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Chapter 4

Profound Amplification of Secretory-Burst Mass and Anomalous Regularity of ACTH Secretory Process in Patients with Nelson’s Syndrome Compared with Cushing’s Disease

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SUMMARY

Objective
As described originally, Nelson’s syndrome is characterized by grossly elevated ACTH concentrations, a sellar mass and skin hyperpigmentation emerging in the course of Cushing’s disease after bilateral adrenalectomy. No detailed studies have defined whether the mechanisms directing ACTH secretion differ in Nelson’s syndrome and untreated Cushing’s disease.

Patients and methods
To address this pathophysiological issue, we studied 9 patients fulfilling the criteria of Nelson’s syndrome receiving glucocorticoid and mineralocorticoid replacement; 9 patients with untreated pituitary-dependent Cushing’s disease and 9 gender- and age-matched controls. ACTH release was appraised by monitoring plasma ACTH concentrations in blood samples collected every 10 min for 24 h. ACTH secretion rates and endogenous decay were quantified by multiparameter deconvolution analysis. The orderliness of the ACTH release process was delineated by the approximate entropy (ApEn) statistic. Diurnal variation in ACTH secretion was appraised by Cosinor analysis.

Results
Basal ACTH secretion was increased 6-fold and pulsatile secretion 9-fold in patients with Nelson’s syndrome compared with Cushing’s disease (P < 0.01 and P<0.001, respectively). The increase in pulsatile secretion was due to an 8-fold augmentation of burst mass. Event frequency was comparable in both patient groups (32 ± 1 vs 28 ± 2 pulses/24h), and higher than in normal controls (22 ± 1 pulses per 24 h, P< 0.0001). Paradoxically, the consistency of subordinate patterns of serial ACTH release, albeit disrupted in active Cushing’s disease, was normal in Nelson’s syndrome (P = 0.014). Normal ACTH secretory-process regularity in Nelson’s syndrome was attributable to a more reproducible (lower ApEn) succession of ACTH secretory-burst mass denoting more uniform amplitude evolution over 24 h (P=0.007, Nelson vs Cushing). On the other hand, the quantifiable regularity of serial interburst intervals (waiting times) was unexpectedly elevated in Nelson’s syndrome (P=0.022). Nelson patients maintained a significant diurnal rhythm in ACTH release, which was marked by a 15-fold greater amplitude (P = 0.0018 vs Cushing’s) and a 4-h acrophase (maximum) delay (P=0.037 vs control).

Conclusion
The present detailed analyses delineate marked ACTH secretory-burst mass amplification and (amplitude-independent) anomalous regularity of successive pulse size and timing in Nelson’s syndrome compared with Cushing’s disease or controls. We postulate that the foregoing novel distinctions are due to unique tumoral
secretory properties, concurrently required glucocorticoid replacement and/or hypothalamic injury associated with prior radiotherapy in Nelson's syndrome.

INTRODUCTION

Nelson's syndrome was first described in 1958 as the constellation of a pituitary macroadenoma, markedly elevated ACTH concentrations, and hyperpigmentation of the skin in a patient after bilateral adrenalectomy for pituitary-dependent hypercortisolism (Cushing's disease) (Nelson et al., 1958). The syndrome develops in 8 - 38% of adults requiring bilateral adrenalectomy for Cushing's disease (Nagesser et al., 2000; Kemink et al., 2001) and occurs infrequently in patients aged 40 yr or more at the time of bilateral adrenalectomy, in contrast to patients treated at an early age (Kemink et al., 1994). The pathogenetic mechanism's underlying tumorigenesis and unrestrained ACTH secretion in Nelson's syndrome are not well understood.

In Cushing's disease, excessive ACTH production is characterized by a marked elevation of basal (nonpulsatile) secretion and secretory-burst mass in association with marked disruption of orderly release and diurnal rhythmicity (Van den Berg et al., 1995). Transsphenoidal adenomectomy normalises most or all alterations in ACTH secretion (Groote Veldman et al., 2000). Cushing's disease and Nelson's syndrome are considered to be distinct pathoaetiological presentations of the same primary biological entity. For example, impaired responsiveness to glucocorticoid enforced negative feedback on ACTH is common to both (Cook et al., 1976). In addition, under in vitro conditions the secretion of POMC-derived peptides was similar in tumoural tissue derived from patients with Cushing's disease and Nelson's syndrome (Westphal & Lüdecke, 1984) However, CRH infusion stimulates greater and more prolonged ACTH secretion in patients with Nelson's syndrome than Cushing's disease (Oldfield et al., 1986). At present, there are relatively few other quantitative comparisons of neurosecretory control of tumoural ACTH secretion in these two clinical pathophysiological entities. The purpose of the present study was to explore and compare the 24-h spontaneous ACTH secretion dynamics in this group of patients with untreated classical Cushing's disease and healthy controls.

Subjects and Methods

Before the availability of transsphenoidal microsurgery for the treatment of Cushing's disease, patients usually underwent bilateral adrenalectomy. In our centre, however, patients were treated by unilateral adrenalectomy and pituitary irradiation until 1978, resulting in remission of the disease in 64% (Nagesser et al., 2000). Nuncured patients underwent complete adrenalectomy, usually after one year of clinical follow-up. Seven of the patients (see Table I) were treated in this way, but developed clinical symptoms of Nelson's syndrome, i.e. hyperpigmentation and grossly elevated ACTH concentrations. Two other patients (patients 7 and 8) underwent bilateral adrenalectomy after unsuccessful pituitary surgery and subsequently developed
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Nelson’s syndrome. We defined Nelson’s syndrome as bilateral adrenalectomy for Cushing’s disease in the past, plasma ACTH-levels of more than 300 ng/L during hydrocortisone replacement therapy (20 mg/day) and hyperpigmentation of the skin. Radiological evidence of a pituitary tumour was found in 7/9 patients. This definition of Nelson’s syndrome agrees with a previous report and discussion (Kasperlik-Zaluska et al., 1996; Kasperlik-Zaluska & Jeske, 2001). In total we studied nine patients with Nelson’s syndrome (7 females, 2 males), nine patients with proven Cushing’s disease and 9 healthy controls matched for gender and BMI.

In order to prevent spurious elevated ACTH concentrations due to low circulating cortisol concentrations under substitution, the medication was switched to dexamethasone. Therefore, starting one day before and during the sampling period, patients with Nelson’s syndrome received a standardized steroid-replacement schedule, consisting of dexamethasone 0.25 mg at 0800 and 1800 h and fludrocortisone 0.125 mg at 0800 h.

Patients with Cushing’s disease were diagnosed by elevated 24-h urinary excretion of free cortisol, subnormal or absent suppression of plasma cortisol after administration of 1 mg dexamethasone overnight, absent or subnormal suppression of urinary cortisol excretion during a low-dose dexamethasone test, suppression of plasma cortisol by 190 nmol/L or more during a 7-h iv infusion of dexamethasone 1 mg/h (Biemond et al., 1990), positive adenoma immunostaining for ACTH and clinical cortisol dependency for several months after selective removal of the adenoma. The mean 24-h plasma cortisol concentration (mean of 145 samples of each series) was 690 ± 140 nmol/L in Cushing’s disease and 206 ± 20 nmol/L in healthy controls (P=0.008).

Table 1 Clinical characteristics of nine patients with Nelson’s syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex (m/f)/</th>
<th>Primary therapy</th>
<th>Interval between ADX and NS (yr)</th>
<th>ACTH (ng/L) (random)*</th>
<th>Pituitary Adenoma</th>
<th>Medication other than adrenal steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/49</td>
<td>UAPI</td>
<td>28</td>
<td>1500</td>
<td>not identified</td>
<td>T4</td>
</tr>
<tr>
<td>2</td>
<td>F/57</td>
<td>UAPI</td>
<td>22</td>
<td>356</td>
<td>present</td>
<td>T4, DDAVP</td>
</tr>
<tr>
<td>3</td>
<td>F/63</td>
<td>UAPI</td>
<td>25</td>
<td>883</td>
<td>present</td>
<td>none</td>
</tr>
<tr>
<td>4</td>
<td>F/54</td>
<td>UAPI</td>
<td>20</td>
<td>505</td>
<td>present</td>
<td>none</td>
</tr>
<tr>
<td>5</td>
<td>F/57</td>
<td>UAPI</td>
<td>20</td>
<td>6685</td>
<td>present</td>
<td>T4</td>
</tr>
<tr>
<td>6</td>
<td>F/49</td>
<td>UAPI</td>
<td>11</td>
<td>372</td>
<td>present</td>
<td>T4</td>
</tr>
<tr>
<td>7</td>
<td>F/43</td>
<td>TSA</td>
<td>9</td>
<td>640</td>
<td>present</td>
<td>none</td>
</tr>
<tr>
<td>8</td>
<td>M/28</td>
<td>TSA and RT</td>
<td>1</td>
<td>1017</td>
<td>present</td>
<td>T4, testosterone</td>
</tr>
<tr>
<td>9</td>
<td>F/39</td>
<td>UAPI</td>
<td>24</td>
<td>1083</td>
<td>not identified</td>
<td>none</td>
</tr>
</tbody>
</table>

UAPI: Unilateral adrenalectomy followed by external pituitary irradiation. TSA: transphenoidal adenectomy. ADX: bilateral adrenalectomy. NS: Nelson’s syndrome. T4: thyroxine. GH: growth hormone. DDAVP: desmopressin.*: Blood samples were taken 1-4 hr after hydrocortisone medication. Mean cortisol concentration was 730 nmol/L, range 480-890 nmol/L. Patient 7 was treated initially by TSA, four years later ADX was performed. Patient 8 was treated initially by TSA and subsequently by pituitary irradiation, because of persisting disease. Recurrence of Cushing’s disease occurred 9 years later, after which ADX was performed.
Neurohormone output in Nelson's syndrome

Methods
Volunteers were admitted to the hospital on the day of the study. An indwelling iv cannula was inserted in a forearm vein at least 60 min before sampling began. Blood samples were withdrawn at 10 min intervals for 24 h, starting at 0900 h. A slow infusion of 0.9% NaCl and heparin (1 U/mL) was used to keep the line open. The subjects were free to ambulate, but not to sleep during the daytime. Meals were served at 0800, 1230 and 1730 h. Lights were turned off between 2200-2400 h. Plasma for ACTH was collected on ice in EDTA-containing tubes, centrifuged at 4°C for 10 min, and stored at ~20°C until later assays. The study was approved by the ethical board of the Leiden University Medical Center and informed written consent was obtained from all the patients and control subjects.

Assays
ACTH concentrations were measured in duplicate by two-site monoclonal immunoradiometric assay (Nichol's Institute, San Clemente, CA) with a detection limit of 2 ng/L. The intraassay coefficient of variation was 2.8-7.5% in the concentration range 3-300 ng/L, and 1.0-2.0 % in the concentration range of 300-1800 ng/L.

Deconvolution Analysis
Multiparameter deconvolution analysis is a technique which resolves the serum hormone concentration profile into its constituent secretory contributions and simultaneously estimates the hormone half-life. This analysis was used to quantify underlying basal and pulsatile ACTH secretion and to estimate the corresponding (endogenous) half-life (Veldhuis et al., 1987). Daily pulsatile secretion is the product of secretory burst (pulse) frequency and the mean mass of hormone released per burst. The mass secreted per burst is the analytical integral of the secretory pulse. The latter is determined by its amplitude (maximal secretory rate) and half-duration (duration of the burst at half-maximal amplitude). Basal secretion was calculated simultaneously as time-invariant interpulse release. Secretory pulse identification for ACTH required that the estimated secretory-burst amplitude exceeded zero by 95% joint statistical confidence intervals (Veldhuis & Johnson, 1992). Based upon ACTH model simulations (Keenan et al., 2001), this statistical requirement affords 95% sensitivity and 93% specificity of ACTH pulse detection for 10-min data (Veldhuis & Johnson, 1995).

Approximate Entropy (ApEn) analysis
A sensitive metric of relative disorderliness of hormone concentration profiles, termed approximate entropy (ApEn), was used to quantify objectively the serial regularity or orderliness of ACTH release patterns over 24 h (Pincus, 1991). This statistic is a finite positive nonzero real number, developed for any single entire hormone pulse profile as an ensemble estimate of the ‘point-by-point’ sub-pattern reproducibility within the data. As such, ApEn provides a scale-invariant and
model-independent quantitation of relative disorderliness, whereas higher ApEn values denote greater relative disorderliness or reduced regularity of the release process e.g., as observed for ACTH in Cushing’s disease, GH in acromegaly, and PRL in prolactinoma (Hartman et al., 1994, Groote Veldman et al., 1999, Van den Berg et al., 1997). Technically, ApEn designates the negative logarithm of the probability that a given pattern of successive hormone measurements is repeated upon next incremental comparison within a tolerance $r$ for a data window length $m$. The parameter $r$ is typically set at 20% of the individual within-series standard deviation to normalise ApEn for unequal mean serum hormone concentrations. For series of lengths < 200, $m$ is typically given as unity. This choice of $m$ and $r$ yields high statistical replicability (Pincus et al., 1999). Thus ApEn is a family of statistics conditional on $m$ and $r$ and relatively insensitive to occasional outliers within the data and to experimental variability (noise) smaller in magnitude than $r$. Results are presented as absolute ApEn values and normalised ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1000 randomly shuffled versions of the same series (Veldhuis & Pincus, 1998). Thus ApEn ratios of unity approach mean empirical randomness for any given sequence, whereas values less than 1.0 denote more orderly sequences. In addition, we applied ApEn to the serial interburst interval and burst-mass values from the deconvolution analysis. Thereby, we quantitate relative randomness of serial interburst interval and burst mass values (Veldhuis et al., 2001*, Farhy et al., 2002). For these measures $m = 1$ and $r = 85\%$ are appropriate (Pincus et al., 1999).

Nyctohemeral (24-h) rhythmicity
The twenty-four-hour variations in ACTH concentrations were analysed using a nonlinear unweighted least squares cosine approximation (cosinor analysis), as reported earlier (Veldhuis et al., 1990). Ninety-five percent statistical confidence intervals were determined for the 24-h cosine amplitude (50% of the nadir-zenith difference), mesor (rhythmic mean) and acrophase (clock-time of maximal value).

Statistical analysis
The primary goal of this investigation was to compare ACTH secretion characteristics with those of patients with proven Cushing disease. Some of the variables of the deconvolution analysis and the cosinor analysis were skewed. Therefore deconvolution and cosinor data were analysed by the Kruskal-Wallis test, followed by the Mann-Whitney test for comparison of groups means. We also used the Kolmogorov-Smirnov test, which gave comparable results. Otherwise, comparison between groups was done with the two-tailed Students t-test for unpaired data. Results are presented as the mean ± SEM. Statistical calculations were performed with Systat, version 10 (SPSS Inc., Chicago, IL). P < 0.05 was considered significant.
RESULTS

ACTH secretion

Figure 1 illustrates representative profiles of 24 h plasma ACTH concentrations over time in patients with Nelson’s syndrome, Cushing’s disease and controls. Deconvolution analysis was used to quantify specific secretory and kinetic features of ACTH output: Table 2. In patients with Nelson’s syndrome, basal ACTH production was increased 6-fold and pulsatile secretion 9-fold compared with values in Cushing’s patients. The increase in pulsatile secretion was attributable to an 8-fold increased mass of ACTH released per event (583 ± 160 ng/L vs 75 ± 20 ng/L, P < 0.001) (figure 2), and the 12-fold increased amplitude (maximal rate of secretion, P<0.0001). In contrast, event frequency (32.3 ± 1 vs 28.3 ± 2, P = 0.1) and the apparent half-life of ACTH (18.8 ± 1.4 vs 17.8 ± 2.0 min, P = 0.51) were comparable to estimates in Cushing’s disease. Neither state of ACTH excess was associated with any change in ACTH half-life. The results of the deconvolution analysis of the controls are listed in Table 2.

ACTH nyctohemeral variation

Cosinor analysis of plasma ACTH concentration time series in Nelson’s syndrome disclosed a 16-fold increase in amplitude and 10-fold elevation in the mesor over values in Cushing’s disease (table 3). The ACTH acrophase in Nelson’s syndrome was delayed compared with controls (1210 ± 113 min vs 0754 ± 20 min, P = 0.037).
Figure 1. ACTH profiles in a patient with Nelson’s syndrome (upper panel), one patient with Cushing’s disease (middle panel) and a healthy control subject. The ACTH concentrations were monitored by sampling blood every 10-min for 24 h. The vertical bars represent the within-assay dose-dependent standard deviation. The fitted continuous curve shows the deconvolution-predicted ACTH profile, the right panels the ACTH secretion rates calculated by multiparameter deconvolution analysis.
Figure 2. Scatter plots of the basal ACTH secretion rate and ACTH burst mass, calculated by multiparameter deconvolution in 9 patients with Nelson's syndrome and in the same number of patients with Cushing's disease (pituitary dependent hypercortisolism) and age-and gender-matched controls. The significance level is shown for the Kruskal-Wallis test. Note that the data are shown on a logarithmic scale.

Figure 3. Scatter plots of Approximate Entropy of the deconvolved plasma ACTH concentration series in 9 patients with Nelson's syndrome, 9 patients with Cushing's disease and 9 age- and gender-matched controls. The ApEn statistic was applied to the burst-intervals (left panel) and to the burst-masses of the deconvolved ACTH concentration series. The horizontal lines reflect the mean. The shown P-value reflects the ANOVA.
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**Approximate Entropy**

ApEn analysis was applied to the 24-h ACTH concentration profiles to quantitate the regularity of the release process: Table 4. ApEn of ACTH release in Nelson’s syndrome did not differ from that in controls, but was significantly elevated in patients with Cushing’s disease, as reported earlier. The latter denotes the highly irregular minute-to-minute ACTH release. To investigate the (unexpected) preservation of pattern regularity of ACTH release in patients with Nelson’s syndrome, ApEn analysis was also applied to the succession (ordered series) of calculated ACTH burst-mass and interburst-interval values. Statistical comparisons revealed that ApEn of serial interburst intervals was lower in Nelson patients than in controls, indicating heightened regularity of tumour secretory event timing (figure 3). In addition, ApEn estimates of sequential ACTH burst mass in Nelson patients was lower than that in Cushing’s patients, but similar to that in controls.

### Table 3 Cosinor analysis of the 24 h plasma ACTH concentration series

<table>
<thead>
<tr>
<th></th>
<th>Nelson M. Cushing</th>
<th>Normal Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mesor (ng/L)</strong></td>
<td>750 ± 350 *</td>
<td>75 ± 14</td>
<td>12.7 ± 1.4</td>
</tr>
<tr>
<td><strong>Amplitude (ng/L)</strong></td>
<td>160 ± 70 ^</td>
<td>10.8 ± 2.1</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td><strong>Acrophase (clock hour ± min)</strong></td>
<td>1210 ± 113</td>
<td>1618 ± 132</td>
<td>0754 ± 20</td>
</tr>
</tbody>
</table>

Data were analysed by the Kruskal-Wallis test (last column). Differences between groups were evaluated with the Mann-Whitney test. Differences between the Nelson and Cushing groups are shown as: a: P=0.0012, b: P=0.0018. Statistical significant differences between Nelson and control groups are given as: c: P=0.0005, d: P=0.037. Data are shown as mean ± SEM. *: mean value about which the 24-h rhythm varies. ^: 50% of the nadir-to-zenith difference in ACTH concentration. ±: time of maximum value.

### Table 4 Approximate Entropy analyses of the relative orderliness of ACTH secretion in patients with Nelson’s syndrome, Cushing’s disease and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Nelson’s syndrome</th>
<th>Cushing</th>
<th>Control</th>
<th>P value Nelson vs Cushing</th>
<th>P value Nelson vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApEn (ACTH)</td>
<td>1.018 ± 0.133</td>
<td>1.420 ± 0.061</td>
<td>0.902 ± 0.049</td>
<td>0.014</td>
<td>0.42</td>
</tr>
<tr>
<td>ApEn ratio (ACTH)</td>
<td>0.576 ± 0.069</td>
<td>0.754 ± 0.029</td>
<td>0.507 ± 0.030</td>
<td>0.031</td>
<td>0.37</td>
</tr>
<tr>
<td>Serial burst interval (ACTH)</td>
<td>0.797 ± 0.065</td>
<td>0.880 ± 0.047</td>
<td>0.998 ± 0.040</td>
<td>0.34</td>
<td>0.022</td>
</tr>
<tr>
<td>Serial burst mass (ACTH)</td>
<td>0.769 ± 0.061</td>
<td>0.974 ± 0.026</td>
<td>0.807 ± 0.066</td>
<td>0.007</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Data were analyzed by the two-tailed Student t-test. Data are shown as the mean ± SEM.
DISCUSSION

Albeit not established previously to our knowledge, an expected and striking feature in patients with Nelson’s syndrome is the multifold elevation of both basal (time-invariant) and pulsatile (episodic) ACTH secretion. This prediction arises from the combined amplification of basal and pulsatile hormonal release by GH- and prolactin-secreting pituitary tumours (Hartman et al., 1994; Groote Veldman et al., 1999). In contrast, we are unaware of any precedence for (paradoxically) accentuated regularity of adenomatous hormone secretion. Indeed, a cardinal property of neuroendocrine neoplasms is marked deterioration of the quantitative consistency of the release process. Accordingly, the present analytical platform establishes joint secretory mechanisms driving elevated mean plasma ACTH concentrations and unique neuroregulatory contrasts in Nelson’s syndrome and Cushing’s disease.

Two hallmarks of Cushing’s disease are diminished suppressibility of ACTH secretion to glucocorticoids and blunted diurnal rhythmicity. These abnormalities are accompanied by increased basal and pulsatile secretion of ACTH and cortisol and marked deterioration of the individual and joint regularity of the release of both hormones (Van den Berg et al., 1995; Roelfsema et al., 1998). The present data in Nelson’s syndrome identify some similitude with more extensively studied Cushing’s disease; viz., elevated ACTH secretory-burst frequency, amplitude (mass) and basal release.; delayed timing of the daily maximum in ACTH secretion; and normal ACTH elimination half-life.

In as much as Nelson’s syndrome occurs primarily in patients with Cushing’s disease after bilateral adrenalectomy a plausible (but unproven) exacerbating factor is therapeutically incomplete restoration of physiological negative feedback by cortisol or synthetic congeners. In this regard, acute metyrapone administration to healthy individuals induces a 12-fold amplification of ACTH secretory burst mass along with a lesser elevation in basal (non-pulsatile) secretion (1.5-fold) and pulse frequency (1.4-fold) (Veldhuis et al., 2001b). However, 60-fold higher basal ACTH secretion in Nelson’s syndrome than controls and a 6-fold higher release than in patients with Cushing’s disease would not be easily attributable to acutely diminished glucocorticoid feedback. Long-term feedback withdrawal might remain relevant to accentuated basal ACTH release. Mechanistically, the latter in principle could reflect the total increase in (tumoural) corticotroph cell mass. For example, unpublished observations in 5 male patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency, who did not use glucocorticoid substitution or were withdrawn from this medication for study purposes, exhibited elevated (8-fold) pulsatile and (6-fold) basal ACTH release. This analogy follows from pituitary cell-specific hyperplasia recognized in patients with congenital primary thyroidal or ovarian failure not receiving early or consistent hormone replacement therapy. In addition, although the precise cellular basis of inferentially constitutive basal ACTH release is not known, adenomatous transformation of corticotroph
cells may heighten this marker of unregulated release. The latter concept has been verified in hyperparathyroidism associated with longstanding renal failure (Schmitt et al., 1998).

Our patients were studied while using a standardized steroid-replacement schedule, consisting of dexamethasone 0.25 mg at 0800 and 1800 h and fludrocortisone 0.125 mg at 0800 h. This schedule was used to obtain stable and approximately physiological systemic glucocorticoid availability. In this regard, Cook et al. (1976) reported that a daily dose of 2 mg dexamethasone does not suppress plasma ACTH concentrations in patients with Nelson’s syndrome, while completely suppressing ACTH secretion in patients with congenital adrenal hyperplasia. Nonetheless, the current data do not explore the potential impact of varying dexamethasone doses on ACTH dynamics. In the latter regard, one patient (of four) with Nelson’s syndrome studied by Karl and colleagues exhibited a mutant glucocorticoid receptor, thereby putatively muting glucocorticoid negative feedback (Karl et al., 1996).

Apparent pulse frequency was elevated comparably in Nelson’s syndrome and Cushing’s disease, as inferred also in patients with somatotropinomas and prolactinomas (Hartman et al., 1994; Groote Veldman et al., 1999). The basis for this general finding is not established. However, curative pituitary adenomectomy typically normalizes this feature (Groote Veldman et al., 2000; van den Berg et al., 1994). The latter data could indicate that accelerated event frequency reflects autonomous properties of adenomatous cells, abnormal tumoural-product feedback on hypothalamic centers, and/or technical overestimation of diminutive release episodes as de facto pulses. Heightened irregularity of tumoural hormone release is an established statistical marker of reduced feedback responsivity (Hartman et al., 1994; Veldhuis et al., 2001), and would concomitantly accentuate the analytical risk of type I (false positive) pulse enumeration (Veldhuis & Johnson, 1995).

A significant delay in diurnal timing of the ACTH concentration maximum of the 24-h rhythm points toward partial hypothalamic supervision of adenomatous secretion. Although the former shift in ACTH acrophase is not observed in patients with CAH withdrawn from glucocorticoid replacement (unpublished), acute blockade of cortisol synthesis does induce 3-h acrophase delay in healthy adults (Veldhuis et al., 2001). Acromegaly and tumoural or functional hyperprolactinaemia appear to differ in this regard. In these disorders either no shift in acrophase or only a modest shift is found. In prolactinomas, but also in functional hypothalamic disconnection, no change in acrophase is present, where in active acromegaly an advance shift of about 3 h was observed (Groote Veldman et al., 1999; van den Berg et al., 1994). These divergent observations do not allow ready generalizations at present.

A striking finding in the present analysis is significantly greater quantitative regularity of serial ACTH release patterns in Nelson’s syndrome than in untreated Cushing’s disease. In fact, approximate entropy analyses could not discriminate between ACTH secretory orderliness in Nelson’s syndrome and that in age- and gender-matched control subjects. The latter mechanistic distinction in regularity
Neurohormone output in Nelson’s syndrome

control was specific to ACTH, since GH output was equivalently irregular in the two hypercorticotropinaemic states (data not shown). Acute reduction in cortisol feedback in healthy adults also significantly increases ACTH orderliness (Veldhuis et al., 2001b). Significantly enhanced ACTH regularity in Nelson’s syndrome compared with Cushing’s disease could therefore reflect greater resistance to glucocorticoid negative feedback in the former case. In addition, secretagogue infusion studies and simpler reductionist mathematical models predict that reduced feedforward signalling can maintain more regular system output (Veldhuis et al., 2001). According to this analytical framework, lesser endogenous CRH and/or AVP drive (for instance as caused by pituitary irradiation), could facilitate more orderly ACTH secretion in Nelson’s syndrome than in Cushing’s disease. Lastly, the higher ACTH concentrations cannot explain this unique distinction, since the statistically normalized ApEn statistic adjusts analytically for markedly unequal mean hormone measurements (Pincus, 1991; Hartman et al., 1994; Pincus, 1994). In addition, ApEn analyses of sequences of (deconvolved) ACTH secretory-burst mass and interburst-interval times corroborate a paradoxical increase in orderliness in patients with Nelson’s syndrome.

We conclude that Nelson’s syndrome is marked by multifold elevation of both basal and pulsatile modes of ACTH secretion and paradoxical regularity of the ACTH release process. These pathophysiological are consistent with increased corticotroph mass and greater tumoural isolation from both (negative) feedback and (positive) endogenous regulatory signals in Nelson’s disease in comparison with in Cushing’s disease.
REFERENCES


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