Circadian rhythms in the chemoreflex control of breathing

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Stephenson, Richard, Ravi M. Mohan, James Duffin, and Tim M. Jaraky. Circadian rhythms in the chemoreflex control of breathing. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R282–R286, 2000.—Mechanisms underlying the circadian rhythm in lung ventilation were investigated. Ten healthy male subjects were studied for 36 h using a constant routine protocol to minimize potentially confounding variables. Laboratory light, humidity, and temperature remained constant, subjects did not sleep, and their meals and activities were held to a strict schedule. Respiratory chemoreflex responses were measured every 3 h using an iso-oxic rebreathing technique incorporating prior hyperventilation. Subjects exhibited circadian rhythms in oral temperature and respiratory chemoreflex responses, but not in metabolic rate. Basal ventilation (i.e., at subthreshold end-tidal carbon dioxide partial pressure \( \text{PETCO}_2 \)) did not vary with time of day, but the ventilatory response to suprathereshold \( \text{PETCO}_2 \) exhibited a rhythm amplitude of \(-25\%\), mediated mainly by circadian variations in the \( \text{CO}_2 \) threshold for tidal volume. We conclude that the circadian rhythm in lung ventilation is not a simple consequence of circadian variations in arousal state and metabolic rate. By raising the chemoreflex threshold, the circadian timing system may increase the propensity for respiratory instability at night.


target influencing variables: body temperature; metabolic rate; constant routine; sleep apnea syndrome; respiratory instability

Many physiological variables exhibit daily oscillations regulated by an endogenous circadian pacemaker located in the suprachiasmatic nuclei of the anterior hypothalamus (21). Although these circadian rhythms are generally assumed to be beneficial (14), they may also have detrimental effects on human health. For example, nocturnal sleep-related breathing disorders, which include the sleep apnea syndromes (SAS), are a major public health problem (20). Although efforts to understand the mechanistic basis of SAS have mainly focused on the effects of sleep state (4, 6, 12), the potential role of the circadian timing system has received scant attention. Indeed, it is unclear whether the circadian timing system has any direct influence on the respiratory control system, either in health or disease.

Breathing rates oscillate over the 24-h day (26). However, it has not yet been determined whether a circadian rhythm in respiration reflects a direct influence of the circadian timing system on respiratory control processes or an indirect consequence of rhythms in related physiological variables such as sleep-wake state, metabolic rate, and body temperature. This study was designed to test the hypothesis that healthy male subjects exhibit circadian rhythms in respiratory chemoreflex control characteristics in the absence of sleep.

MATERIALS AND METHODS

Experimental protocol. Twelve male subjects, all healthy nonsmokers aged 19–37 yr (body mass 72.9 ± 8.0 kg), were studied in three groups of four using procedures approved by the University of Toronto Committee for Human Experimentation. After giving their informed written consent, four subjects arrived at the laboratory at 8:45, 9:30, 10:15, and 11:00 Eastern Standard Time. Thus the subjects within a group were staggered to optimize use of the respiratory test apparatus. They were asked to abstain from food, alcohol, or caffeinated drinks for at least 12 h before the experiment. In an effort to reduce the effects of factors that might mask (i.e., induce or hide) a circadian rhythm in respiration, the subjects were placed on a constant-routine protocol (17) for the next 36 h. Two subjects, whose respiratory responses were irregular due to persistent swallowing, changes in posture or apparent brief changes in arousal state during the rebreathing tests, were excluded from subsequent analyses. Circadian rhythmicity was examined in the pooled data from the remaining 10 subjects after eliminating intersubject differences in both the timing of the internal circadian clock and in the average magnitudes of respiratory variables (see Data analysis). Experiments began on Friday mornings. After arriving at the laboratory, each subject was immediately placed on a repeating 3-h cycle. Each cycle began with a 15-min seated rest period terminating with measurement of oral temperature and immediately followed by a rebreathing test. At exactly 45 min after the start of the cycle, the subjects ate a small meal consisting of measured quantities of fruit juice,
low-fat sandwiches, and proprietary nutritional supplements (solid and liquid). Total energy content of the meals varied from 1,000 to 1,600 kJ between subjects but was held constant over time for each individual. Subjects had access to bottled water at all times except for the first 45 min of each cycle. Laboratory conditions remained constant (ambient temperature, 20–22 °C; relative humidity, 25–27%; fluorescent light intensity, 450–600 lx), and for each subject, all 13 rebreathing tests were conducted by the same research personnel. Exposure to sunlight was prevented, and the subjects were not allowed to sleep at any time during the study. The subjects were ambulatory, but physical activity was discouraged, and they occupied their time between tests by reading, watching movies, and playing computer games.

Assessment of respiratory chemoreflexes. Rebreathing tests were conducted following the procedure described in detail elsewhere (18). Briefly, subjects wore a noseclip and sterile mouthpiece. They voluntarily hyperventilated room air for 5 min to lower their internal stores of CO₂ below the chemoreflex thresholds, then rebreathed from a 6-liter plastic bag connected to a spirometer. During rebreathing, end-tidal oxygen partial pressure (PETO₂) was held constant at 61 ± 1 mmHg (groups 1 and 2) or 48 ± 2 mmHg (group 3). Differences in PETO₂ had no statistically significant effect on any aspect of the respiratory response (Student’s t-tests, P > 0.05) and data from all three groups were pooled. End-tidal carbon dioxide partial pressure (PETCO₂) increased linearly over time during rebreathing (see Fig. 2A), and the rate of accumulation of CO₂ (i.e., ΔPETCO₂/Δt) was used as an index of metabolic rate. Rebreathing concluded when PETCO₂ had risen above 55 mmHg. During rebreathing, tidal volume (VT, liters BTPS) and breathing frequency (FR, breaths/min) remained steady at their basal values until PETCO₂ exceeded their respective thresholds (see Fig. 2). As PETCO₂ continued to rise, VT and FR increased approximately linearly (see Fig. 2, C and D) until PETCO₂ reached a point at which the slope of VT often (but not always) changed. The slopes of the least-squares regression lines fitted through the linear part of the suprathreshold data (see Fig. 2) were interpreted as the sensitivities of VT (l/mmHg) and FR (breaths·min⁻¹·mmHg⁻¹) components of the ventilatory chemoreflex. Thresholds were initially estimated by eye and then defined as the intersection of regression lines fitted through the data above and below this first approximation (see Fig. 2).

Data analysis. Data were analyzed for tests 3-10, covering an interval of 24 h beginning 6 h after the start of the study. Data were adjusted to “circadian time” by aligning the oral temperature minima to the observed intersubject average time of 06:20 h. For each variable of interest in each subject, a 24-h mean value was calculated. The data were then reexpressed (in their original units) as deviations from the mean value. The resulting time-adjusted deviations were then combined across subjects and fitted with a least-squares sine function (Sigmaplot, SPSS, Chicago, IL)

\[ y = y_o + a \sin(2\pi x/b + c) \]

where y is the physiological variable (time-adjusted deviation), y₀ is the 24-h fitted mean value (equal to 0 for time-adjusted deviations), a is the rhythm amplitude (one-half the peak-to-trough difference), x is time of day (hours), b is the rhythm period (constrained to 24 h in this analysis), and c is the time of fitted peak value (acrophase).

Circadian rhythms were inferred from a significant (P < 0.05) regression analysis of variance and confirmed using paired t-tests to compare data at the times of the peak and trough of the fitted sine function. Data are presented as means ± SD.

RESULTS

The subjects exhibited robust circadian rhythms in oral temperature and respiratory responses to suprathreshold levels of CO₂ (Fig. 1). Oral temperature oscillated about a 24-h mean (± SD) value of 36.4 ± 0.2°C (Fig. 1A). On average, the nadir of the temperature rhythm occurred at 06:20 h, varying from 02:20 to 07:15 h between subjects. The amplitude of the oral temperature rhythm was 0.3 ± 0.1°C, varying from 0.1 to 0.5°C between subjects. The rate of rise of the PETCO₂
within the rebreathing apparatus (an index of metabolic rate) did not oscillate with a 24-h period \( [F(3,73) = 0.458, P > 0.7; \text{Fig. 1B}] \).

The circadian rhythm in chemoreflex responses is illustrated in Fig. 1C by plotting minute ventilation (\( V_E, \text{L/min} \)) at low (45 mmHg) and high (53 mmHg) levels of \( \text{PETCO}_2 \) attained during the rebreathing tests vs. time of day. These \( \text{PETCO}_2 \) values were chosen because 53 mmHg was above the thresholds for both \( V_T \) and \( F_R \) (Fig. 1D), whereas 45 mmHg fell between the \( V_T \) and \( F_R \) thresholds in seven of the subjects (45 mmHg was below both thresholds in 2 subjects, e.g., Fig. 2, and above both thresholds in 1 subject). The 24-h mean \( V_E \) was 18.9 ± 8.8 L/min at 45 mmHg and 69.9 ± 38.9 L/min at 53 mmHg. Amplitudes of the circadian rhythms were 26.5% of the 24-h mean for \( V_E \) at 45 mmHg \( [F(3,73) = 11.162, P < 0.0001] \) and 24.2% of the 24-h mean for \( V_E \) at 53 mmHg \( [F(3,73) = 9.164, P < 0.0001] \). Fitted acrophases (adjusted time of peak values) occurred at 19:20 h for \( V_E \) at 45 mmHg and 18:00 h for \( V_E \) at 53 mmHg. Basal ventilation had a 24-h mean value of 11.1 ± 4.1 L/min and did not oscillate significantly with a circadian period \( [F(3,72) = 1.373, P = 0.258; \text{Fig. 1C}] \).

The circadian rhythm in the response of \( V_E \) to suprathreshold levels of \( \text{PETCO}_2 \) was composed of parallel circadian variations in the responses of both \( V_T \) and \( F_R \) (Fig. 2). For example, at a \( \text{PETCO}_2 \) of 53 mmHg, \( V_T \) varied \( [F(3,73) = 9.922, P < 0.0001] \) about a 24-h mean value of 3.1 ± 1.1 liters, with a rhythm amplitude of 0.39 l (12.6%), and \( F_R \) varied \( [F(3,73) = 6.543, P = 0.0006] \) about a 24-h mean of 22.6 ± 8.0 breaths/min, with a rhythm amplitude of 2.4 breaths/min (10.6%). \( V_E \) at a \( \text{PETCO}_2 \) of 53 mmHg was not correlated with metabolic rate (Pearson correlation coefficient \( r = -0.002, P > 0.05 \)).

The rhythm in \( V_T \) was mediated by a low amplitude (3.7% of the mean value of 43.0 ± 1.3 mmHg) but highly significant \( [F(3,73) = 8.377, P < 0.0001] \) oscillation in the threshold of the response of \( V_T \) to changing \( \text{PETCO}_2 \) (Fig. 1D). The peak of the rhythm in \( V_T \) threshold was at 06:10 h, which is in antiphase with the oral temperature rhythm. The rhythm in \( F_R \) was mediated by variations in both threshold and sensitivity of \( F_R \) to changing \( \text{PETCO}_2 \) (Fig. 1, D and E), neither of which, however, achieved statistical significance \( [F(3,73) = 2.332, P = 0.081 \text{ and } F(3,73) = 1.799, P = 0.155, \text{ respectively}] \).

To examine the effects of sleep deprivation on respiratory chemoreflexes, the data for three consecutive rebreathing tests conducted between 12:00 and 20:15 h were averaged in each subject, and data from days 1 and 2 were then compared using paired t-tests. Increases in the \( \text{CO}_2 \) thresholds for both \( V_T \) (day 1, 41.7 ± 1.8 mmHg; day 2, 43.0 ± 1.8 mmHg; \( t_{22} = -2.957, P = 0.016 \)) and \( F_R \) (day 1, 45.3 ± 2.7 mmHg; day 2, 46.9 ± 2.7 mmHg; \( t_{22} = -3.049, P = 0.014 \)) were observed. Response sensitivities (i.e., \( V_E/\text{PETCO}_2, V_T/\text{PETCO}_2, \text{and } F_R/\text{PETCO}_2 \)) were not affected by prolonged wakefulness. 

**DISCUSSION**

This study supports the hypothesis that circadian modulation of human respiratory chemoreflex characteristics occurs in the absence of concurrent changes in arousal state and metabolic rate. Subthreshold (i.e., basal) ventilation did not vary as a function of time of day, but ventilation at suprathreshold levels of \( \text{PETCO}_2 \) exhibited a substantial circadian rhythm with an amplitude of ~25% of the mean. That is, for a given level of hypercapnia, \( V_T \) at 18:00 h was 67% greater than that at 06:00 h. By using a rebreathing procedure in which the partial pressure of oxygen was held constant at moderately hypoxic levels, we assessed the effects of circadian time on combined central and peripheral chemoreceptor stimulation. This ensured that both major components (i.e., central hypercapnic and peripheral hypoxic/hypercapnic) of the respiratory chemosensory system were assessed. Since a significant circa-
CIRCADIAN RHYTHMS IN RESPIRATION

The circadian rhythm in respiratory chemoreflex responses occurred in the absence of a circadian rhythm in metabolic rate (Fig 1), indicating that the former was not dependent on the latter. Human resting metabolic rate has been found to be quite variable over short (e.g., hourly) time intervals, and the circadian rhythm is typically of low amplitude (<10% in nonsleeping subjects), even when studied under more rigidly controlled conditions than we used (16). Therefore, the absence of a detectable rhythm in energy metabolism in our subjects was not surprising. A similar temporal dissociation between respiratory chemosensitivity and metabolic rate has been observed in birds (30). By contrast, the ventilatory responses to CO₂ of awake adult rats (19) and to hypoxia in 6-day-old rat pups (24) were greater at night (when these nocturnal rodents are normally awake) than during the day (when they are normally sleeping). However, in these cases the differences between day and night were largely due to differences in CO₂-induced and hypoxia-induced hypometabolism, and the metabolism-specific ventilatory responses (V̇E/V̇CO₂ and V̇E/V̇O₂) were similar at both times of day.

Basal ventilation has been shown to be independent of both CO₂ and O₂ (9, 18) and, therefore, represents a nonchemical stimulus to breathe, often referred to as a “wakefulness stimulus.” The absence of any systematic circadian variations in basal V̇E, V̇T, or ḞR indicates that the circadian rhythm in the chemoreflex response is not simply an indirect effect of variations in the wakefulness stimulus and, therefore, is likely to have functional consequences during sleep as well as wakefulness.

The transition from wakefulness to sleep is accompanied by depression of the respiratory chemoreflexes by way of increased thresholds and decreased sensitivity to CO₂ (7, 10). In this study we have found that a nocturnal decline in respiratory responsiveness occurs even in the absence of sleep (Fig 1). Because human sleep is normally restricted to nighttime, susceptible individuals may be at heightened risk of sleep-related respiratory problems, especially if the circadian- and sleep-dependent effects on respiratory regulation are additive. Conversely, sleep-disordered breathing may be attenuated during midday naps compared with nocturnal sleep. Furthermore, our observations raise the intriguing possibility that sleep-disordered breathing could arise in people with high-amplitude circadian modulation and normal sleep mechanisms, in which case interventions aimed at the circadian timing system would be indicated.

Many sleep-related breathing disorders induce sleep fragmentation, but short-term (1–3 nights) sleep fragmentation has been shown to have minimal effects on human or canine respiratory chemoreflex responses (3, 8). Nevertheless, judging from the excessive daytime somnolence in SAS patients and pronounced sleep rebound during the early stages of nasal continuous positive airway pressure (CPAP) treatment (15, 28), SAS patients may suffer cumulative sleep deprivation after prolonged periods (months to years) of sleep...
disruption. The findings that reduced respiratory responses to CO₂ in SAS patients can be reversed following treatment by CPAP (2) or tracheostomy (11) are consistent with this suggestion.

In our subjects, a single day of sleep deprivation induced significant increases in the CO₂ thresholds for both VT and FR, but response sensitivities were unaltered. Others have reported reduced hypercapnic ventilatory responses following 24 h of continuous wakefulness (5, 25, 29) that they ascribed to reduced sensitivity; however, none of the previous studies employed hyperventilation before rebreathing, which prevented direct measurement of chemoreflex thresholds. As discussed earlier, this issue is important because reduced hypercapnic ventilatory responses can increase or decrease the tendency for periodic breathing, depending on whether it is mediated by increased thresholds or decreased chemosensitivity, respectively. Our results suggest that sleep deprivation, like the day-to-night transition (and wake-to-sleep transitions (4, 7, 10)), will, by elevating response thresholds, increase the tendency for respiratory instability. Further research is needed to determine whether these effects are additive.

In conclusion, our data provide evidence for a direct role of the circadian timing system in the regulation of breathing, independent of any indirect effects it may have via modulation of metabolic rate and arousal state. This effect is primarily mediated by a nocturnal increase in the chemoreflex threshold, a change that is predicted by control theory to contribute to respiratory instability, with implications for the pathogenesis of periodic breathing and sleep apnea (4, 13). The circadian timing system may, therefore, play a vital role in the etiology of some respiratory disorders.

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