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Circadian rhythms in the chemoreflex control of breathing

Stephenson, Richard, Ravi M. Mohan, James Duffin, and Tim M. Jarsky. 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 isocyclic rebreathing technique incorporating prior hyperventilation. Subjects exhibited circadian rhythms in arterial oxygen tension and respiratory chemoreflex responses, but not in metabolic rate. Basal ventilation [i.e., at subthreshold end-tidal carbon dioxide partial pressure (PETCO2)] did not vary with time of day, but the ventilatory response to suprathreshold PETCO2 exhibited a rhythm amplitude of ~25%, mediated mainly by circadian variations in the CO2 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.

MANY PHYSIOLOGICAL VARIABLES exhibit daily oscillations regulated by an endogenous circadian pacemaker located in the suprachiasmatic nuclei of the anterior hypothalamic (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%; fluores-
cent 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 chemore-
flex threshold, 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). Differ-
ces in PETCO2 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 data above
represented 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 reex-
pressed (in their original units) as deviations from the mean
value. The resulting time-adjusted deviations were then
crossed across subjects and fitted with a least-squares sine
function (SigmaPlot, SPSS, Chicago, IL)

\[ y = y_0 + a \sin[(2\pi \times \text{b}) \times c] \]

where \( y \) is the physiological variable (time-adjusted devia-
tion), \( y_0 \) 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), \( c \) 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 supra-
threshold 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 tempera-
ture 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

![Fig. 1. Circadian rhythms in body temperature and respiratory chemoreflex characteristics. *Statistically significant (ANOVA, P <
0.05). Sample size = 77 for all variables. Data are time-adjusted deviations (see text for details). For purposes of illustration, a constant
equal to 24-h mean value has been added to data. A: oral temperature. B: rate of accumulation of CO2 (ΔPETCO2/Δt, mmHg/s)
during rebreathing, used as an index of metabolic rate. C: minute ventilation (BTPS) measured at basal (subthreshold) PETCO2, and 2
specified suprathreshold levels of CO2 (PETCO2). D: threshold PETCO2 above which respiratory tidal volume (VT) and breathing
frequency (FR) increased in response to increases in PETCO2. E: sen-
sitivities of tidal volume (ΔVT/ΔPETCO2, L/mmHg) and respiratory frequency (ΔFR/ΔPETCO2, breaths·min⁻¹·mmHg⁻¹) to suprathreshold PETCO2.

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**A**

![Graph A]

**B**

![Graph B]

**C**

![Graph C]

**D**

![Graph D]

**E**

![Graph E]
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; Fig. 1B].

The circadian rhythm in chemoreflex responses is illustrated in Fig. 1C by plotting minute ventilation (V̇E, l/min) at low (45 mmHg) and high (53 mmHg) levels of PETCO₂ attained during the rebreathing tests vs. time of day. These PETCO₂ values were chosen because 53 mmHg was above the thresholds for both VT and FR (Fig. 1D), whereas 45 mmHg fell between the VT and FR 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 V̇E 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 V̇E at 45 mmHg [F(3,73) = 11.162, P < 0.0001] and 24.2% of the 24-h mean for V̇E at 53 mmHg [F(3,73) = 9.164, P < 0.0001]. Fitted acrophases (adjusted time of peak values) occurred at 19:20 h for V̇E at 45 mmHg and 18:00 h for V̇E 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; Fig. 1C].

The circadian rhythm in the response of V̇E to suprathreshold levels of PETCO₂ was composed of parallel circadian variations in the responses of both VT and FR (Fig. 2). For example, at a PETCO₂ of 53 mmHg, VT 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 FR 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%). V̇E at a PETCO₂ of 53 mmHg was not correlated with metabolic rate (Pearson correlation coefficient r = −0.002, P > 0.05).

The rhythm in VT 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 VT to changing PETCO₂ (Fig. 1D). The peak of the rhythm in VT threshold was at 06:10 h, which is in antiphase with the oral temperature rhythm. The rhythm in FR was mediated by variations in both threshold and sensitivity of FR to changing PETCO₂ (Fig. 1, D and E), neither of which, however, achieved statistical significance [F(3,73) = 2.332, P = 0.081 and F(3,73) = 1.799, P = 0.155, 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 CO₂ thresholds for both VT (day 1, 41.7 ± 1.8 mmHg; day 2, 43.0 ± 1.8 mmHg; t(21.9) = −2.957, P = 0.016) and FR (day 1, 45.3 ± 2.7 mmHg; day 2, 46.9 ± 2.7 mmHg; t(21.9) = −3.049, P = 0.014) were observed. Response sensitivities (i.e., V̇E/PETCO₂, VT/PETCO₂, and FR/PETCO₂) 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 PETCO₂ exhibited a substantial circadian rhythm with an amplitude of −25% of the mean. That is, for a given level of hypercapnia, V̇E 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-
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Dian influence was observed in this study, further research is warranted to determine whether the circadian timing system exerts its effects on one or both of these chemosensory pathways.

An important finding is that the nocturnal decline in the hypercapnic ventilatory response was primarily a result of a right shift of the chemoreflex response curve. That is, the threshold PETCO2 was 3.2 mmHg higher at 06:00 h than at 18:00 h. This morning-to-evening difference in chemoreflex threshold is of the same order of magnitude as the differences observed between wakefulness and sleep (7, 10). An increased response threshold can increase the propensity for respiratory instability (4). Of equal significance in this context is the observation that chemosensitivity (i.e., the slope of the suprathreshold ventilatory response, or “controller gain”) was not markedly reduced at night. This implies that short-term respiratory oscillations (i.e., breath-to-breath instability) arising as a result of a nocturnal rise in response threshold would not be counteracted by a concurrent reduction in controller gain. These circadian changes in chemoreflex characteristics, therefore, have important implications for the development, and perhaps also for the treatment, of some types of nocturnal respiratory dysfunction.

Oral temperature and ventilatory chemoreflex responses varied in parallel in our subjects; on average, both peaked in the early evening and then fell through the night to reach a nadir just before (subjective) dawn. Raschke (22) and Raschke and Möller (23), in a study of 10 male subjects, also observed this. However, their results were confounded by a protocol that allowed subjects to sleep before some of the rebreathing tests. In contrast, Spengler et al. (26) reported rhythms in resting VE and metabolic rate (VO2 and VCO2) that peaked 8 h before body temperature. The latter authors interpreted this observation as evidence that circadian changes in ventilation are unlikely to be causally related to temperature changes. Although our data cannot exclude the possibility that the respiratory rhythm is mediated by the temperature rhythm, we tend to concur with the conclusion of Spengler et al. (26), because, as is discussed below, current evidence is not very supportive of such a mechanism.

An acute rise in body temperature has been shown to stimulate breathing (1, 27), although temperature had to rise by 1°C or more before a ventilatory response was detected, and unmasked circadian temperature change is usually less than this in human subjects. In addition, hyperthermia caused an elevation of basal ventilation (i.e., V̇E at subthreshold levels of CO2) and the sensitivity (slope) of the V̇E response to suprathreshold levels of CO2, with no effect on the response threshold itself (1). In contrast, we found that circadian rhythms were primarily associated with changes in threshold, with no changes in basal ventilation and only small changes in sensitivity. Furthermore, thermal stimulation of breathing was mainly effected by increases in FR (1, 27), but in our subjects the circadian rhythm was due in large part to changes in V̇E.

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 (VE/VCO2 and V̇E/VO2) 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 VE, VT, or FR 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 CO\textsubscript{2} 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 CO\textsubscript{2} thresholds for both \(V_T\) and \(F_{\text{Ea}}\), but response sensitivities were unaffected. 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 (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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REFERENCES


