]. Indeed, medical therapy failure aimed at TSH normalization may lead to the correct (additional) diagnosis.

A limitation of the study is the small number of investigated patients with this rare disorder. In addition, we could only evaluate patients with TSH concentrations mostly within the normal range. Therefore, the present conclusions cannot be extrapolated to patients with much higher TSH levels.

All our patients had a significant diurnal TSH rhythm with a mean phase delay of 2.7 h with unchanged amplitude. Few TSH serum profiles of patients with TSH-secreting adenomas have been published, but not of co-secreted GH and prolactin. Samuels et al. described a female patient with a TSH-secreting tumor, who had pulsatile TSH secretion, but no diurnal rhythm when monitored by 15-min sampling for 24-h [43]. Later, the same group reported absent diurnal variation of TSH in two other thyrotropinoma patients [44]. In another report two male patients were sampled at 2-h intervals: one patient showed a clear diurnal rhythm with a significant phase delay, as observed here, whereas the other patient had no clear rhythm [45]. Finally, a male patient was described with no apparent TSH rhythm, but the rhythm was restored during treatment with bromocriptine and octreotide. Paradoxically, the mean 24-h TSH concentration increased during medical treatment [46].

This limited patient series also demonstrates abnormalities in GH and prolactin secretion. One patient had overt acromegaly, and two others exhibited a definitely abnormal GH secretion pattern (Fig. 5), which resembled the TSH pattern. In addition, the results of the deconvolution

analysis also revealed (moderately in two) increased GH secretion in 3 patients, consistent with a high incidence of GH excess. Indeed, IGF-I concentrations and the SD scores were elevated in these patients, but clinical symptoms were not apparent in two individuals. Other indirect biochemical clues of hormone cosecretion were significant cross-correlations between TSH and GH (3 out of 5 patients), and between TSH and prolactin (5 out of 5 patients) and the phase delay of TSH and PRL diurnal rhythms. Somewhat unexpectedly, a significant correlation between TSH and α-subunit was found in only two patients, one of whom had clinically severe acromegaly. The lack of correlation between TSH and α-subunit suggests that their secretion is not coordinately regulated in the thyrotrope or that some tumors may consist of a mixed population of thyrotropes and purely α-subunit-secreting cells [47].

Recent histological studies of the pituitary gland have revealed that growth hormone-secreting cells form a large-scale network. This functional three-dimensional GH-network consists of cells linked with focal adherent junctions, shows robustness across the lifespan despite reversible plasticity of the architecture, for instance at puberty when GH secretion is amplified [48]. Such a network can produce rapid and synchronized hormone pulses in response to physiological needs. If a similar network exists for thyrotropes, disruptions of functional organization in TSH adenomas could explain disorganized TSH secretion, as here observed with increased ApEn in concert with the increased burst frequency. In addition, folliculo-stellate cells might be involved coordinating hormone secretion in the three-dimensional network [49]. These cells participate in scavenger activity, paracrine regulation of endocrine cells, as a source of stem cells and large-scale intercellular communication by means of long cytoplasmatic processes and gap junctions that mediate intercellular Ca^{2+} waves [49–53]. Observed loss of folliculostellate cells might thus contribute to abnormal hormone secretion patterns by pituitary adenomas.

In summary, TSH secretion by thyrotropinomas is characterized by increased pulse frequency with normal interpulse variability, decreased secretory regularity, enhanced hormone release and a delayed phase of the diurnal rhythm. In addition, GH and prolactin secretion also exhibit various abnormalities, ranging from decreased regularity to overt hypersecretion. We hypothesize that aberrant secretory characteristics are caused by histological and cellular alterations of the network of thyrotrophic tumor cells.

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