Increased episodic release and disorderliness of prolactin secretion in both micro- and macroprolactinomas

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Abstract

To quantify prolactin (PRL) secretion patterns, ten untreated (female) microprolactinoma patients and six (male) macroprolactinoma patients underwent repetitive blood sampling every 10 min over 24 h. PRL release activity was analyzed from plasma PRL concentration (immunofluorimetric assay) profiles via a model-independent discrete peak detection program (Cluster) and a waveform-independent deconvolution technique (Pulse). Diurnal variations were analyzed by cosinor analysis. The number of distinct PRL pulses (mean±S.E.M.) was increased in patients: microprolactinoma 18.6±6.2/24 h versus female controls 12.4±0.6 (P=6.7×10^{-5}), and macroprolactinoma 18.0±0.9 versus male controls 13.5±0.8/24 h (P=0.003). In patients, PRL pulse height, amplitude, pulse area and interpeak nadir concentrations were each greatly elevated compared with gender-matched controls. By 2-component deconvolution analysis, the mean nadir PRL secretion rate in microprolactinoma patients was augmented 20-fold at 0.408±0.089 μg/l per min versus in female controls 0.019±0.009 μg/l per min (P<0.001); and in macroprolactinoma by 130-fold at 2.067±0.693 μg/l per min versus male controls 0.016±0.001 μg/l per min (P=0.001). Corresponding 24 h mean PRL secretion rates were in women, 0.658±0.147 and 0.044±0.018 (P<0.001), and in men, 3.30±1.356 and 0.035±0.010 μg/l per min (P=0.001), being respectively 15- and 94-fold increased in tumors. The estimated PRL production per day was 160±54 μg in male and female controls respectively. PRL production was 2860±640 μg in female patients with microadenomas (P<0.001), and 37 800±5900 μg in male macroadenoma patients (P=0.001). Cosinor analysis of the plasma concentrations revealed a significant rhythm in nine of ten patients with a microadenoma, and in five of six with a macroadenoma. The same method applied to pulse height and amplitude disclosed a significant rhythm for PRL pulse height, but not for pulse amplitude, suggesting preserved rhythmicity of baseline interpulse nadir PRL concentrations. Approximate entropy (ApEn), a scale- and model-independent regularity statistic, averaged 1.65±0.028 in microprolactinoma patients versus 0.81±0.079 in female controls (P=1.7×10^{-5}), ApEn in macroprolactinomas was 1.56±0.054 versus male controls 0.87±0.026 (P=1.7×10^{-3}), signifying greater secretory irregularity in the patients. Compared with microadenomas, macroadenomas exhibited a higher mean plasma concentration, overall mean PRL secretion rate, nadir secretion rate and pulse area, but similar peak frequency. We conclude that PRL secretion by prolactinomas is characterized by increased plasma PRL episodicity of release, increased total (15- to 100-fold) and basal (20- to 130-fold) secretion rates, and increased disorderliness of minute-to-minute secretion. These abnormalities of secretory control are very similar to those for GH and ACTH identified earlier in acromegaly and Cushing’s disease respectively, thus suggesting mechanistic generality of pituitary tumor secretory derangements, independent of the particular hormone.

Introduction

Prolactin (PRL) secretion in man is regulated by stimulatory and inhibitory factors derived from the hypothalamus. Although detailed knowledge of the precise contribution of these factors to the synthesis and release of PRL into the circulation is not well established, dopamine is the major inhibiting substance. However, dopamine does not contribute primarily to the pulsatile mode of release and the diurnal variation. Various other hormones have been proposed as PRL-releasing hormones, including thyrotropin-releasing...
hormone (TRH), vasoactive intestinal polypeptide (VIP) etc., but the physiological contribution of each of these stimuli is not known in detail (1).

Hyperprolactinemia is a rather common clinical condition in the human, arising physiologically in pregnancy and lactation and pathophysiologically elicited by drugs. Another very well known cause of hyperprolactinemia is a PRL-secreting pituitary adenoma. In other pituitary adenomas, e.g. growth hormone (GH)- and adrenocorticotropin (ACTH)-secreting tumors, the secretory patterns of the corresponding hormones have been studied thoroughly. Most often tumor release is characterized by an increased number of secretion episodes, and increased total pulsatile and basal release, but also by quantitatively irregular release (2–4). Studies of plasma PRL secretory patterns in hyperprolactinemic patients are scarce. In one study, 6 h plasma PRL profiles in normal female subjects during the menstrual cycle and in the postmenopausal state, and in patients with functional hyperprolactinemia, were analyzed with Detect (5). In another study, the plasma PRL profiles of five patients were reported before and during bromocriptine therapy, although this study unfortunately lacked a control group (6). Also not established is whether PRL secretory patterns in micro- and macroprolactinoma differ along with their distinct growth characteristics. We therefore undertook an investigation of PRL secretory activity both in female patients with microprolactinomas and in male patients with macroprolactinomas, along with sex- and age-matched controls.

Methods

Patients

Twelve healthy men and 15 healthy women of normal height and body mass index served as matched controls for the hyperprolactinemic patients. Controls and patients originated from the same community, and were evaluated in an identical sampling paradigm and PRL 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 40±2.6 years (mean ± S.E.M.) and of the female controls 44±3.7 years. The two groups of patients investigated included ten untreated female subjects with a microprolactinoma and six male patients with a macroprolactinoma. All patients had a pituitary tumor on MRI scanning. The age of the patients with a microprolactinoma was 37±2.6 years and of the patients with a macroadenoma 40±2.2 years (NS vs controls). None of the patients used hormone substitution therapy. The postmenopausal women studied here did not use estrogen therapy. Premenopausal female control subjects were studied in the early follicular phase of the menstrual cycle. The endocrine tests performed for assessing pituitary reserve function included i.v. bolus corticotropin-releasing hormone, TRH and gonadotropin-releasing hormone tests on separate days.

Assays

Plasma PRL was measured with a sensitive time-resolved fluoro-immunoassay (Wallac, Turku, Finland). The standards were calibrated against the WHO 3rd International Standard for Prolactin 84/500 (to convert μg/l to mU/l multiply by 36). The limit of detection (defined as the value –2 S.D. above the mean value of the zero standard) was 0.04 μg/l. The intra-assay coefficient of variation varied from 2.0 to 3.3% in the assay range from 3.0 to 80 μg/l, and the interassay coefficient of variation was 3.4–6.2% in the same range.

Study sampling protocol

Patients and volunteers were hospitalized the evening before the sampling studies. On the following morning, an indwelling i.v. cannula was inserted into a large vein of the forearm, and blood samples were withdrawn at 10 min intervals for 24 h starting at 0900 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 daytime. Meals were served at 0800, 1230 and 1730 h. Lights were turned off between 2200 and 2400 h, depending on the sleeping habits of the patient. No sleep monitoring was carried out.

Cluster analysis

Discrete peak detection was undertaken with Cluster analysis using a power function fit of all intrasample variances within the 24 h (145 samples) series plotted against mean sample concentrations (7). We used a 2×2 test cluster configuration (two samples in the test nadir and two in the test peak) and t-statistics of 2.0 for significant up- and down-strokes for PRL peaks to constrain the false positive rate to less than 5% on signal-free noise. The locations and durations of all significant plasma hormone peaks were identified and the total number counted. In addition, the following pulse parameters were determined: mean maximal peak height (highest value attained in the peak), mean incremental peak height (amplitude), mean area under the peak (above baseline), and mean interpulse valley PRL concentration.

Deconvolution analysis

For this analysis, we used a waveform-independent deconvolution method, which calculates hormone secretion rates at each time point given an estimated 2-component half-life and its associated variance without making any assumptions about the nature of
underlying secretory events and/or basal secretion rates (8, 9). For the present study, we used the plasma PRL decay curves in healthy controls by Sievertsen et al. (10). From the original data set, we recalculated the plasma half-life by nonlinear regression. The half-life of the fast component was 18.4±4.0 min, and that of the slow component 139±25 min, with a fractional contribution of the slow component to the overall decay amplitude of 49.5±15.0%. From this analysis, we calculated the mean 24 h PRL secretory rates, the basal secretory rates and the distribution of sample secretory rates in patients and controls. The total 24 h production rate was calculated by multiplying the mean secretion rate by 1440 and the distribution volume. We assumed a distribution volume of 7.9% of the body weight, similar to that of GH (11).

**Approximate entropy quantification of episodicity**

To quantify episodicity, we utilized ApEn, approximate entropy, a scale- and model-independent regularity statistic that has provided new insights both mathematically and in various biological applications (2, 3, 12, 13). ApEn is complementary to pulse detection algorithms widely employed to appraise hormone secretion time-series (14). ApEn evaluates both dominant and subordinant patterns in data; notably, it will detect changes in underlying episodic behavior not reflected in peak occurrences or amplitudes (15). Additionally, ApEn provides a direct barometer of feedback system change in many coupled systems (16).

ApEn assigns a non-negative number to a time-series, with larger values corresponding to greater apparent process randomness, and smaller values corresponding to more instances of recognizable patterns of recurring features in the data. Below, we apply ApEn with parameter choices m=1, r=20% s.d., a standard set of choices utilized in virtually all endocrinological applications. Briefly, ApEn measures the logarithmic likelihood that runs of patterns that are close (within r) for m contiguous observations remain close (within the same tolerance width r) on next incremental comparisons. Smaller ApEn values indicate a greater likelihood of successive values remaining close, i.e. there is greater regularity, and conversely. By assigning to each of the original data points a random variation within 2 s.d. determined by the within-assay coefficient of variation via a Monte Carlo approach, we thus create a new series reflecting the variability within the assay. This process was then repeated 300 times giving 300 individual ApEn values, from which the mean and s.d. were calculated (17).

**Nyctohemeral rhythms**

Twenty-four hour variations in plasma PRL concentrations were analyzed by cosinor analysis. The following parameters were calculated: mesor (mean value around which the 24 h oscillation occurs), amplitude (half the difference between highest and lowest values), and acrophase (time of maximum concentration).

**Statistical analysis**

Data are given as the mean±s.e.m., unless otherwise mentioned. Statistical analyses were done by using two-tailed t-tests for unpaired data, ANOVA and regression techniques. 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**

The mean 24 h plasma PRL concentration in (female) patients with a microprolactinoma was 74±16 μg/l, and in the female controls the mean concentration was 4.8±0.5 μg/l (P=0.002). By Cluster analysis, patients with a microprolactinoma had significantly increased pulse height, greater pulse amplitude, shorter width of the pulses, and an increased pulse area (Table 1). The interpeak nadir concentration was greatly increased in patients compared with controls. The number of discrete PRL pulses per 24 h in female controls was 12.4±0.6 versus 18.8±0.6 in patients (P=6.7×10⁻⁸).

In the male patients with a macroprolactinoma, comparable differences with sex-matched controls existed as (above) for the female counterparts. The mean 24 h plasma PRL concentration was 364±127 μg/l in patients and 3.7±0.3 μg/l in controls (P=0.001). Pulse height, pulse amplitude and pulse area were all increased in patients compared with the controls, whereas pulse width was about 20% shorter in patients than in controls. Nadir concentrations were greatly increased in patients (Table 2). Similar to patients with a microprolactinoma, the macroprolactinoma patients had an increased number of pulses per 24 h compared with controls, i.e. 18.0±0.9 vs 13.5±0.8 (P=0.001). The quantitative differences between patients and their respective control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (μg/l)</td>
<td>74.2±16.7</td>
<td>4.8±0.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Pulse height (μg/l)</td>
<td>80.6±18.2</td>
<td>5.7±0.5</td>
<td>0.003</td>
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<tr>
<td>Pulse amplitude (μg/l)</td>
<td>10.5±2.5</td>
<td>2.1±0.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Pulse width (min)</td>
<td>53±2</td>
<td>85±6</td>
<td>0.0005</td>
</tr>
<tr>
<td>Pulse area (μg/l × min)</td>
<td>369±77</td>
<td>121±22</td>
<td>0.002</td>
</tr>
<tr>
<td>Percent increase</td>
<td>115±1</td>
<td>161±8</td>
<td>0.0005</td>
</tr>
<tr>
<td>Nadir concentration (μg/l)</td>
<td>67.8±15.1</td>
<td>3.5±0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>No. of pulses/24 h</td>
<td>18.8±0.6</td>
<td>12.4±0.6</td>
<td>6.7×10⁻⁸</td>
</tr>
</tbody>
</table>

Table 1 Cluster analysis of PRL release in women with a microprolactinoma. Cluster analysis was applied to plasma samples obtained at 10 min intervals for 24 h. The female patients were compared with female controls by a two-tailed Student’s t-test for unpaired data.
are displayed in Fig. 1, showing the 24 h plasma profiles for representative patients and controls.

By waveform-independent deconvolution analysis, the calculated mean sample PRL secretion rate in patients with the microprolactinoma was about 15 times higher than in controls and amounted to 0.658 ± 0.147 μg/l per min, whereas in female controls 0.044 ± 0.018 μg/l per min was secreted (P<0.001). Similarly, nadir PRL secretion (i.e. interpulse nonpulsatile secretion) was much higher in patients than in controls (0.408 ± 0.089 vs 0.019 ± 0.007 μg/l per min (P<0.001). The statistical distribution of the sample secretion rates (for each of the 145 ten-minute samples) was highly skewed to the left in controls, but was almost symmetrical in patients. This is illustrated for both patient groups and controls in Fig. 4. The calculated skewness in microprolactinoma was 0.72 ± 0.12, and in controls 3.25 ± 0.37 (P<0.001). The respective values for kurtosis were 0.31 ± 0.23 and 15.74 ± 3.84 (P=0.01).

In patients with macroadenomas, the mean PRL secretion rate was almost 100 times higher than in controls; namely, in patients 3.309 ± 1.156 μg/l per min versus controls 0.035 ± 0.010 μg/l per min (P=0.001). Nadir secretion was also greatly increased in macroadenoma patients: 2.067 ± 0.693 vs 0.016 ± 0.001 μg/l per min in controls (P=0.001). The mean skewness of the PRL secretion rate distributions in patients was 0.91 ± 0.08 and in controls 3.65 ± 0.43 (P<0.001). For kurtosis the respective values were 0.88 ± 0.34 and 20.05 ± 5.03 (P=0.003).

For the estimation of total 24 h PRL production, we assumed that the PRL distribution volume was comparable to that found for GH (10). For male controls, the calculated 24 h secretion was then 324 ± 32 mg and for female controls 327 ± 36 mg (males vs females, NS).

Table 2 Cluster analysis of PRL release in men with a macroprolactinoma. Cluster analysis was applied to plasma samples obtained at 10 min intervals for 24 h. The male patients were compared with male controls. Statistical comparisons were made with a two-tailed Student’s t-test for unpaired data.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (μg/l)</td>
<td>364 ± 127</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Pulse height (μg/l)</td>
<td>397 ± 139</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Pulse amplitude (μg/l)</td>
<td>56 ± 22</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Pulse width (min)</td>
<td>57 ± 3</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>Pulse area (μg/l x min)</td>
<td>1986 ± 803</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>Percent increase</td>
<td>114 ± 1</td>
<td>156 ± 6</td>
</tr>
<tr>
<td>Nadir concentration (μg/l)</td>
<td>330 ± 113</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>No. of pulses/24 h</td>
<td>18.0 ± 0.9</td>
<td>13.5 ± 0.8</td>
</tr>
</tbody>
</table>

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Twenty-four hour serum PRL concentration profiles illustrated in one male and one female control subject and in two patients with micro- (female) and macro- (male) prolactinomas.
patients, the mesor concentrations were elevated, similar to the mean 24 h concentrations, i.e. 74.2±16.7 vs 4.85±0.56 μg/l in controls (P=0.001). The mean cosine amplitudes in patients and controls were 4.84±0.95 and 2.76±0.54 μg/l (P=0.054), and hence the ratio of amplitude/mesor was greatly diminished in patients compared with controls (0.073±0.0086 and 0.57±0.07, P<0.001). The time of maximum, or acrophase occurred slightly, but not significantly, later in patients than in controls (0615 h±31 min vs 0408 h±65 min, P=0.057). In the male patients with microprolactinomas, the mean 24 h mesor was 308±139 μg/l and in controls 3.66±0.30 μg/l (P=0.001). The amplitude in patients was 35.3±18.2 μg/l and in controls 1.52±0.20 μg/l (P=0.009). The ratio amplitude/mesor was therefore diminished in patients, i.e. 0.097±0.014 vs 0.43±0.06 in controls (P=0.002). The acrophases of controls and macroadenoma patients were similar, occurring in patients at 0512 h±89 min, in controls at 0513 h±16 min (NS).

Complementary to the cosinor analysis of all the plasma sample concentrations, we also explored the diurnal variation of the height and amplitude of the pulses as determined by the Cluster analysis. To this end, the 24 h cycle was divided into 6 h blocks and the average values were calculated for each block. The results are shown in Figs 2 and 3. For pulse height, highly significant ANOVAs were found for the control group and patients with a microprolactinoma (P<0.005). For these subjects a significant statistical contrast between the last period (i.e. the period from 0300 to 0900 h) and the other three combined periods was present (P<0.004). For patients with a macroprolactinoma the ANOVA was less striking (P=0.035), with no significant contrast of the fourth period compared with the other time periods. Pulse amplitude exhibited a significant variation only in controls, but not in patients. The ApEn(1, 20%), in control females was 0.8128±0.079 and was significantly larger (signifying greater disorderliness) in patients, with a microprolactinoma 1.6559±0.028 (P=1.7×10⁻⁵). In male controls, ApEn(1, 20%) was 0.8773±0.076 and in the patients with the macroprolactinoma it was 1.5674±0.054 (P=1.7×10⁻⁵). The results for individual subjects (plotted as the mean±s.d. by Monte Carlo perturbation) are shown in Fig. 5.

Patients with a macroadenoma secreted more PRL than patients with a microadenoma, as reflected by differences in the mean 24 h concentrations, mean secretion rates, pulse height, pulse amplitude, pulse area and total 24 h secretion. No statistically significant differences between these two groups were found for the number of pulses per 24 h, the ApEn, the statistical distribution of the sample secretion rates, the relative amplitude of the diurnal variation, and the time of acrophase.

**Discussion**

This study was designed to analyze and compare the pulsatile, nyctohemeral and entropic secretory characteristics of PRL-producing pituitary (micro- and macroadenoma) patients. To date, the plasma PRL profiles of (micro- or macroprolactinoma) patients have not been studied in detail, although some data are available on luteinizing hormone and follicle-stimulating hormone secretion patterns in these patients (6, 18).

We used several complementary analytical strategies to explore the nature of the presumably deranged PRL secretion patterns, namely pulse analysis, cosinor

**Table 3** Deconvolution analysis of PRL secretion in prolactinoma patients. Secretion rates were calculated with a waveform-independent deconvolution method (8, 9, 32). Plasma samples were obtained at 10 min intervals for 24 h. For each individual, secretion rates are the mean of 145 sample rates. In addition, 24 h secretion was also estimated, see Methods. Patients were compared with a sex-matched control group. Statistical comparisons were made by the two-tailed Student’s t-test for unpaired data.

<table>
<thead>
<tr>
<th></th>
<th>Microprolactinoma</th>
<th>Female controls</th>
<th>P value</th>
<th>Macroprolactinoma</th>
<th>Male controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretion rate at nadir (μg/l per min)</td>
<td>0.408 ± 0.089</td>
<td>0.019 ± 0.007</td>
<td>&lt;0.001</td>
<td>2.067 ± 0.693</td>
<td>0.016 ± 0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean secretion rate (μg/l per min)</td>
<td>0.658 ± 0.147</td>
<td>0.044 ± 0.018</td>
<td>&lt;0.001</td>
<td>3.309 ± 1.156</td>
<td>0.035 ± 0.010</td>
<td>0.001</td>
</tr>
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</table>
analysis and the ApEn statistic to define respectively the pulsatile and diurnal variations, and subordinate pattern reproducibility in the plasma concentrations. By way of pulse analysis, Cluster and deconvolution algorithms, both model-free methods, were applied as complementary, but mathematically independent tools.

Few quantitative data on PRL secretion profiles are available. In the present study, we calculated for our female controls a normalized (per square meter body surface area) mean PRL production rate of 187 μg per day and for males 160 μg/day. The only directly measured data in the literature on human PRL secretion are from Sievertsen et al. (10). They established with isotopically labeled PRL a metabolic clearance rate of 40±2.5 ml/min per m² in six female control subjects. Applying this figure to the mean plasma PRL concentration in our female controls, the total daily production rate of PRL in our female controls

Figure 3 Diurnal variation of the maximal serum PRL pulse height, as calculated with Cluster. The variation in patients is smaller than in controls (see Results).

Figure 4 Distributions of the calculated sample PRL secretory rates in one male and one female control subject and two patients. Note the highly left-skewed distribution in controls compared with patients with prolactinomas.
either men or animals (25, 26). Episodic low-amplitude release does not inhibit the pulsatile mode of PRL secretion in episodic release, since potent antidopaminergic drugs do not contribute exclusively to PRL inhibiting factor. However, it is likely that this neurohypophysis contains a PRL-releasing factor, and experimental evidence in rats indicates that the PRL-producing cells to stimulatory factors (1, 20–22).

Pulsatile PRL secretion is amplitude regulated, as suggested earlier in men (31, 32). In contrast, patients with PRL-secreting pituitary tumors lost the diurnal rhythm of pulse amplitude, although pulse height still showed a (weak) diurnal variation. This observation is not easily explained, since in other pituitary tumoral hypersecretory states we found a GH diurnal rhythm in most of our acromegalic patients with active untreated disease, and a significant ACTH rhythm in two-thirds of our patients with Cushing’s disease (3, 33). One of the many possibilities is that the sum of all stimulatory factors or hormones acting on the lactotrope population (either normal or tumoral) is time-invariant over 24 h, and that the physiological variation observed is caused by changing sensitivity (intrinsic circadian rhythm) of the lactotropes towards stimulatory and inhibitory signals, a property lost by tumoral cells. At the same time, basal (i.e. nonpulsatile, interpeak nadir) PRL secretion is much more is known concerning GH physiology and pathophysiology. Recently, Straume and coworkers developed a dynamical network model of GH feedback regulation, in order to simulate GH secretion patterns under both basal conditions and pharmacological manipulations (30). In principle, a similar model approach to appraise PRL release is feasible if all physiologically important (stimulating or inhibitory) factors are characterized. Such a strategy might be helpful in better understanding PRL secretion in physiological and pathophysiological states.

In our controls, we found a very strong diurnal rhythm of PRL release in both men and women, consisting of large pulses in the early morning hours. This was corroborated by ANOVA of the Cluster results showing that the large pulses were especially evident in the 0300–0900 h period, without a time-dependent variation in pulse frequency. Thus, the diurnal rhythm is amplitude regulated, as suggested earlier in men (31, 32). In contrast, patients with PRL-secreting pituitary tumors lost the diurnal rhythm of pulse amplitude, although pulse height still showed a (weak) diurnal variation. This observation is not easily explained, since in other pituitary tumoral hypersecretory states we found a GH diurnal rhythm in most of our acromegalic patients with active untreated disease, and a significant ACTH rhythm in two-thirds of our patients with Cushing’s disease (3, 33). One of the many possibilities is that the sum of all stimulatory factors or hormones acting on the lactotrope population (either normal or tumoral) is time-invariant over 24 h, and that the physiological variation observed is caused by changing sensitivity (intrinsic circadian rhythm) of the lactotropes towards stimulatory and inhibitory signals, a property lost by tumoral cells. At the same time, basal (i.e. nonpulsatile, interpeak nadir) PRL secretion retained its diurnal properties. Previous studies of PRL secretion in prolactinoma patients invariably reported the loss of diurnal variation, but these studies lacked a mathematical analysis (34, 35).

In the present study, we also established a significantly increased ApEn in prolactinoma patients, indicating that the hormone release is markedly more irregular than in the controls. Similar changes in ApEn have been described in other endocrine tumors, such as acromegaly (33), cortisol and ACTH secretion in Cushing’s disease (4), aldosterone secretion in patients with Conn’s disease (36), and also GH secretion in

![Figure 5](image)

**Figure 5** ApEn of 24 h PRL release in controls and patients with prolactinomas. Patients had greater ApEn values compared with controls, but no difference in ApEn was present between microprolactinoma and macroprolactinoma patients. Each calculated ApEn value is represented by its mean and 1 s.d. Higher ApEn denotes greater disorderliness of release.
fasting (3) and elderly people (37), and hypopituitarism (38, 39). The last two categories indicate that increased ApEn (signifying greater disorderliness) is not dependent on hypersecretion per se, but rather reflects disrupted feedback and feedforward neuroendocrine control (14, 17). An independent statistical network-based analysis of predictability corroborated the notion of diminished orderliness of GH release in active acromegaly (40).

In summary, the present study shows remarkable similarities in disrupted PRL secretion between patients with prolactinomas and those with other hyperfunctioning pituitary tumors. Tumoral PRL hypersecretion is characterized by augmented basal release, an increased number of secretory events, an amplified mass of PRL secreted per pulse, relative loss or attenuation of the diurnal properties, and highly irregular secretion. Such generality of altered secretory properties among diverse pituitary tumors suggests common mechanisms by which regulated hormone secretion is disrupted in tumoral pathophysiology.

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