Altered Setting of the Pituitary-Thyroid Ensemble in Hypocretin Deficient Narcoleptic Men

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Running title: pituitary-thyroid axis in narcolepsy.

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Keywords for indexing:
narcolepsy, TSH, orexin, sleep, entropy
Abstract

Narcolepsy is a sleep disorder caused by disruption of hypocretin (orexin) neurotransmission. Injection of hypocretin-1 acutely suppresses TRH and TSH release in rats. In contrast, subchronic administration does not appear to affect the hypothalamo-pituitary-thyroid (HPT) ensemble in animals. We explored (in 7 patients and 7 controls) whether hypocretin deficiency impacts circulating TSH levels and circadian timing of TSH release in narcoleptic humans. Plasma TSH concentration profiles (blood samples taken at 10 min intervals during 24 h) and TSH levels in response to TRH injection were analyzed by Cluster, robust regression, approximate entropy (ApEn) and deconvolution. Circulating TSH levels were lower in patients, which was primarily attributable to lower pulse amplitude and nadir concentrations. TSH secretion correlated positively with mean 24-h leptin levels ($R^2 = 0.46, P = 0.02$) and negatively with the amount of sleep ($R^2 = 0.29, P = 0.048$). Pattern-synchrony between 24-h leptin and TSH concentrations was demonstrated by significant cross-correlation and cross-ApEn analyses with no differences between controls and patients. The onset of sleep was closely associated with a fall in circulating TSH. The features of diurnal rhythmicity of circulating TSH fluctuations were similar in patients and controls, with the acrophase occurring shortly after midnight. Thyroxine and triiodothyronine concentrations were similar in patients and controls and did not display a diurnal rhythm. The response of plasma TSH levels to TRH was also similar in both groups. Sleep patterns in narcoleptics were significantly disorderly compared with controls, as measured by ApEn ($P=0.006$). In summary, circulating TSH concentrations are low in hypocretin-deficient narcoleptic men, which could be attributable to their low plasma leptin levels and/or their abnormal sleep-wake cycle.
Introduction

Narcolepsy is a neurological disorder, characterized by excessive daytime sleepiness, hypnagogic hallucinations, cataplexy and sleep paralysis (41) which affects 20 to 60 of every 100,000 subjects in the U.S.A. and Europe (32). Disruption of hypocretin (orexin) neurotransmission underlies the disease in animals and man (28,39). Hypocretins (hypocretin-1 and -2, also called orexin A and B) are produced by a small group of neurons in the lateral hypothalamus which project widely throughout the central nervous system (8,56). The paraventricular nucleus (PVN) is among the various brain sites that receive hypocretin input signals (7). This nucleus harbors neurons that synthesize thyrotropin-releasing hormone (TRH), which is involved in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis (24). During starvation, thyroid hormone secretion is suppressed (2,52), while prepro-hypocretin mRNA is up-regulated in the lateral hypothalamus (4). This led us to hypothesize, that hypocretin neural circuits can modulate the activity of hypothalamic TRH neurons and thereby impact the HPT axis.

Studies of the putative effects of hypocretin peptides on the HPT axis in animals have been incomplete and results are conflicting. A single intravenous or intracerebroventricular (icv) injection of hypocretin-1 in rats acutely decreased plasma TSH concentrations and hypothalamic TRH release (34). In contrast, several other papers report that hypocretin-1 has no measurable effect on the pituitary thyroid axis (16,20,53,54), whereas circulating TSH was shown to increase in response to central administration of hypocretin-2 (20). Thus, although the topography of hypocretin- and thyrotrope neural circuits suggests that TRH neuronal activity is governed by hypocretin input, the nature of the signal (i.e. excitatory or inhibitory) remains unclear (where
hypocretin-1 and 2 may have differential effects as a result of their distinct chemical structure).

We have previously reported that circulating leptin levels are low in (male) narcoleptic patients (22). Since leptin has stimulatory effects on the HPT axis, it is conceivable that (relative) hypoleptinemia downregulates HPT-axis activity in narcolepsy. In addition, the typical disruption of the sleep-wake cycle associated with narcolepsy may also impact TSH release, as sleep inhibits TSH secretion in healthy subjects (42).

To gain insight in the regulation of the HPT axis in hypocretin deficient humans we analyzed 24 h plasma TSH and thyroid hormones concentrations in relation to leptin and sleep in 7 narcoleptic men and 7 healthy controls.

**Methods**

**Subjects**

We included seven male patients (mean age 46.1 yr, range 26-71) from the outpatient clinic of the Department of Neurology. The diagnosis of narcolepsy with cataplexy was made on clinical grounds by a physician experienced with narcolepsy (GJL). Multiple sleep-latency testing showed results typical for narcolepsy (33). All patients were HLA-DR2/DQB1*0602 positive, and lacked hypocretin-1 in their CSF (measurements as previously described (39). All subjects were free of medication, or (in three patients) discontinued medication for at least two weeks prior to study. Two patients used psychostimulants (methylfenidate and modafinil) and one a tricyclic antidepressant (clomipramine) as treatment of narcolepsy.

Weight and height of the subjects were measured, as well as waist and hip circumference. The waist-to-hip ratio (WHR) was used as a relative measure of
abdominal fat mass. Total body fat mass was determined by dual-energy X-ray absorptiometry (Hologic QDR4500, Waltham, MA). Seven male control subjects (mean age 46.9 yr, range 26-72) were recruited through advertisements in local newspapers. They were matched for age, body mass index (BMI), total fat mass, and WHR, since narcoleptics are (moderately) obese (23).

Subjects were eligible for the study after exclusion of hypertension (defined as a repeated blood pressure measurement of systolic > 160 mm Hg or diastolic > 90 mm Hg), any known (history of) pituitary disease, recent weight change (> 3 kg weight gain or loss within the last 3 months), and fasting blood glucose > 7.0 mmol/l. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the Leiden University Medical Center.

**Experimental protocol**

Subjects were admitted to the Clinical Research Center in the morning of the 24 h study occasion. During the 24 h study occasion, three standardized meals were served, at 0900, 1300 and 1800 (Nutridrink 1.5 kcal /ml, 1500-1800 kcal/day; macronutrient composition per 100 ml: 5 g protein, 6.5 g fat, 17.9 g carbohydrate; Nutricia, Zoetermeer, The Netherlands). Subjects remained sedentary throughout the study, except for bathroom visits. Lights were switched off at 2300. Upon arrival, an intravenous cannula was inserted in an antecubital vein one hour before the start of blood sampling. Blood samples were collected with S-monovetten (Sarstedt, Etten-Leur, The Netherlands) from a 3-way stopcock, that was attached to a 0.9% NaCl and heparin (1 U/ml) infusion (500 ml/24 h) to keep the cannula from clotting. Blood was collected at 10 minute intervals for 24 h. It was sampled through a long line to prevent sleep disturbance by investigative
manipulations. Samples were clotted, centrifuged at 4°C for 20 min, and serum was frozen at -20°C until assay.

Plasma TSH concentration was measured in every sample. Total thyroxine and triiodothyronine concentrations were measured hourly, because of their relative long plasma half lives. The plasma free thyroxine concentration was measured only once in the morning.

On a separate occasion, a TRH stimulation test was carried out in 5 patients and 5 controls. In the fasting state, 200 μg TRH was injected iv and blood samples were taken at 10 min intervals for 3 h.

**Assays**

Thyroid hormone assays were performed on automated systems: total thyroxine (T₄) was measured by fluorescence immuno-enzymometric assay (FIEMA, Abbott, Diagnostics Division, Hoofddorp, The Netherlands) and triiodothyronine (T₃) by fluorescence enzyme-immunoassay (FEIA, Abbott, Diagnostics Division, Hoofddorp, The Netherlands). The free thyroxine concentration was measured by electrochemoluminescence immunoassay (Roche Diagnostics Nederland BV, Almere, The Netherlands). Thyrotropin (TSH) was measured by time resolved immunofluorometric assay (Wallac, Turku, Finland). The detection limit of the TSH assay is 0.05 mU/L and the interassay coefficient of variation is < 5%. In addition, the plasma leptin concentration was measured in every 20-min sample by RIA (Linco Research, St Charles, MO). The detection limit of the assay was 0.5 μg/L, the CV was 3.4-8.3 % in the concentration range of 4.9-25.6 μg/L. A detailed description of circulating leptin levels in these narcoleptic men was previously published (22). Here we
just correlate the 24-h mean leptin concentration and TSH secretion and also 20-min leptin data in relation to the reconstituted 20-min plasma TSH profile.

**Sleep recording and analysis**

During the 24 h sampling procedure, sleep was recorded polygraphically using a Porti-5 ambulant electroencephalogram recording system (Twente Medical Systems International, Enschede, The Netherlands) and scored on EEG-2100 review software (Nihon Kohden, Tokyo, Japan). An experienced technician, blinded for the subject under study, visually scored the sleep recordings at 30-s epochs by means of standardized criteria (49). For the present study we only used the distribution and amount of sleep across the 24 h.

**Calculations and statistics**

**Cluster analysis**

Cluster was used for the detection of discrete TSH peaks. This computerized pulse algorithm is largely model-free, and identifies statistically significant pulses in relation to dose-dependent measurement error in the hormone time series. For the present analysis a 2x1 test cluster configuration was used, two data points for the test nadir and one for the test peak, and a t-statistic of 2.0 for the up- and down-strokes, which minimizes both false positive and false negative peaks. The locations and widths of all significant concentration peaks were identified, the total number of peaks was counted, and the mean peak interval was calculated in minutes. In addition, the following pulse parameters were determined: peak height (highest value attained within the peak), incremental peak amplitude (the difference between peak height and prepeak nadir), and area under the peak. Interpulse valleys were identified as regions embracing nadirs with no intervening
up-strokes. The total area under the curve was also calculated, as well as the summed pulse areas (59).

**Deconvolution analysis**

The 24-h TSH secretion rate was calculated with Pulse. This deconvolution method is waveform independent and can be used for the estimating the hormone secretion rate at each time point as well as the total 24-h rate (60). The method requires a priori specification of the plasma half-life. For the present analysis we used a two-compartment model with a fast component of $18 \pm 3$ min and a slow component of $90 \pm 5$ min, with a fractional contribution of 0.32 of the slow component to the overall decay (data kindly provided by Dr J.D. Veldhuis, Mayo Clinic, Rochester, MN). The mass of TSH released after TRH injection was also calculated by deconvolution. On a separate occasion, patients received 200 μg TRH iv. in the morning, and blood samples were drawn just before the injection and at 10 min intervals for 180 min.

**Approximate Entropy**

The univariate approximate entropy (ApEn) statistic was developed to quantify the degree of irregularity, or disorderliness, of a time series (44,62). Technically, ApEn quantifies the summed logarithmic likelihood that templates (of length $m$) of patterns in the data that are similar (within $r$) remain similar (within the same tolerance $r$) on next incremental comparison and has been formally defined elsewhere (45). The ApEn calculation provides a single non-negative number, which is an ensemble estimate of relative process randomness, wherein larger ApEn values denote greater irregularity. Cross-ApEn (X-ApEn) quantifies joint pattern synchrony between two separate but parallel time series after standardization (z-score transformation) (47,51). In the present
analysis, we calculated cross-ApEn between leptin and TSH and between sleep and TSH, assuming $r = 20\%$ of the SD of the individual time-series and $m = 1$, and hence use the designation X-ApEn $(1, 20\%)$. This parameter set affords sensitive, valid and statistically well replicated ApEn and cross-ApEn metrics for assessing hormone time series of this length (46). Both ApEn and cross-ApEn results are reported as absolute value and the ratio of the absolute value to that of the mean of 1000 randomly shuffled data series, values approaching 1.0 denoting complete randomness.

Nyctohemeral (24-h) rhythmicity

Diurnal variations in plasma TSH concentrations were calculated after fitting the data with a robust curve fitting algorithm (3,5). The acrophase was the maximal value of the fitted curve. The amplitude of the rhythm was defined as half the difference of the nocturnal zenith and the day-time nadir. The relative amplitude was the maximal percentage increase of the nadir value.

Sleep and TSH concentrations

The relation between sleep onset and decrease in TSH concentration was quantified by the hypergeometric (joint binomial) distribution (61). This program calculates the probability that events (pulses) in time series (two or more) occur randomly.

Statistical analysis

Results are presented as the mean ± SEM unless stated otherwise. Comparisons were made with the two-tailed Student’s t-test for unpaired data, if necessary after logarithmic transformation to limit the variability. In addition, we used the Kolmogorov-
Smirnov test. Relations between variables were analyzed by regression techniques. Calculations were performed with Systat version 11 (Systat Inc, Richmond, CA). P < 0.05 was considered significant.

**Results**

*Plasma hormone concentrations*

Figure 1 shows the average 24-h serum TSH concentration vs. time of the narcoleptic subjects and their matched controls. The mean 24-h plasma TSH concentration was significantly lower in narcoleptic patients (1.58 ± 0.27 (N) vs. 2.88 ± 0.36 (C) mU/L, P = 0.014). In contrast, mean (hourly) plasma total T₄ and T₃ were not different in patients and controls (Table 1). Also, basal free T₄ measurements were similar in both groups (14.4 ± 0.40 (N) vs. 15.0 ± 0.93 (C) pmol/L, P = 0.55). The circadian plasma T₄ and T₃ levels did not display a significant diurnal rhythm.

*Cluster analysis*

The results of the Cluster analysis are summarized in Table 2. TSH pulse frequency and mean pulse interval did not differ between patients and controls. Mean pulse area was smaller in patients than in controls, which was primarily caused by diminution of pulse height and pulse amplitude. The integrated total area under the TSH curve (AUC) was also lower in patients than in controls: 2260 ± 390 mU/L vs. 4140 ± 520 mU/L (P = 0.013). The mean nadir concentration was about 50% lower in patients than in controls.

*Diurnal variation of TSH*
The acrophase (time of maximal TSH) occurred at 24.39 ± 0.78 h in patients and at 24.28 ± 0.48 h in controls (P = 0.91). Nadir concentrations were reached at 15.27 ± 1.14 h in patients and at 15.18 ± 0.61 h in controls (P = 0.94). The amplitude of the rhythm was smaller in patients than in controls: 0.48 ± 0.13 mU/L vs. 1.00 ± 0.26 mU/L (P=0.009), but the percentage increase in TSH concentration was similar in the two groups (93 ± 16% vs. 115 ± 21 %, P = 0.43). Examples of representative TSH profiles and their fitted curves are shown in Figure 2.

**TRH test**

On a different occasion, the response of TSH to TRH was investigated. The total mass of TSH released, calculated with deconvolution analysis, was 27.1 ± 5.0 mU/L in patients and 24.4 ± 4.9 mU/L in controls (P = 0.39, Figure 3). Basal levels were 2.0 ± 0.17 mU/L in patients and 3.25 ± 0.38 mU/L in controls (P = 0.02). Maximal TSH values, which were reached 20-30 min after TRH injection, were not different between patients and controls (20 min P = 0.24, 30 min P = 0.30).

**TSH secretion and leptin**

Figure 4 shows the correlation between leptin and TSH concentrations. The synchronicity between leptin and TSH series was similar in patients and controls (Cross-ApEn score between leptin (leading) and TSH: 1.750 ± 0.024 in patients and 1.650 ± 0.067 in controls (P = 0.23)). Expressed as the ratio to a random series, the X-ApEn ratio in patients was 0.902 ± 0.024 and in controls 0.870 ± 0.016 (P = 0.31). The ratios differed significantly from random series by 4 SD’s. The linear relation between mean 24-h leptin concentration and the 24-h TSH secretion rate (as determined by deconvolution analysis) is depicted in Fig 5.
Sleep and TSH secretion

Narcoleptic patients slept longer during the sampling protocol. The median duration was 7.61 h, range 4.1-20.7 h and in controls 3.6 h, range 3.0-6.6 h (P = 0.047). In Figure 2 the sleep patterns, expressed as percentage awake, with the TSH concentration profiles are displayed for 2 patients and controls. In all patients and all controls, except one, a highly significant concordance of sleep onset and a decrease of TSH concentrations was present (P values ranging from 0.002 to < 0.000001). Total sleep duration correlated negatively with the 24 h TSH secretion (R^2 = 0.287, P = 0.048). The relation of sleep to TSH concentrations was also explored with approximate entropy analyses. Sleep ApEn (1, 20%) and ApEn (1, 20%) ratio were significantly increased compared with controls, denoting increased sleep disorderliness in narcoleptic patients (Figure 6). Cross-ApEn analysis demonstrated a coupling of sleep and TSH in controls and patients, with no mutual differences.

Discussion

The present paper presents the first detailed description of plasma TSH concentration profiles in hypocretin deficient narcoleptic humans. The average TSH concentration was significantly lower in narcoleptic men, which was specifically attributable to a reduction of TSH pulse amplitude, while the number of TSH pulses was similar in patients and controls. The circadian fluctuation of plasma TSH concentrations was comparable in narcoleptics and controls. Circulating leptin and TSH secretion appeared to correlate positively, whereas sleep was inversely associated with TSH release. Despite the significantly lower plasma TSH concentration in narcoleptics, thyroxine and triiodothyronine concentrations were similar in patients and controls.
TSH synthesis and secretion are primarily regulated by TRH feedforward input and feedback inhibition by thyroid hormones. A host of other hormones and neurotransmitters, including somatostatin, catecholamines, various cytokines and leptin modulate this neuroendocrine ensemble (13,58). In addition, sleep has an inhibitory effect on TSH secretion (1,42). TRH is synthesized in the thyrotrophic area of the PVN and in the anterior pituitary, where it acts to promote TSH secretion through endocrine and paracrine mechanisms respectively (58). Although hypocretin neuronal cell bodies heavily project to the PVN (43), it is currently unknown if hypocretin axons actually synapse with TRH neurons. It is therefore uncertain whether hypocretins can directly modulate TRH synthesis and release and thus affect TSH secretion. Also, experiments investigating the impact of (exogenous) hypocretin peptides on TSH release in rodents have yielded inconclusive results. Icv or iv injection of hypocretin-1 either reduced plasma TSH levels or showed no effect on TSH and/or TRH release (16,34,53,54). Thus, it seems unlikely that hypocretin deficiency per se inhibits TRH secretion to reduce TSH levels in narcoleptic humans.

We previously showed that circulating leptin levels are approximately 50% lower in narcoleptic patients than in controls matched for age and body fat mass (22). Here we show that circulating leptin levels and TSH secretion rates are correlated positively in narcoleptic men. Other studies have revealed that leptin is intimately involved in the regulation of the HPT-axis. Specifically, a reduction of circulating leptin mediates the inhibition of HPT-axis activity during starvation (13). Leptin primarily acts to integrate the activities of neuronal circuits that orchestrate energy balance (40). In this context, leptin activates α-melanocyte stimulating hormone (α-MSH) and cocaine- and amphetamine- regulated transcript (CART), while it inhibits neuropeptide Y (NPY) and agouti related peptide (AgRP). TRH neurons receive functional inputs from all of these
neurotransmitters (10-12,25,26,57): NPY/AgRP inhibit TRH release (21), whereas α-MSH/CART promote TRH secretion (11,21). In addition, leptin can directly stimulate pre-pro-TRH mRNA synthesis and processing and promote TRH release via activation of leptin receptors on TRH neurons (37). Moreover, the fact that leptin and leptin receptors are expressed in rodent anterior pituitary cells suggests that leptin may also impact TSH secretion directly (19). Diurnal pulsatile leptin and TSH levels exhibit strong pattern synchrony in healthy humans, whereas TSH release gets disorganized and its circadian rhythm phase-shifted in leptin deficient human subjects (31). In the present study we also demonstrate strong pattern synchrony between leptin and TSH both in patients and in controls. These data clearly suggest that leptin is involved in the regulation of TSH release in rodents and humans. Thus, the fact that TSH release rates were positively correlated with circulating leptin levels in the present study supports, but certainly does not prove, the contention that hypoleptinemia blunts TSH secretion in narcoleptic patients.

Diminished dopaminergic tone may also blunt TSH secretion in narcoleptic subjects through diminution of TRH release. Studies using the canine model of narcolepsy suggest that hypoactivity of dopaminergic and noradrenergic circuits are among the major pathophysiological mechanisms in narcolepsy (38). Recent in vivo brain imaging studies in humans support this notion (9) and the disease is currently treated symptomatically with drugs that enhance monoaminergic (i.e. dopamine, noradrenaline) neurotransmissions (38). In vitro and in vivo studies clearly show that hypocretin peptides stimulate dopaminergic neurons in various brain sites (16,36), which also suggests that hypocretin deficiency may silence dopaminergic circuits. Dopamine promotes TRH release via hypothalamic dopamine D2 receptors (27). Therefore, hypocretin deficiency may blunt TRH (and thereby TSH) release through diminution of dopaminergic
neurotransmission in the hypothalamus. However, the role of dopamine in the regulation of TSH secretion is complex, in view of the fact that this monoamine inhibits TSH release at the level of the pituitary (6,14). This observation implies that reduced dopaminergic tone would lead to an increase of circulating TSH in narcoleptic subjects. In this context, it seems important to mention that prolactin secretion, which is also controlled by dopamine at the pituitary level, is not altered in narcoleptic patients (SW Kok, unpublished observation). Thus, the role of dopamine in the pathophysiology of low plasma TSH levels in narcoleptic humans remains uncertain.

Other clinical features of narcolepsy also suggest that TRH deficiency (through hypoleptinemia or other (unknown) causes) may be involved in the pathophysiology of the disease. Excessive daytime sleepiness (EDS) and cataplexy are reflections of depressed activity of the central nervous system (CNS). Accordingly, the current options for treatment of narcolepsy include CNS stimulants for EDS and antidepressants for cataplexy (41). TRH receptors are distributed widely in the central nervous system (CNS)(15) and TRH has diverse neuromodulating actions (15,48). Among many other features, it has CNS stimulant and antidepressant effects (18,29). Interestingly, chronic administration of a TRH analog increases wakefulness and ameliorates cataplexy in narcoleptic dogs (50), which may imply that diminished TRH tone is involved in the pathogenesis of these narcoleptic symptoms. However, the fact that circulating prolactin levels are normal in narcoleptic humans (Kok et al, unpublished) argues against (but does not exclude) a primary TRH deficiency in these patients, as TRH is involved in the regulation of prolactin release.

Sleep has an inhibitory effect on TSH release, as demonstrated in the human under a normal light-dark cycle and during sleep and light-dark cycle manipulations (1,17). The underlying neuroendocrine mechanisms are not known. In the present study, sleep and
plasma TSH levels were inversely associated and sleep periods were invariably followed by a reduction of TSH concentration in both patients and controls, suggesting that hypocretins are not involved in the coupling of sleep and TSH release. The present data also suggest that the fact that patients slept longer compared with controls may have contributed to the reduction of their TSH levels.

Hypersensitivity of the thyroid gland to TSH stimulation may also contribute to the alterations of the pituitary thyroid ensemble described here. Despite the fact that TSH levels were reduced in narcoleptic subjects, plasma thyroxine and triiodothyronine concentrations were similar to those in healthy controls and completely within the normal range. This may imply that the thyroid is more sensitive to TSH stimulation in narcoleptics (unfortunately our study does not yield further data to support this notion). Thyroid hypersensitivity to TSH may be brought about by the reduction of sympathetic tone in these patients (55), as TSH-induced thyroxine release is inhibited by alpha-1 adrenergic receptor stimulation (30,35). This putative resetting of the pituitary-thyroid axis could fully explain our observations. Anyhow, the fact that thyroid hormone levels are within the normal range in narcoleptics indicates that these patients are euthyroid. However, it remains to be established if the ensemble acts appropriately under conditions of greater metabolic demands, such as growth, cold adaptation or pregnancy.

Our data clearly show that the approximate entropy statistic can appropriately detect disorderliness of sleep patterns in narcoleptic humans. Thus, it is conceivable that the application of ApEn analysis to sleep patterns and the effect of drug therapy in other sleep disorders and to physiological studies of sleep and related neuroendocrine phenomena might contribute to further understanding of the complicated networks involved.
In conclusion, the present paper shows that circulating TSH concentrations are reduced in the face of normal plasma thyroid hormone concentrations in hypocretin deficient narcoleptic humans. Hypoleptinemia and disorderly sleep episodes of longer duration may be involved in the pathophysiology of this neuroendocrine feature of narcolepsy.
Acknowledgements

The authors gratefully acknowledge all the work performed by the following collaborators: E.J.M. Ladan-Eygenraam and E.C. Sierat-van der Steen for the technical support. M. van Dijk-Besling and H.G. Haasnoot-van der Bent for performing the TSH, T4 and T3 assays.
Legends to figures

Figure 1. Twenty-four-hour TSH concentrations in 7 narcoleptic patients (triangles) and 7 controls (circles). Data are shown as mean with SE bars.

Figure 2. Plasma TSH concentration series in two patients (upper panels) and two controls (lower panels). The locally weighted robust regression line of the data points is also shown. The percentage wakefulness calculated for 10-min bins is displayed in the top of the panels.

Figure 3. TSH increase after injection with 100 μg TRH in 5 narcoleptic patients (triangles) and in 5 controls (circles). Basal TSH concentrations differed significantly (P=0.02). Maximal values at 20 and 30 min were not different, and the total TSH mass secreted was similar in patients and controls. Data are shown as mean ± SEM.

Figure 4. Regression between TSH and leptin concentrations in 7 narcoleptic patients (triangles) and 7 controls (circles). Data represent the samples obtained at 20 min intervals during the 24-h study.

Figure 5. Linear regression between the mean 24-h leptin concentration and TSH secretion estimated with deconvolution analysis. Narcoleptic patients are shown as circles, and controls as triangles.
Figure 6. Approximate entropy and cross-approximate entropy for sleep and the joint synchrony of sleep and TSH in 7 narcoleptic patients and 7 age- and body fat mass-matched controls. Statistical comparisons were made with the two-tailed Student’s t-test for unpaired data.
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Table 1. Mean twenty-four-hour serum TSH and thyroid hormone concentrations in narcoleptic patients and healthy control.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (mU/L)</td>
<td>1.58 ± 0.27</td>
<td>2.88 ± 0.36</td>
<td>0.014</td>
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<tr>
<td>T4 (nmol/L)</td>
<td>85.9 ± 3.5</td>
<td>90.6 ± 4.7</td>
<td>0.44</td>
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<tr>
<td>T3 (nmol/L)</td>
<td>1.58 ± 0.04</td>
<td>1.62 ± 0.05</td>
<td>0.58</td>
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</tbody>
</table>

Data are mean ± SEM. Statistical comparisons between patients and controls were carried out with the two-tailed Student’s t-test.
Table 2. Cluster analysis of the twenty-four-hour serum TSH concentration series in narcolepsy and controls

<table>
<thead>
<tr>
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<th>Narcolepsy</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulses/24 h</td>
<td>17 ± 1</td>
<td>14 ± 1</td>
<td>0.10</td>
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<tr>
<td>Mean pulse interval (min)</td>
<td>83 ± 6</td>
<td>94 ± 9</td>
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<td>Mean pulse width (min)</td>
<td>56 ± 5</td>
<td>69 ± 8</td>
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<tr>
<td>Mean pulse height (mU/L)</td>
<td>1.81 ± 0.30</td>
<td>3.29 ± 0.44</td>
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<tr>
<td>Mean pulse amplitude (mU/L)</td>
<td>0.41± 0.08</td>
<td>0.69 ± 0.12</td>
<td>0.049</td>
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<tr>
<td>Mean pulse area (mU/L/min)</td>
<td>21.0 ± 6.4</td>
<td>46.0 ± 8.5</td>
<td>0.03</td>
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<tr>
<td>Summed pulse areas (mU/L/min)</td>
<td>337 ± 85</td>
<td>602 ± 95</td>
<td>0.03</td>
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<tr>
<td>Mean nadir (mU/L)</td>
<td>1.34 ± 0.23</td>
<td>2.49 ± 0.32</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Statistical comparisons between patients and controls were carried out with the two-tailed Student’s t-test.
Figure 1

- controls
- narcolepsy
Figure 2
Figure 3

![Graph showing TSH levels over time.](image)
Figure 4

R^2 = 0.23, P < 0.0001
Figure 5

Leptin (µg/l)

TSH secretion (mU/l/24h)

R² = 0.46, P = 0.02
Figure 6

Sleep

Sleep-TSH

P=0.006

P=0.01

P=0.36

P=0.39

ApEn (1,20%) ApEn ratio X-ApEn (1,20%) X-ApEn ratio

N C C N C N C