Neuroendocrine perturbations in human obesity

Kok, P.

Appendix A

Abbreviations

ACTH	adreno corticotropin hormone
ApEn	approximate entropy
AVP	arginine vasopressin
AUC	area under the curve
BF	body fat
BMI	body mass index
CART	cocaine- and amphetamine-regulated transcript
CRH	corticotropin releasing hormone
CSF	cerebro spinal fluid
CV	inter-assay coefficients of variation
D2R	dopamine 2 receptor
DA	dopamine
DEXA	dual energy X-ray absorptiometry
E2	estrogen
EEG	electro encephalo gram
FFAs	free fatty acids
fT_4	free thyroxine
GH	growth hormone
GHRH	growth hormone releasing hormone
HOMA	homeostatic model assessment
HPA	hypothalamic-pituitary-adrenal
HPT	hypothalamic pituitary thyroid
ICV	intra cerebroventricular
IGF-1	insulin like growth factor type 1
IV	intra venous
LPS	lipopolysacharide
LUMC	leiden university medical centre
MET	metoclopramide
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
N	nitrogen
NE	norepinephrine
NPY	neuropeptide Y
POMC	pro-opiomelanocortin
PRL	prolactin
T_3	triiodothyronine
TG	triglyceride
TSH	thyroid stimulating hormone
TRH	thyrotropin releasing hormone
TBFM	total body fat mass
T_4	thyroxine
SEM	standard error of the mean
SFM	subcutaneous fat mass
REE	resting energy expenditure
VCO_2	volume of carbon dioxide
VD	distribution volume
VFM	visceral fat mass
VLCD	very low calorie diet
VMH	ventromedial hypothalamus
VO_2	volume of oxygen
WHO	world health organization

Appendix B:

Analysis 24 h hormone profiles

Endocrine glands secrete hormones in a temporal manner that is of major importance to achieve appropriate physiological functioning, e.g. the cellular response in target tissues. Quantifying secretion profiles rather then merely inspecting plasma hormone concentration patterns reveals additional information about pulse duration, pulse shape, pulse height, pulse timing and clearance rates (1). The prominent intermittency of hormonal release can be either rhythmic (regularly repeating secretion episodes over time) or episodic (apparently randomly scattered secretion events over time). Calculation of regularity and circadian rhythmicity of hormone concentration time series data provides additional insight of hormonal release (2). Various validated mathematical techniques have been developed to appraise these parameters from hormone concentration patterns (for review see (3)). Diurnal concentration patterns of different neuroendocrine systems in obese premenopausal women enrolled in the clinical studies described in this thesis were analyzed using Cluster, Deconvolution, Cosinor, Cleveland robust fitting and Approximate Entropy (ApEn) algorithms. The operating principles and a general introduction of these mathematical techniques will be discussed in this appendix.

Cluster Analysis

The first developed pulse detection method by Johnson and Veldhuis was the Cluster analysis method (4). This method uses a sliding pooled t-test to identify data points within the hormone time series that correspond to statistically significant increases and decreases in hormone concentrations (changes at the edges of times series are not identified). Thus, the Cluster program identifies locations and durations of significant plasma hormone peaks. In performing the analysis, one has to specify individual test cluster sizes for the nadir and the peak (i.e., number of points to be used in testing nadirs against peaks), a minimum and maximum of intra series coefficient of variation, a t-statistic to identify a significant increase and a t-statistic to identify a significant decrease. The following parameters are estimated: mean concentration, total area under the curve, peak frequency, mean peak height (maximum value attained within a peak), peak amplitude (mean incremental peak height), incremental peak height as a percentage of nadir, mean peak area (above the baseline) and mean inter peak valley concentration (nadir).

Deconvolution Analysis

As the Cluster program does not provide information about the secretion and elimination of hormones, the deconvolution analysis was developed (1). Deconvolution analysis is a statistically based algorithm that estimates hormone kinetics and secretion rates from hormone concentration time-series (5;6). The general approach of the deconvolution technique is to derive a mathematical model for the form of a hormone concentration pulse an then, using nonlinear least-square methods, fit the actual experimental data to a series of these mathematical forms (secretory bursts) occurring at various times. Each secretory burst has a specific waveform (shape), which is dependent on the appearance, distribution and the clearance of the hormone from plasma or serum. Disappearance of hormones from plasma is best described by a two compartment model, characterized by a fast component half-life, a slow component half-life and a fractional contribution of the slow component to the overall decay. The Pulse algorithm is a waveform-independent deconvolution method, which can be used for calculation of hormonal secretion, without specifying shape, number and time of secretory events (1). The technique

requires a priori specification of hormonal half-life in plasma, although the algorithm is relatively insensitive to the assumed values of elimination half-lives. Thus, before running the Pulse program, the fast and a slow component half-life and the fractional contribution of the slow component to the overall decay are entered for the hormone analyzed. Pulse can thus be used to quantify hormonal secretion. Secretion rates are expressed per liter distribution volume (VD). One limitation of this method is that the program does not identify small secretory events and a large number of tenuous assumptions must be imposed to propagate the uncertainties of the experimental observations into the uncertainties of the secretory profile. However, Pulse can be used to assess the initial parameters required for the waveform- dependent deconvolution method, as amplitudes and locations of large secretory events are easily defined. After the initial guess by waveform-independent estimates of hormonal secretion using Pulse, subsequent analysis with a waveform-dependent multi-parameter deconvolution method is performed. Mean best fit values and statistical confidence limits for each secretory and clearance parameter are taken into account by the program. Thus, the probability that a secretory burst has a significant amplitude is estimated. Furthermore, all underlying relevant secretory events are evaluated simultaneously, which enhances the statistical power of the deconvolution procedure. This technique also requires a priori specification of hormonal half-life in plasma. The fast and a slow component half-lifes and the fractional contribution of the slow component to the overall decay have to be specified for the specific hormone analyzed. This technique estimates the combined rates of basal release, number, duration, amplitude and mass of randomly ordered secretory bursts and the subject-specific half-life. The daily pulsatile secretion is the product of secretory burst frequency and mean mass released per event. Total secretion is the sum of basal and pulsatile secretion. Results are expressed per liter distribution volume. For the calculation of production rates per liter, the distribution volume of the hormone has to be calculated.

Cosinor and Cleveland robust fitting

Nyctohemeral characteristics of hormone concentration patterns can be determined using cosinor or Cleveland robust fitting analysis. The cosinor test is the oldest method proposed for and applied to 24 h rhythms. Cosinor analysis entails trigonometric regression of a cosine function on the full 24 h plasma hormone concentration profile vs. time: $y(t) = M + A \cos(\omega t + \phi) + e(t)$ with y(t) the value at time t of the periodic function of angular frequency ω (degrees per time unit, 360 degrees = complete circle), defined by parameters M (mesor), A (amplitude) and ϕ (acrophase). The cycle duration (τ) is fixed for each fit (e.g. 24 hours). This function is fitted to the data (using least squares method) to derive rhythm parameters estimates; the acrophase (clock time during 24 h at which hormone concentration is maximal), mesor (midline estimated statistic of rhythm, or the average value of the rhythmic cosine curve) and the amplitude (half of the total predictable change in the rhythm). The major disadvantage of this technique is that it assumes that the observed 24 h rhythm is best described by a symmetric sinusoidal curve. However, most 24 h concentration patterns have asymmetrical wave-shapes. Therefore, the robust curve (LOWESS) fitting algorithm described by Cleveland was used to determine the zenith, nadir and mesor of the day long hormone rhythms. The technique is described in more detail in ref (7). This program provides a more adequate description of asymmetrical wave shapes, based on periodogram calculations or non-linear regression procedures.

Approximate Entropy Analysis

Approximate entropy (ApEn) can be used to quantify the orderliness or regularity of serial hormone concentrations over 24 h (2;8). ApEn is a scale- and model-independent statistic developed and formulated by Pincus (9), which is applicable to a wide variety of physiological and clinical time-series data. ApEn measures the logarithmic likelihood that runs of patterns in a time series that are close for m consecutive observations remain close when considered as m + 1 consecutive observations. Greater regularity (higher probability to remain close) yields smaller ApEn values. Higher absolute ApEn values denote greater relative randomness or lower regularity of hormone patterns. Calculation of ApEn requires prior definition of two parameters: m (length of the run to be compared) and r (filter or the magnitude that will discern “close” and “not close”). For optimal statistical validity ApEn is typically implemented in hormone time series by using m values of 1 or 2 and r values of approximately 0.2 SD of the series being considered. The ApEn metric thus evaluates the consistency of recurrent subordinate (nonpulsatile) patterns in a time series. Regularity of hormonal secretion patterns mirrors the net result of feed forward signaling and feedback restraint (8). Thus, ApEn yields information about hormonal time series, distinct from and complementary to Cluster, deconvolution, cosinor and Cleveland analyses (2).

Some potential pitfalls of these mathematical techniques have been described (10). For example, the computer models require input of prolonged serial measurements of circulating hormone concentrations obtained by highly intensive sampling regimens. Furthermore, hormone concentration series can be noisy and experimental conditions may contribute to the uncertainty in the data (blood loss, subject manipulation, sample processing, assay methods). Another problem is that the waveform-independent deconvolution requires a priori knowledge of half-life. It is not possible to estimate secretion and clearance rates using waveform-dependent deconvolution analysis, without an initial assumption of the waveform. Finally, fluctuations of basal or tonic hormone secretion within the time series analyzed are not taken into account by the program, which in turn may yield different estimates of basal secretion rates. Secretion rates that are called basal (tonic secretion or inter-pulse secretion), cannot be interpreted as necessarily significant (i.e. distinct from zero or above assay noise and/or experimental uncertainty). Unfortunately, at present there is no useful and critical information regarding the analysis of low levels of tonic hormonal secretion.

Nevertheless, these techniques provide a way to obtain additional (physiologically relevant) information from hormone time series and these analyses are important non-invasive methods to calculate the temporal distribution of hormone pulses and secretion rates. In addition, the regularity of secretion (ApEn) can be measured, giving insight into the feedforward and feedback signaling of the particular neuroendocrine system. Finally, insight into the diurnal (circadian) properties of the neuroendocrine system can be obtained.

Reference List

1. Johnson ML, Veldhuis JD. Evolution of deconvolution analysis as a hormone pulse detection period. Methods in neurosciences 1995; 28:1-24.
2. Veldhuis JD, Pincus SM. Orderliness of hormone release patterns: a complementary measure to conventional pulsatile and circadian analyses. Eur J Endocrinol 1998; 138(4):358-362.
3. Urban RJ, Evans WS, Rogol AD, Kaiser DL, Johnson ML, Veldhuis JD. Contemporary aspects of discrete peak-detection algorithms. I. The paradigm of the luteinizing hormone pulse signal in men. Endocr Rev 1988; 9(1):3-37.
4. Veldhuis JD, Johnson ML. Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. Am J Physiol 1986; 250(4 Pt 1): E486-E493.
5. Veldhuis JD, Johnson ML. Deconvolution analysis of hormone data. Methods Enzymol 1992; 210:539-575.
6. Veldhuis JD, Carlson ML, Johnson ML. The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. Proc Natl Acad Sci U S A 1987; 84(21):7686-7690.
7. Cleveland WS. Robust locally weighted regression and smoothing scatter plots. J Am Stat Assoc 1979; 74:829-836.
8. Veldhuis JD, Straume M, Iranmanesh A et al. Secretory process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans. Am J Physiol Regul Integr Comp Physiol 2001; 280(3):R721-R729.
9. Pincus SM. Quantification of evolution from order to randomness in practical time series analysis. Methods Enzymol 1994; 240:68-89.
10. Veldhuis JD, Johnson ML. Returning to the roots of endocrinology: the challenge of evaluating in vivo glandular secretory activity. Endocrinology 1990; 127(6):2611-2617.