Gender Differences in the Temporal Organization of Prolactin (PRL) Secretion: Evidence for a Sleep-Independent Circadian Rhythm of Circulating PRL Levels—A Clinical Research Center Study*

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ABSTRACT

Although a nocturnal rise in PRL secretion is well known, it has long been presumed to be evoked by sleep. To determine whether PRL secretion was driven by a sleep-independent circadian rhythm, we studied 12 men and 10 women using a constant routine protocol.

Under the constant routine conditions of continuous semirecumbent wakefulness in constant indoor room light with hourly meals distributed throughout the day and night, a persistent circadian rhythm of PRL secretion was present in men and in women at the follicular and luteal phases of the menstrual cycle. Furthermore, the amplitude of this rhythm in women was significantly greater than that in men.

The present data demonstrate the presence of a robust sleep-independent endogenous circadian rhythm of PRL secretion in humans. We hypothesize that this endogenous component of the circadian rhythm of PRL secretion together with those of body temperature, urine production, and cortisol, TSH, and melatonin secretion are driven by the central circadian pacemaker located in the suprachiasmatic nucleus of the hypothalamus. (J Clin Endocrinol Metab 81: 1483-1487, 1996)

MORE THAN 20 yr ago, Sassin and colleagues (1) described a sleep-related augmentation of prolactin secretion. Although the mechanism by which sleep stimulates PRL secretion is not known, this diurnal pattern has been confirmed by a number of researchers (1–9). Sleep reversal and sleep deprivation studies initially suggested that, unlike the endogenous circadian rhythms of ACTH and cortisol that persist in the absence of sleep, the diurnal rhythm of PRL was entirely sleep related (9–16). However, an early evening rise in PRL before sleep onset was noted (13, 17). More recently, Van Cauter et al. (18) confirmed this observation of an early evening, presleep rise in PRL secretion, and Desir et al. (19) reported an influence of transmeridian travel on PRL secretory patterns. This has led to the suggestion that there may be a sleep-independent endogenous circadian rhythm of PRL secretion (18, 19). We used the constant routine (CR), as first proposed by Mills et al. (20) and later refined by Czeisler et al. (21), to unmask the endogenous circadian rhythm of PRL secretion in men and women while controlling for sleep, activity, posture, light exposure, meals, and menstrual cycle phase.

Subjects and Methods

Subjects

Twelve healthy drug-free men, aged 19–29 yr (mean ± SEM, 21.6 ± 0.9 yr), and 10 such women with regular menstrual cycles, aged 20–32 yr (mean ± SEM, 24.9 ± 1.4 yr), were recruited for study. Only those women with ovulatory menstrual cycles, confirmed by biphasic basal body temperature charts and luteal phase progesterone levels, were included. The body mass index for each group was calculated from self-reported height and weight [men, 25.1 ± 1.04 (n = 9); women, 21.4 ± 0.67 (n = 10)] from subjects for whom the information was available.

Study protocol

The experimental protocols were approved by the Brigham and Women's Hospital human research committee. All subjects gave signed informed consent before beginning the study.

Subjects were instructed to maintain a regular sleep-wake schedule and record their sleep and wake times during the week preceding the in-patient study. Core body temperature, heart rate, and activity were continuously recorded during that time using an ambulatory monitoring device (FMS-8 Recorder, Vitalog Monitoring, Redwood City, CA). Subjects who were found to have an irregular sleep-wake schedule were disempaneled from the study. Their average self-reported bedtimes and wake times for this week were used as the bed and wake times during the in-patient study. Among the female subjects, seven studies were performed between days 2–7 of the follicular phase, and seven studies were performed between days 2–10 of the luteal phase. Four women were studied twice, once during each of the two phases of the menstrual cycle. The follicular phase studies were dated using the onset of menses as day 1, and the luteal phase studies were dated using an urinary ovulation test kit (Ovustix; Monoclonal Antibody, Mountain View, CA) or OvuQuick (Quidel, San Diego, CA) to detect the day of the LH surge, which was designated as luteal phase day 0.

Subjects were admitted to the General Clinical Research Center for the in-patient study. After an adaptation night, subjects underwent 1 day of baseline monitoring with activity confined to their rooms, followed by a 28- to 40-h CR, described below. Subjects were maintained on an
Blood sampling

An indwelling iv catheter with side port holes (Angiocath, Becton Dickinson Vascular Access, Sandy, UT) was used to collect samples approximately three times per h. The length of the 12-foot long tubing (VMR Products, Fruit Heights, UT) was sufficient to allow sampling to be performed from outside the room during scheduled sleep episodes and the CR. Blood sampling began 1 h before wake time on the day before the CR, and continued until the end of the CR. Each sample was later assayed for prolactin (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intraassay coefficient of variation ranged from 3.5–4.6%, and the interassay coefficient of variation ranged from 6.7–8.0%.

Due to technical difficulties, blood sampling could not be continued during the baseline sleep episode in one man and during the follicular phase study in one woman. Blood sampling during the luteal phase studies on the scheduled day was also found in some subjects. The afternoon increase seen in many of the men (follicular phase) developed nausea and vomiting after approximately 25 h of her CR; therefore, subsequent data were excluded from analysis. All other aspects of these studies conformed to the protocol described above.

Data analysis

Data were averaged across subjects with reference to each subject’s habitual bedtime or wake time rather than to clock hour. To facilitate this, linear interpolation was used to estimate PRL levels between the times at which blood samples were collected. Interpolated data from all studies in each of the three groups (follicular phase women, luteal phase women, and men) were averaged together, minute by minute, to generate average waveforms with respect to either their habitual bedtimes or wake times.

To determine whether there was a significant circadian rhythm in PRL levels during the CR and to determine the phase and amplitude of this rhythm, the data were fit with a harmonic regression model (24). For this analysis, data were not interpolated, and data from the first 5 h of the CR were eliminated to exclude residual masking effects of sleep (25). ANOVA with Scheffe’s multiple comparison test was used to compare the mean amplitudes of the groups (women vs. men or follicular women vs. luteal women vs. men).

Results

Scheduled day

During the baseline day, PRL levels varied in a pulsatile and diurnal manner (Figs. 1–3). An overall elevation of PRL levels after sleep onset (Fig. 2) and a subsequent decline upon awakening (Fig. 3) were observed in all subjects. This diurnal variability was more prominent in women than in men (Figs. 1–3). An afternoon/evening increase in PRL was seen in 9 of the 10 studies in women in whom blood samples were collected during the afternoon/evening of the scheduled day. In contrast, only 6 of the 12 studies in men showed a similar afternoon/evening rise. These features of the data are evident in the average PRL waveforms shown in Figs. 2 and 3.

CR

Examination of the data obtained during the CR revealed that there was a persistent endogenous circadian rhythm of PRL secretion in women at both phases of the menstrual cycle and in men (Figs. 1–3). In 12 of the 14 studies in women, there was an increase in nighttime PRL levels at some point during the habitual sleep time despite the fact that the subjects did not change their posture or actually sleep. This sleep-independent nocturnal rise was detectable in only 5 of the 12 men, and when seen, the magnitude of the rise was smaller. A nadir in PRL levels after habitual wake time was seen in nearly all subjects. The afternoon increase seen in many of the studies on the scheduled day was also found in some subjects during the afternoon of the CR.

Although there was considerable variability in PRL data among individuals, differences between the groups emerge when the average waveforms are compared. Figures 2A and 3A demonstrate the robust elevation in the average PRL waveform recorded in the evening and night among follicular phase women. In those women, average PRL levels
during the CR rose in the late afternoon and remained elevated until the habitual wake time, after which time they dropped to a nadir in the late morning/early afternoon. Figures 2B and 3B show that average PRL levels rose at essentially the same time (late afternoon) in luteal phase women, but the elevated levels were not sustained throughout the night. Instead, there was a late afternoon/evening peak in average PRL levels, which began to decline several hours before the habitual bedtime during the CR. Subsequently, average PRL levels in these luteal phase women reached a second peak during the latter half of the habitual sleep time, even though these subjects were kept awake throughout the CR. As in the follicular phase women, this was followed by a nadir in the late morning/early afternoon. Figures 2C and 3C reveal a more modest circadian rhythm in average PRL levels among men during the CR. The most prominent feature of this rhythm in the men is a nadir in average PRL levels that occurred in the late morning/early afternoon, followed by a modest rise in average PRL levels, which reached a plateau throughout the late afternoon, evening, and nighttime during the CR.

Fitting the CR data from each study with a harmonic regression model revealed the presence of a significant circadian rhythm of PRL secretion in 13 of the 14 studies in women and in 10 of the 12 men ($P < 0.01$). The mean amplitude of the circadian PRL profile in women was significantly higher than that in men (women, $2.8 \pm 0.3 \, \mu\text{g/L}$; men, $1.5 \pm 0.3 \, \mu\text{g/L}$; $P < 0.01$). There was no significant difference in mean amplitude between women at the two menstrual phases. However, when women were separated into follicular and luteal phases and compared with men, there was a
significant difference only between the follicular women and the men (follicular, 3.3 ± 0.3 µg/L; luteal, 2.4 ± 0.5 µg/L; men, 1.5 ± 0.3 µg/L; P < 0.01).

Discussion

The daily rhythm of PRL secretion as originally described was thought to be a passively dependent response to a periodically recurring behavior, i.e. sleep (7, 9, 11–15). The present data demonstrate for the first time a robust sleep-independent endogenous circadian rhythm of PRL secretion in humans, which is more prominent in women. This gender difference in the regulation of PRL secretion was previously overlooked. Our data are consistent with an earlier report in male transmeridian travelers that suggested the possibility of a sleep-independent component to the pattern of PRL secretion (19). However, as activity, meal times, and exposure to light were not controlled in that study, those results could still have been attributed to external factors (26), rather than to an underlying rhythm. Furthermore, as only men were included in those prior studies, few data were available to address this issue in women. The present data, in which waking, behavior, posture, and meal timing were carefully controlled, demonstrated that there is an endogenous circadian component of the daily PRL rhythm. We hypothesize that this endogenous PRL rhythm, like other endogenous endocrine rhythms such as those of cortisol, TSH, and melatonin (21, 23, 27, 28), is driven by the human circadian pacemaker located in the suprachiasmatic nucleus of the hypothalamus.

The present findings are not consistent with earlier studies which concluded that the rhythm of PRL secretion was sleep dependent, without an endogenously rhythmic component (7, 11, 13–15). There are several possible explanations for this. Nearly all subjects in prior studies were men, whereas the present data indicate that men exhibit only a modest circadian PRL rhythm that may not have been detected in the absence of strict control of environmental and behavioral conditions. In addition, many prior investigators relied on shifted sleep and sleep inversion studies to differentiate between an endogenous circadian and a sleep-related rhythm (9–13) by comparing average data obtained during nocturnal wakefulness with corresponding levels during daytime sleep. As sleep exerts such a strong influence on PRL secretion, the sleep-independent component of the PRL rhythm was not as easily detected with those protocols. The CR procedure, which controls for wakefulness throughout the circadian cycle, enabled us to unmask the endogenous circadian rhythm of PRL secretion and observe the entire 24-h endogenous component of the rhythm. In fact, the present data are consistent with the earlier finding that PRL levels during nocturnal sleep are higher than those during nocturnal wakefulness and with a recent study in which Wehr et al. (29) found an evening increase in PRL levels after the onset of darkness in subjects chronically exposed to long nights. By maintaining subjects in constant conditions across the entire circadian cycle, it was possible in the present study to unmask an endogenous circadian component of the rhythm. Furthermore, these data demonstrate that PRL levels during nocturnal wakefulness increase compared to PRL levels during daytime wakefulness, although not to the same extent as during nocturnal sleep. One possible explanation for the increase in PRL secretion at night may be due to enhanced sensitivity to GnRH stimulation at night, which is further enhanced during sleep (30).

The present data reveal that one of the most prominent features of the endogenous component of the circadian PRL rhythm is a previously unreported nadir that consistently occurs after habitual wake time in both men and women. A prominent negative influence on PRL secretion at this time of day has also been demonstrated in lactating women, in whom PRL levels rise with breastfeeding at all times except 0800 h (31).

The observed gender differences in the endogenous rhythm of PRL secretion may result from the influence of circulating hormones or neurotransmitters or from an inherent difference in circadian rhythmicity. Estrogen has a well described promotive effect on PRL secretion in men and women (7, 32–34). Although estrogen-induced increases in PRL levels may explain the higher PRL levels in women than in men and the higher levels during sleep, they may not explain the current findings of an increased amplitude of the sleep-independent endogenous circadian rhythm of PRL secretion. An alternative explanation for the observed sexual dimorphism in the pattern of PRL secretion could be a difference in the nature of the circadian signal arising from the suprachiasmatic nucleus. This, in turn, may result from an influence of ambient, perinatal, or peripubertal hormone levels or an underlying gender difference in the transduction of the circadian signal from the suprachiasmatic nucleus (36, 37).

In summary, frequent sampling of PRL levels in men and women during the follicular and luteal phases of the menstrual cycle have demonstrated an endogenous circadian sleep-independent rhythm of PRL secretion that is robustly expressed in women and present, but more weakly expressed, in men. Under baseline conditions, this rhythm is synchronized to the 24-h light-dark cycle. Sleep augments PRL secretion and masks detection of the underlying circadian rhythm. Only after studying subjects under constant conditions, controlling for sleep and other factors that affect circadian rhythms and PRL levels, were it possible to unmask the circadian rhythm of PRL secretion. This study stresses the importance of controlling for both environmentally and behaviorally evoked effects when studying the physiology of circadian processes in humans and for the careful examination of gender differences.

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References


