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ORIGINAL ARTICLE

The hypothalamic-pituitary-thyroid axis and melatonin in humans: Possible interactions in the control of body temperature

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Abstract

OBJECTIVE: Melatonin plays a role in the regulation of biological rhythms, body temperature presents circadian variations with lower levels during nighttime, when melatonin levels are very high, and thyroid hormones influence shiver independent thermogenesis. We have investigated on possible interactions between the hypothalamic-pituitary-thyroid axis and melatonin in the control of body temperature in humans. **METHODS:** Peripheral blood samples for thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), free-thyroxine (FT_4), melatonin levels determination and body temperature measurements were obtained every four hours for 24-hours starting at 0600h in a controlled temperature and light-dark environment from ten healthy males, aged 38–65 (mean age ±s.e. 57.4±3.03, mean body mass index ±s.e. 25.5±0.75). We calculated fractional variation and correlation on single time point hormone serum levels and tested whether the time-qualified data series showed consistent pattern of circadian variation. **RESULTS:** A statistically significant difference was evidenced for the fractional variation of daytime TSH serum levels (0600h–1000h vs. 1000h–1400h, *p=0.01*, 1000h–1400h vs. 1400h–1800h, *p=0.0001*, 1400h–1800h vs. 1800h–2200h, *p=0.001*) and for the fractional variation of FT4 serum levels at 1800h–2200h vs. 2200h–0200h *(p=0.02)*. FT_4 serum levels correlated positively with TRH serum levels at 1000h (*r=0.67, P=0.03*) and at 1400h *(r=0.63, p=0.04)*, negatively with TSH serum levels at 2200h *(r=–0.67, p=0.03)*, negatively with melatonin serum levels at 2200h *(r=–0.64, p=0.04)* and at 0200h *(r=–0.73, p=0.01)*. TRH serum levels correlated positively with TSH serum levels at 0200h *(r=0.65, p=0.04)* and at 0600h *(r=0.64, p=0.04)*. Body temperature correlated positively with FT4 serum levels at 1000h *(r=0.63, p=0.04)* and negatively with melatonin serum levels at 0200h *(r=–0.64, p=0.04)*. A clear circadian rhythm was validated for body temperature (with acrophase in the morning) and melatonin, TRH and TSH secretion (with acrophase at night), while FT_4 serum level changes presented ultradian periodicity (with acrophase in the morning). **CONCLUSION:** Changes of TSH serum levels are smaller and those of FT4 are greater at night,

when melatonin levels are higher, so that the response of anterior pituitary to hypothalamic TRH and of thyroid to hypophyseal TSH may be influenced by the pineal hormone that may modulate the hypothalamic-pituitary-thyroid axis function and influence the circadian rhythm of body temperature

Introduction

Melatonin, hormone secreted by the pineal gland, has been demonstrated to play an important role in the regulation of biological rhythms [1]. The secretion of melatonin by the pineal gland is regulated by a neural pathway including the retina, a specific retino-hypothalamic tract leading to the suprachiasmatic nucleus, which projects fibers to the superior cervical ganglion, that innervates the pineal with sympathetic fibers [2]. Activity of the retino-hypothalamic-pineal system is influenced by environmental lighting conditions, with an inhibitory effect of light on melatonin secretion, whose variations are able to convey within the organism information about photoperiodic changes [3]. The melatonin mediated photoperiodic message is a fundamental cue for the circadian and seasonal coordination of biological functions [4]. The body temperature in humans presents a circadian rhythm, influenced but dissociable from the sleep/wake cycle, with a minimum during nighttime, when melatonin levels are at their maximum [5,6]. Thyroid hormones increase thermogenesis by enhancement of mitochondrial oxidative metabolism [7]. In the pineal gland a type II thyroxine 5'-deiodinating activity (5'–D II) with nighttime increase has been found, melatonin stimulates this 5'–D II activity in the brown adipose tissue (BAT) and a complex interaction among light, pineal, fat body mass and serum thyroxine has been demonstrated in experimental animals [8,9]. We investigated on possible interactions between the pineal gland and the hypothalamic-pituitary-thyroid axis in the control of body temperature in human subjects.

Materials and methods

Subjects, study protocol and hormone measurements

Ten healthy males, aged 38–65 years (mean age ±s.e. 57.4±3.03) gave their informed consent and took part in this study, approved by the local Scientific and Ethical Committee. The mean body mass index (±s.e.) was 25.5±0.75 and the subjects were studied in hospital between October and November, submitted to the same social routine, with identical mealtimes and sleep/wake cycle in a controlled temperature and light-dark environment (ambient temperature 20°C, lights on at 0630h and lights off at 2130h, 15:9 L:D). An indwelling catheter, kept patent with a slow infusion of 0.9% NaCl, was inserted in an antecubital vein and blood samples were drawn at 4-hour intervals for 24 hours starting at 0600h, together with oral body temperature measurement; at night samples were collected with a dim red light source. Blood was centrifuged, separated and stored at –20°C, until assayed for serum melatonin, TRH, TSH and FT_4. All samples were analyzed in duplicate in a single assay; the intrassay and interassay coefficients of variation were below 13% and 16% respectively for melatonin, 5% and 6% for TRH, 8% and 7% for TSH, 4% and 6% for FT4. Standard curves were run with every assay and the experimental values were derived from the curves. We measured melatonin by radioimmunoassay (Melatonin Radioimmunoassay Kit, Nichols Institute Diagnostics), TRH by radioimmunoassay ("Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary), TSH by immunoenzymatic assay (Enzymun-Test TSH, Boehringer Mannheim Immunodiagnostics), FT_4 by immunoenzymatic assay (Enzymun-Test FT_4, Boehringer Mannheim Immunodiagnostics).

Statistical analysis

We calculated the fractional variation (*FV*) on single time point TRH, TSH and FT4 serum levels according to the formula

$$FV = \frac{y_{(t)} - y_{(t+n)}}{y_{(t)}} \%$$

where y is the hormone serum level and t is the sampling time. The data were statistically evaluated by not inferential descriptive biometric analysis (Pearson's product moment correlation coefficients calculated for hormone serum levels at each sampling time and one way analysis of variance and Kruskal-Wallis one way analysis of variance on ranks, as indicated, followed by Student-Newman-Keuls method for pairwise multiple comparison procedure on fractional variations) and by inferential temporal descriptive biometric analysis, using the methods named Single Cosinor and Population Mean Cosinor, which entail fitting sine curves to the data from individual subjects and from groups respectively, testing whether the time-qualified data series showed consistent pattern of circadian variation and quantifing the parameters (MESOR, amplitude and acrophase) of the circadian rhythm [10]. A p value less than *0.05* was regarded as significant.

MESOR, acronym for Midline Estimating Statistic of Rhythm, defines the rhythm-determined average. Amplitude is the measure of one half the extent of rhythmic change in a cycle estimated by the function used to approximate the rhythm. Acrophase, measure of timing, is the phase angle of the crest time in the function appropriately approximating a rhythm, in relation to the specified reference timepoint. Rhythms with a frequency of 1 cycle per 20–28 h are designated circadian and frequencies higher than 1 cycle per 20 h are designated as ultradian.

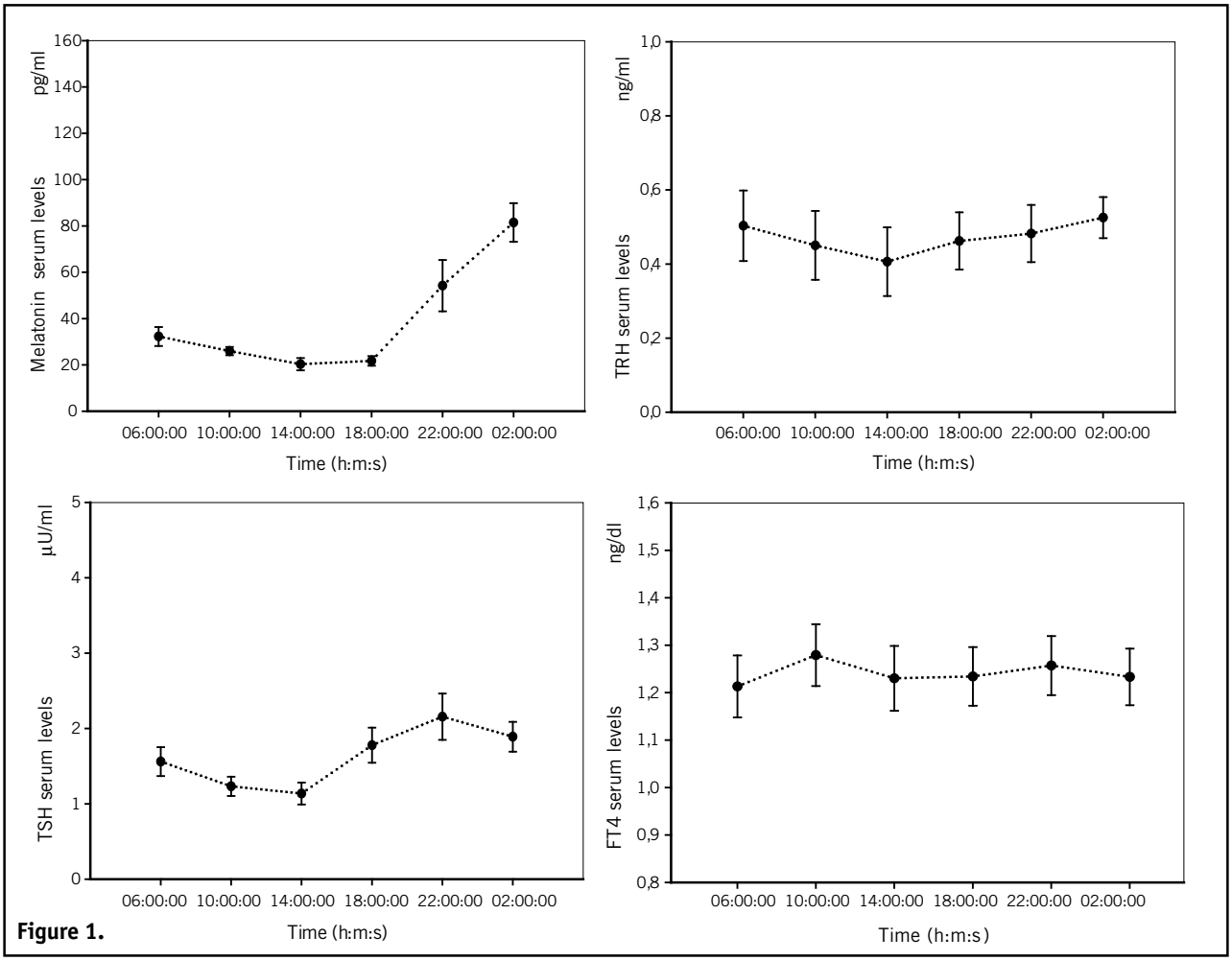

Figure 1.

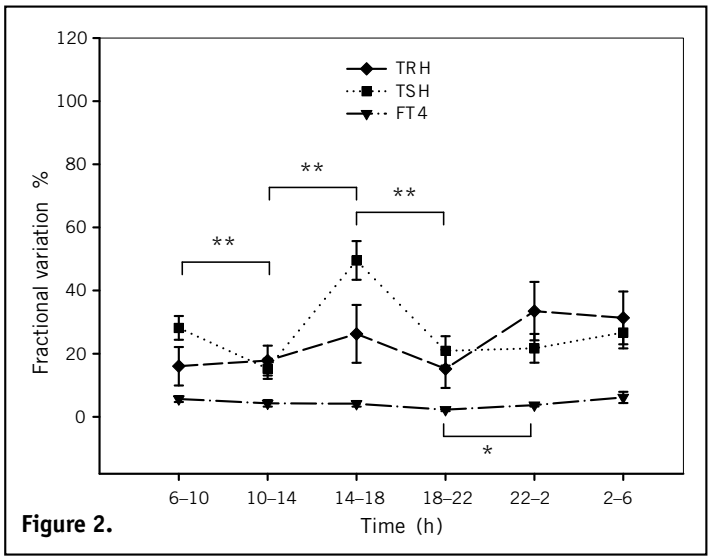

Figure 2.

Figure 1. Twenty-four hour profiles of serum melatonin, TRH, TSH and FT4 levels in ten healthy subjects (mean±s.e.)

Figure 2. Time related fractional variation of TRH, TSH and FT4 serum levels in 24-hour profiles (** p<0.05, ** p≤0.01)*

Results

Figure 1 shows twenty-four hour profiles of serum melatonin, TRH, TSH and FT4 levels in the studied subjects (mean±s.e.). Figure 2 shows time related fractional variation of TRH, TSH and FT4 serum levels. Table 1 shows chronobiological data derived from best fitting sine curves (fitted period: 24 hours).

A statistically significant difference was evidenced for the fractional variations of daytime TSH serum levels (0600h–1000h vs. 1000h–1400h, $p=0.01$, 1000h–1400h vs. 1400h–1800h, $p=0.0001$, 1400h–1800h vs. 1800h–2200h, $p=0.001$) and for the fractional variation of FT4 serum levels at 1800h–2200h vs. 2200h–0200h ($p=0.02$).

Pearson Product Moment Correlations showed that FT_4 serum levels correlated positively with body temperature at 1000h ($r=0.63$, $p=0.04$) and with TRH serum levels at 1000h ($r=0.67$, $p=0.03$) and at 1400h ($r=0.63$, $p=0.04$), negatively with TSH serum levels

Table 1: Chronobiological data derived from best fitting sine curves (fitted period: 24 hours = 360°)

Factor	*p*	MESOR±s.e.	Amplitude±s.e.	Acrophase±s.e. (°)	Peak time±s.e.(h.m)
Body temperature	0.009	36.60±0.40	0.32±0.05	–188.0±25.3	12.32±1.40
Melatonin	0.010	41.89±4.97	32.36±7.18	–20.9±12.2	01.21±0.48
TRH	0.011	0.46±0.01	0.04±0.01	–41.6±11.7	02.44±0.44
TSH	0.002	1.37±0.04	0.43±0.05	–343.8±8.6	22.53±0.36
FT_4	0.752	1.24±0.01	0.01±0.01	–236.5±71.3	15.44±4.44

Units: °C for body temperature, pg/ml for melatonin, ng/ml for TRH, μU/ml for TSH, ng/dl for FT_4

at 2200h *(r=–0.67, p=0.03)* and with melatonin serum levels at 2200h *(r=–0.64, p=0.04)* and at 0200h *(r=–0.73, p=0.01)*. TRH serum levels correlated positively with TSH serum levels at 0600h *(r=0.64, p=0.04)* and at 0200h *(r=0.65, p=0.04)*. Melatonin correlated negatively with body temperature at 0200h *(r=–0.64, p=0.04*).

A clear circadian rhythm was validated for the time-qualified changes of body temperature (with acrophase in the morning) and melatonin, TRH and TSH secretion (with acrophase at night). FT_4 serum level changes showed rhythmicity with a 12 hour period with acrophase in the morning (P=0.02, MESOR±s.e. 1.24±0.001, Amplitude±s.e. 0.02±0.001, Acrophase±s.e. –307.3±14.2, Peak time±s.e. 10.14±0.28).

Discussion

Core body temperature and melatonin secretion show circadian periodicity with opposite phase and melatonin has sleep inducing and hypothermic effects: its nocturnal secretion generates about 40% of the amplitude of body temperature circadian rhythm [11–13]. Thyroid hormones play an important role in thermoregulation, enhancing cellular respiration and thermogenesis, after intracellular metabolic transformation by type I 5'-deiodinase (found in thyroid, kidney, liver, white adipose tissue and peripheral mononuclear blood cells) [14] and type II 5'-deiodinase (restricted to central nervous system, pineal gland, anterior pituitary, BAT and placenta) [15,16]. BAT plays a major role in nonshivering thermogenesis [17] and two pathways for the regulation of thyroid hormone-dependent thermogenesis in BAT have emerged: thyroid hormones increase the sensitivity of BAT to β-adrenergic stimulation, enhancing catecholamine-induced respiration, and α-adrenergic stimulation of 5'-D II results in increased production of triiodothyronine (T_3) by BAT [18,19]. Melatonin exerts a stimulating effect on 5'-D II activity in BAT especially at night and this may contribute to its chronomodulatory role in thermoregulation. A strong correlation exists between the serum concentrations of TSH and thyroid hormones, such that the amounts of thyroid hormone available to peripheral tissue are maintained within narrow limits; the sensitivity of the thyrotrophs is such that reductions in serum T_3 and T_4 concentrations of as little as 10% to 15% result in 50% to 100% increases in serum TSH concentrations and the feed-back mechanisms that regulate TRH, TSH,T3 and T4 concentrations act very rapidly (in a time varing from half an hour to few hours) [20,21].

The data obtained in the current study agree with those evidenced by previous studies [22–27] and show that FT_4 serum level changes do not present circadian periodicity ; this observation must be correlated with TRH, TSH and melatonin circadian rhythms. Free thyroxine levels are high in the morning, when we find low levels of TRH and TSH and low levels of melatonin, and rather lower at night, when we find high levels of TRH and TSH and very high levels of melatonin. This phenomenon and the observation that TSH serum level changes are more evident during daily hours, while those of FT4 serum levels are more evident during nightly secretion, are not fully explained by feed-back mechanisms and may be related to a modulatory action of melatonin on hypothalamic-pituitary-thyroid axis function. In our study thyroid secretion is positively correlated with TRH serum levels during the day and negatively correlated with TSH and melatonin serum levels at night, while TRH and TSH serum levels are positively correlated late at night and in the morning. Body temperature is positively correlated with FT4 serum levels in the morning and negatively correlated with melatonin serum levels at night. Melatonin may infuence thermoregulation increasing vasodilation and peripheral heat loss and may also regulate thermogenesis [28–30] Thyroid hormones are photoperiod-dependent in animals [31] and an inhibitory action of the pineal gland on hypothalamic-pituitary-thyroid axis (with reduced thyroid response to TSH in the presence of elevated melatonin levels) has been evidenced in hamsters and rats and in vitro [32].

The pineal gland may act by a "feed-sideward" effect similar to that described for the interactions between the pineal gland and the hypothalamic-pituitary-adrenal axis [33,34], functioning as a servomechanism that diminishes or increases responses when stimuli are respectively too high or too low.

In conclusion, the response of anterior pituitary to hypothalamic TRH and of thyroid to hypophyseal TSH may be influenced by melatonin levels, so that melatonin may modulate the function of the hypothalamic-pituitary-thyroid axis and influence the circadian variability of body temperature.

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