Effects of Leuprolide-Induced Hypogonadism and Testosterone Replacement on Sleep, Melatonin, and Prolactin Secretion in Men

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ABSTRACT

The possible role of gonadal steroids in regulating sleep and circadian rhythms in humans has received relatively little attention despite the importance of the topic to several clinical syndromes. Pharmacologically induced hypogonadism, with and without gonadal steroid replacement, provides an opportunity to examine these questions within a controlled experimental design. We used leuprolide acetate, with and without testosterone replacement, to study the role of testosterone in the regulation of sleep and of melatonin, PRL, and TSH secretion in men. Results from 10 men revealed significant decreases in 24-h PRL levels and in the percentage and time of stage 4 sleep in the hypogonadal state compared with testosterone replacement. There were no differences in melatonin or TSH secretion or in the timing or duration of sleep between the two hormonal conditions. These results indicate that testosterone has relatively specific and discrete effects on sleep and hormonal rhythms in men. (J Clin Endocrinol Metab 82: 3203–3207, 1997)

MANY STUDIES have examined the role of the hypothalamic-pituitary-gonadal (HPG) axis in the regulation of sleep, activity, and circadian rhythms in animals (1–3). In humans, it is unclear whether regulatory links exist between the HPG axis and the retino-hypothalamic-pineal system controlling melatonin secretion; our first goal in this study was to explore this question. Investigators working with several clinical syndromes [hypothalamic amenorrhea (4–6), idiopathic hypogonadotropic hypogonadism (7–9), and delayed puberty (8)] have noted an association between hypogonadism and elevated melatonin levels. Conversely, investigators have reported an association between central precocious puberty (CPP) and low melatonin levels (10–13). However, the consistency of such findings remains unclear; for example, studies of melatonin levels in male primary hypogonadism have yielded mixed results (9, 14, 15).

Our second question was whether gonadal steroids modulate the sleep-activity cycle in men. Two studies indicate that estrogen replacement in postmenopausal women may increase rapid eye movement (REM) sleep (16, 17). The effects of gonadal steroids on the sleep-wake cycle in men have not been studied, although there is evidence that the androgen dehydroepiandrosterone may increase both REM sleep and sleep spindles (18). In addition to considering these direct effects of gonadal steroids on sleep, our third question was whether PRL and TSH could serve as links between the HPG axis and the sleep-activity cycle. PRL secretion is modified by gonadal steroid levels (19, 20) and may alter the microarchitecture of sleep (21–23). Similarly, the hypothalamic-pituitary-thyroid and HPG axes interact at multiple levels (24, 25), and variations in TSH secretion are associated with fluctuations in the sleep-wake cycle (26).

Although questions concerning the effects of gonadal steroids on circadian regulation or on the sleep-wake cycle can be addressed by studying patients with relevant clinical syndromes, the interpretation of such studies is limited by the inability to establish causal relationships, the variability in gonadal steroid levels, and the question of generalizability. A complementary research strategy involves the use of the GnRH agonist leuprolide acetate (Lupron), to reversibly induce hypogonadism in normal volunteers. When administered for more than 2–4 weeks, Lupron down-regulates GnRH receptors in the pituitary and inhibits gonadotropin secretion, thereby suppressing gonadal steroid secretion (27). As part of a larger study of the effects of gonadal steroids on brain physiology and behavior (Schmidt, P. J., unpublished data), we used Lupron administration and testosterone replacement to study the effects of hypogonadism and gonadal steroid replacement in men on the timing, duration, and microarchitecture of sleep and on the 24-h secretory profiles of melatonin, PRL, and TSH.

Subjects and Methods

Subjects

We recruited men between the ages of 18–45 yr with unremarkable physical and neurological examinations and normal values on screening laboratory tests (thyroid, liver, and hepatic functions; complete blood count; urinalysis; testosterone and gonadotropin levels; electrolytes; glucose; acid phosphatase; and prostate-specific antigen). The Structured Clinical Interview for DSM-III-R (28) was used to exclude subjects with a current or prior history of Axis I psychiatric disturbance. Individuals performing shift work or suffering from sleep disorders were...
also excluded. The study was approved by the investigational review board of the National Institute of Mental Health. Subjects gave informed consent for all procedures and were paid for their participation.

Hormonal treatments

Subjects were given Lupron (leuprolide acetate, TAPP Pharmaceuticals, Chicago, IL; 7.5 mg, im, monthly) for 3 months. After 1 month of Lupron alone, testosterone and placebo injections were administered for 1 month each in a double-blind, randomized order, cross-over design. Subjects received two injections, 2 weeks apart, of either testosterone enanthate (Covyn Pharmaceuticals, Vernon Hills, IL; 200 mg, im) or sesame oil. Blood samples for gonadal steroid levels were drawn every 2 weeks.

Data collection

Early in the study, all subjects were admitted to an in-patient research unit for an adaptation night, during which they slept with sham electroencephalograph (EEG) leads and a rectal temperature monitor. Subjects were then readmitted for approximately 48 h during week 3 or 4 of each hormonal condition. On day 1 of each procedure, subjects entered the unit at approximately 1700 h and were allowed to choose their own bedtime and awakening time.

EEG recordings were obtained during sleep. Throughout the hospital stay, rectal temperature was measured by an indwelling probe (Yellow Springs Instrument Co., Yellow Springs, OH) connected to a portable electronic monitor that measured temperature every minute (Squirrel model 1206, Science Electronics, Dayton, OH; accuracy and resolution, 0.05°C, range, −50 to 150°C). On day 2, an iv line was inserted, and subjects were placed in a dim room (<10 lux) at 1100 h. Constant routine conditions (29, 30) were maintained to eliminate the masking effects of sleep, light, and meals on circadian rhythms. In these conditions, subjects remained awake, sitting in a reclining chair, and were fed bland, isocaloric meals every 2 h. Subjects completed the Stanford Sleepiness Scale hourly throughout the procedure (31). Beginning at 1300 h, blood samples to measure melatonin were drawn every 30 min, and blood samples to measure PRL and TSH were drawn every 60 min. Blood for free testosterone total testosterone total TSH was drawn at 1400 h. Samples were stored on ice and centrifuged within 3 h. The plasma was then extracted and frozen at −20°C. In addition to these in-patient procedures, subjects completed a sleep log with 15-min resolution for 2 weeks before the in-patient procedure.

Hormonal assays

Plasma melatonin was measured by RIA at Stockgrand Laboratories (Surrey, UK). The detection limit of the assay was 5 pg/mL. The interassay coefficient of variation (CV) was 9.3% at 11.0 pg/mL, 7.8% at 21.0 pg/mL, 5.3% at 59.9 pg/mL, and 3.1% at 252.0 pg/mL. The intraassay CV was 8.0% at 10.0 pg/mL, 5.5% at 22.0 pg/mL, 4.9% at 57.0 pg/mL, and 2.2% at 246.0 pg/mL. (n = 20 for all quality control values). Two subjects had unusually high daytime melatonin levels, and their samples were reassayed using an extraction method. Plasma PRL, TSH, T₃, T₄, free T₄, and free T₃ were measured at Covance Laboratories (Vienna, VA). Detection limits were 0.056 ng/mL, 0.08 µU/mL, 20 ng/dL, 1.0 µg/dL, 0.5 pg/mL, and 0.05 ng/dL, respectively. PRL, TSH, and free T₄ were measured by immunoenzymometric assay using reagents supplied by TOSOH Corp. (Tokyo, Japan). For PRL, interassay CVs were 4.6% at 9.2 ng/mL, 6.0% at 18.3 ng/mL, and 9.0% at 39.4 ng/mL. Intraassay CVs were 9.2% at 9.9 ng/mL, 4.8% at 18.7 ng/mL, and 4.2% at 39.6 ng/mL. For TSH, interassay CVs were 6.1% at 1.5 µU/mL, 2.2% at 8.7 µU/mL, and 5.2% at 29.8 µU/mL. Intraassay CVs were 8.4% at 1.5 µU/mL, 2.0% at 8.9 µU/mL, and 2.8% at 30.9 µU/mL. For free T₄, interassay CVs were 10.9% at 0.43 ng/dL, 7.7% at 1.62 ng/dL, and 3.9% at 6.15 ng/dL. Intraassay CVs were 10.1% at 0.436 ng/dL, 3.9% at 1.63 ng/dL, and 3.7% at 6.19 ng/dL. Total T₃ and total T₄ were measured by RIA using a commercial kit supplied by Coat-A-Count. For T₃, interassay CVs were 5% at 75.50 ng/dL, 2% at 148.73 ng/dL, and 4% at 321.42 ng/dL. Intraassay CVs were 8.9% at 56 ng/dL, 5.9% at 166 ng/dL, and 3.1% at 398 ng/dL. For T₄, interassay CVs were 9% at 2.64 µg/dL, 5% at 8.06 µg/dL, and 5% at 13.85 µg/dL. Intraassay CVs were 3.8% at 2.4 µg/dL, 2.7% at 7.4 µg/dL, and 2.8% at 13.8 µg/dL. For free T₄, interassay CVs were 12% at 2.24 pg/mL, 10% at 4.56 pg/mL, and 4% at 11.28 pg/mL.

Testosterone levels were measured by RIA at Mayo Laboratories (Rochester, NY). Interassay CVs were 16% at 33 ng/dL and 8% at 1003 ng/dL. Intraassay CVs were 10% at 80 ng/dL and 6% at 1257 ng/dL.

Data analysis

The times of melatonin onset and offset were defined as the times of appearance and disappearance (respectively) of sustained detectable melatonin levels, with the duration being the interval between them. The peak value of melatonin was used to determine amplitude (as all subjects had undetectable daytime melatonin levels). Paired t tests were used to compare these variables and to compare levels of free T₃, free T₄, total T₃, and total T₄ in the two hormonal conditions. PRL and TSH profiles were analyzed using repeated measures ANOVA with Greenhouse-Geisser corrections.

EEG records were scored for wakefulness and sleep stages in 30-s epochs using conventional criteria by technicians blind to hormonal condition. Sleep logs for the 2 weeks before each in-patient procedure were analyzed by calculating the time of wake onset (the time at the start of the first run of three consecutive 15-min awake intervals from 0300–1200 h), time of sleep onset (the time at the start of the first run of three consecutive 15-min sleep episodes after 1900 h), and sleep duration (number of hours between sleep onset and wake onset). Paired t tests were used to compare sleep data from the two hormonal conditions.

Temperature data were smoothed using a five-point moving average, and the minimum, maximum, and amplitude were determined. The duration of the nocturnal temperature trough was defined as the interval between the downward and upward midrange crossings of the smoothed curve. Data were analyzed using paired t tests.

All means are presented ±s, and the significance level was set at P < 0.05.

Results

Ten subjects with an average age of 29.3 ± 7.2 yr completed the study. Mean trough testosterone levels (drawn within 0–4 days of each study) were 42.6 ± 37.2 ng/dL in the Lupron plus placebo condition and 474.2 ± 416.9 ng/dL in the Lupron plus testosterone condition.

24-h hormonal profiles

There was no difference between hormonal conditions in the times of melatonin onset (2133 h ± 1.34 during Lupron plus placebo vs. 2154 h ± 1.17 during Lupron plus testosterone) or offset (0833 h ± 0.57 during Lupron plus placebo vs. 0845 h ± 1.30 during Lupron plus testosterone), or in melatonin amplitude (49.8 ± 20.8 pg/mL during Lupron plus placebo vs. 51.2 ± 19.5 pg/mL during Lupron plus testosterone; Fig. 1). For the PRL analysis, analyzable data were available from eight subjects (two subjects had large numbers of missing samples in one of the hormonal conditions). There was a significant main effect for condition, with the mean value being higher in the Lupron plus testosterone condition compared with Lupron plus placebo (mean, 13.4 ± 9.9 vs. 7.8 ± 5.6 ng/mL; F = 9.6; df = 7; P = 0.02; Fig. 2). Neither the main effect for time nor the interaction between time and condition was significant. Total T₃ and total T₄ were both significantly increased in the Lupron plus placebo condition compared with those in the Lupron plus testosterone condition (total T₃ 77.1 ± 12.1 vs. 63.0 ± 20.8 ng/dL (t = 2.36; df = 9; P = 0.04); total T₄ 6.5 ± 1.0 vs. 5.8 ± 1.0 mg/dL (t = 2.58; df = 9; P = 0.03)). However, there were no significant differences between the two conditions in levels of free T₃ or free T₄ or in TSH secretion.
Sleep

EEG data are shown in Table 1. Because a paper jam occurred during one recording, data are available from nine subjects. There was a significant increase in stage 4 sleep (in terms of both minutes and percentage) during the Lupron plus testosterone condition compared with that during the Lupron plus placebo condition, with a trend in the opposite direction for stage 3 sleep. There was also a trend for REM latency to lengthen during Lupron plus testosterone treatment compared with that during Lupron plus placebo. In the self-reported sleep log data, there was no difference between conditions in the time of sleep or wake onset or in sleep duration. There was also no difference between conditions in the ratings on the Stanford Sleepiness Scale obtained during the constant routines.

Temperature

Because of probe slippage in two subjects, temperature data are available from eight subjects. There was no difference between conditions in the minimum, maximum, amplitude, or time of the temperature fall. However, there was a trend for the time of the upward midrange crossing to be later in the Lupron plus placebo condition than in the Lupron plus testosterone condition (mean, 0940 h ± 2:25 vs. 0753 h

Fig. 1. Mean 24-h profiles of melatonin in 10 men treated with Lupron plus placebo and Lupron plus testosterone. Samples were obtained every 30 min in dim (<10 lux) light under constant routine conditions. There are no significant differences between hormonal conditions. See text for details.

Fig. 2. Mean 24-h profiles of PRL in eight men treated with Lupron plus placebo and Lupron plus testosterone. Samples were obtained hourly in dim (<10 lux) light under constant routine conditions. There is a significant (P = 0.02) main effect for hormonal condition. See text for details.
TABLE 1. Mean ± SD for polysomnographic variables in men receiving Lupron plus placebo and Lupron plus testosterone (n = 9) 

| Variables       | Lupron + placebo | Lupron + testosterone | t      | df | P     |
|-----------------|------------------|------------------------|--------|----|-------|
| Sleep continuity|                  |                        |        |    |       |
| Total            | 517.39 ± 49.73   | 540.06 ± 48.62         |        |    |       |
| Recording period (min) | 464.89 ± 46.43  | 474.89 ± 47.16         |        |    |       |
| Time awake (min) | 20.83 ± 18.78   | 25.18 ± 38.47          |        |    |       |
| Sleep            |                  |                        |        |    |       |
| Efficiency (%)   | 89.89 ± 3.73     | 88.36 ± 9.80           |        |    |       |
| Sleep latency (min) | 21.72 ± 13.18   | 18.89 ± 20.23          |        |    |       |
| Sleep architecture|                |                        |        |    |       |
| Stage            |                  |                        |        |    |       |
| 1, min (%)       | 16.11 ± 10.22    | 14.00 ± 7.42 (3.00 ± 1.73) |        |    |       |
| 2, min (%)       | 291.50 ± 28.29   | 306.44 ± 26.60 (64.78 ± 5.38) |        |    |       |
| 3, min (%)       | 35.11 ± 18.68    | 23.56 ± 13.72 (5.00 ± 3.28) |        |    |       |
| 4, min (%)       | 13.17 ± 19.10    | 25.27 ± 26.05 (5.22 ±5.36) |        |    |       |
| 3 + 4, min (%)   | 48.28 ± 27.65    | 48.72 ± 28.34 (10.22 ± 6.22) |        |    |       |
| REM, %           | 23.56 ± 3.28     | 22.44 ± 5.08           |        |    |       |
| REM measures     |                  |                        |        |    |       |
| Latency (min)    | 79.56 ± 26.64    | 109.28 ± 48.84         | 1.929  | 8  | 0.090 |
| Density          | 1.61 ± 0.35      | 2.00 ± 0.77            |        |    |       |
| Index            | 186.17 ± 52.84   | 219.67 ± 87.32         |        |    |       |
| Time (min)       | 111.17 ± 23.78   | 107.61 ± 33.28         |        |    |       |
| No. of REM periods | 4.22 ± 0.83      | 4.11 ± 1.17            |        |    |       |

Statistics are included where P < 0.10.

In this study, short term, pharmacologically induced hypogonadism in men caused significant decreases in the amount of stage 4 sleep and in PRL levels, compared with measures taken during testosterone replacement. In addition, in the Lupron plus placebo condition, there were trends toward increased stage 3 sleep, decreased REM latency, a delayed temperature rise, and an extended nocturnal temperature trough. There were no differences in melatonin or TSH secretion between the hypogonadal and the testosterone-replaced conditions.

In our study, PRL levels were lower during Lupron plus placebo treatment compared with those during Lupron plus testosterone at all times of the day. The effect of testosterone levels on PRL secretion has received little study, although androgens have been shown to induce PRL production by human endometrial cells in vitro (19). Estrogen appears to act at a number of physiological levels to facilitate PRL secretion (20), so a possible mechanism for our finding could involve the aromatization of testosterone to estradiol. The only study comparable to ours is that by Stoffel-Wagner et al. (32), in which PRL levels were measured in male control subjects before, during, and after treatment with a GnRH agonist. In that study, PRL levels were elevated during the posttreatment period at a time when testosterone levels were high normal. Thus, the data reported by both Stoffel-Wagner et al. (32) and our group are consistent with the interpretation that testosterone and/or its metabolites facilitate PRL secretion.

Increased PRL secretion in the Lupron plus testosterone condition may be physiologically linked with our second finding, a significant increase in the amount of stage 4 sleep in the testosterone-replaced condition. This finding, combined with the trend we observed toward a decrease in stage 3 sleep during Lupron plus testosterone, indicates that although the total amount of slow wave (i.e. stages 3 and 4) sleep is unchanged between the two hormonal conditions, Δ counts per min are probably higher in the testosterone-replaced state. A recent study using spectral analysis of EEG recordings found a positive correlation between PRL secretory rates (calculated by a deconvolution procedure) and relative power in the Δ band in men (23). Because the interval between PRL and Δ-wave pulses in this study (23) ranged from 0–20 min (with PRL secretion lagging behind Δ sleep), these researchers suggest that common regulatory mechanisms are involved in the two phenomena. Our results, in which increased PRL and increased stage 4 sleep are both associated with the testosterone-replaced state, indicate that testosterone levels may modulate the set-point of this common regulatory mechanism. Additionally, one can speculate that age-related declines in testosterone levels in normal men may contribute to the decline in slow wave sleep (33) that is seen with age.

Our study revealed no differences in melatonin and TSH secretion between the two hormonal conditions. As free T3 and free T4 levels did not differ with hormonal state, the differences we detected in total T3 and total T4 levels are probably secondary to alterations in binding protein levels. With respect to melatonin, the lack of a baseline melatonin profile means that suppression of endogenous gonadotropin secretion by Lupron may have caused melatonin secretion to increase (relative to baseline) in both conditions. However, the amplitude of the melatonin profiles that we obtained is well within the range of values commonly seen in control subjects (34), so this possibility seems unlikely.
Therefore, the most probable conclusion from our results is that gonadal steroid manipulations in postpubertal males do not affect the profile of nocturnal melatonin secretion. What is the relevance of this negative finding to the clinical literature, which shows a possible association between CPP and low melatonin levels and between high melatonin levels and hypogonadism (see introduction)? Our experimental design cannot speak to one possible explanation for these clinical associations; namely, that melatonin has antigonadal effects in humans. Alternatively, these clinical associations might indicate that gonadal steroid manipulations can alter melatonin secretion, a possibility that is addressed both by treatment studies in patients and by our study. The former have produced inconsistent results. For example, although Luboshitzky et al. (35) reported a decline in melatonin levels after testosterone treatment in men with idiopathic hypogonadotrophic hypogonadism, these findings were not replicated by Ozata et al. (9). However, the latter study relied on a single daytime measurement of plasma melatonin (36, 37). More germane to our results (because they involve pharmacological attempts to reverse puberty) are studies in which GnRH agonists were used to treat patients (mostly girls) with CPP. In these studies, GnRH agonists clearly caused pituitary-gonadal suppression, but melatonin levels [which in at least some studies were reported to be abnormally low at baseline (10–12)] either did not change (38) or declined further (10). One possible explanation for these seemingly disparate results is that maturity in the hypothalamic-pineal system is a one-way street; although treatment with gonadal steroids might lower melatonin levels in patients who have never undergone puberty (35), neither suppression of precocious puberty (10, 38) nor pharmacologically induced hypogonadism (as in our study) restores melatonin to its high prepubertal levels.

In summary, we found that testosterone replacement had relatively discrete effects on sleep and hormonal rhythms in hypogonadal men. Testosterone replacement caused robust effects on sleep and hormonal rhythms in prepubertal levels.

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