Circadian pattern of ventilation during acute and chronic hypercapnia in conscious adult rats

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Seifert, Erin L., and Jacopo P. Mortola. Circadian pattern of ventilation during acute and chronic hypercapnia in conscious adult rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R244–R251, 2002; 10.1152/ajpregu.00290.2001.—Because metabolism is a determinant of the ventilatory chemosensitivity, we tested the hypothesis that the ventilatory response to acute and prolonged hypercapnia is adjusted to the circadian oscillations in oxygen consumption (V˙O2). Adult rats were instrumented for measurements of body temperature (Tb) and activity by telemetry. Pulmonary ventilation (V˙E) was measured by the barometric method and V˙O2 by the flow-through method. In the acute experiments, 16 conscious rats entrained to a 12:12-h light (L)-dark (D) cycle (lights on 7:00 AM) were exposed to air, 2%, and then 5% CO2 in normoxia (30–45 min each) at 11:00 AM and 11:00 PM. In a separate group of seven rats, simultaneous recordings of all variables were made continuously for 3 consecutive days in air followed by 3 days in 2% CO2 in normoxia, in a 12:12-h L-D cycle (lights on 7:00 AM). In air, all variables were significantly higher at night, whether rats were studied acutely or chronically. Acute CO2 exposure had similar significant effects at 11:00 AM and 11:00 PM on V˙E (~25 and 100% increase with 2 and 5% CO2, respectively) and V˙O2 (~8% drop with 5% CO2), such that the hyperventilatory response (% increase in V˙E/V˙O2 from air) was similar at both times. Chronic CO2 breathing increased V˙E at all times of the day, but less so during the L phase (~15 vs. 22% increase in L and D, respectively), when activity was lower. However, V˙O2 was reduced from the air level (~10% drop) in the L, such that the V˙E/V˙O2 response was similar between L and D. The same result was obtained when the V˙E/V˙O2 response was compared between the L and D phases for the same level of activity. These results suggest that, throughout the day, the hypercapnic hyperventilation, whether during acute or prolonged CO2, is perfectly adjusted to the metabolic level.

control of breathing; chemosensitivity; barometric method; oxygen consumption

IN MAMMALS AND BIRDS, blood gas homeostasis depends on the matching of pulmonary ventilation (V˙E) to metabolism. Indeed, a close coupling between V˙E and oxygen consumption (V˙O2) has been found under a variety of conditions where V˙O2 is either raised or lowered (20). Variations in metabolism are also associated with changes in V˙E chemosensitivity (19). A higher V˙O2, as during muscular exercise (35) or cold exposure (12), is accompanied by a greater absolute increase in V˙E in response to acute hypoxia or hypercapnia, whereas reflex hyperpnea is lower when V˙O2 is reduced, such as with myxedema (36) or semi-starvation (6). Hence, the V˙E chemoreflex response seems to track V˙O2. This would tend to minimize fluctuations in blood gases in normoxia and when chemosensory drive is elevated.

Oxygen consumption presents a circadian oscillation. Although the daily high values of V˙O2 generally occur when an animal is more active, the oscillation persists without changes in activity (1, 3, 33). In conscious rats in which the breathing pattern was measured continuously for several days, we found a daily oscillation in V˙E resembling that commonly obtained for V˙O2, body temperature (Tb), and activity (32). It would therefore seem reasonable to suggest that the absolute increase in V˙E during chemoreceptor stimulation should be greater when V˙O2 and V˙E are higher, such that the degree of hyperventilation (increase in V˙E/V˙O2) remained similar throughout the day. Indeed, in awake newborn rats maintained under a 12:12-h light-dark cycle, the V˙E response relative to V˙O2 was similar in the morning and evening during acute exposure to hypoxia (30), despite differing levels of hyperpnea. A similar conclusion can be reached from data from adult rats in acute hypercapnia, although the interpretation is complicated by inconsistencies in the normocapnic data to which the hypercapnic values were compared (25). On the other hand, in humans, awake and under constant ambient conditions for 40 h, the acute hypercapnic V˙E response showed a circadian pattern that could not be linked to changes in metabolism (33, 34). The persistence of this situation with chronic hypercapnia would imply the presence of significant oscillations in the coupling between air convection and metabolism and, therefore, in blood gases.

In the present study, we further examined the question of whether the daily oscillation in V˙O2 influences the V˙E response to hypercapnia, following the hypothesis that the hyperpnea is greater when V˙O2 is higher. In conscious rats, we compared V˙E, V˙O2, and Tb during...
acute CO₂ exposure, using two CO₂ concentrations, in the morning and in the evening. In addition, these variables, along with activity, were monitored continuously for 3 days in air followed by the same period of CO₂ breathing under a 12:12-h light-dark cycle. Different from the acute experiments, the chronic exposure allowed us to determine whether an increase in chemosensory drive alters the effectiveness of VE-V̇O₂ coupling throughout the day.

METHODS

Experiments were performed on Sprague-Dawley adult male rats. The study was approved by the Animal Ethics Committee of McGill University. When not under study, rats were housed individually, with voluntary access to rat chow and water, and maintained in a 12:12-h light-dark cycle (lights on 7:00 AM-7:00 PM). Separate groups of animals were used for the acute and chronic exposures to hypercapnia.

Protocols. For the acute measurements, 2 h before recordings began rats \( n = 16 \), body wt = 240 ± 3.4 (SE) g were placed in a 1,700-ml Plexiglas chamber that was continuously flushed at 1,200 ml/min STPD. In each rat, \( \dot{V}_E \), \( \dot{V}_{O_2} \), and \( T_b \) were measured twice, at 11:00 AM and 11:00 PM. The time of the first experiment was randomized, and 24–48 h separated the trials, between which rats were returned to their cages. All experiments were conducted in the light at an ambient temperature of 23 ± 0.1°C. Data were collected only after the rat appeared quiet but awake for ≥10 min. Measurements were taken in air, 2% CO₂, and finally 5% CO₂ in normoxia (delivered from calibrated pressurized tanks), always in that order. The CO₂ exposures lasted 45 min, with no return to air between them. Data were collected 30–45 min after the onset of each exposure, well beyond the chamber washout time (≈2 min).

A separate group of rats \( n = 7 \), starting body wt = 198 ± 6 g was studied chronically in a 10-liter chamber supplied with rat chow and water ad libitum and flushed continuously at ∼2 l/min STPD. \( \dot{V}_E \), \( \dot{V}_{O_2} \), \( T_b \), and activity were monitored simultaneously in air for 3 consecutive days and then in 2% fractional concentration of inspired CO₂ in normoxia (FiCO₂) for a further 3 consecutive days in a 12:12 h-light-dark cycle (lights on 7:00 AM-7:00 PM, light intensity 20–30 lx) at 23.4 ± 0.1°C. The switch of the inspired gas to the CO₂ mixture occurred at 6:00 PM. The experimental setup was placed in an isolated quarter and was left undisturbed except for cage cleaning every ∼24–36 h and equipment checks, both done at arbitrary times.

\( T_b \) and activity. A few days before the measurements, rats were instrumented with an intra-abdominal transmitter. In the acute experiments, a frequency signal proportional to \( T_b \) was emitted by the transmitter (VM-PH, Mini-Mitter) and monitored by a receiver (RLA3000, Data Sciences International) connected to a multimeter. In the chronic experiments, the transmitter was powered by an energizer-receiver unit (series 4000E, Mini-Mitter) for measurements of \( T_b \) and activity. \( T_b \) was obtained from the frequency of the transmitter, and activity was the total score of counts registered by the radiating coils of the energizer-receiver platform over a period of 2 min. These were recorded simultaneously (sampling rate 1 Hz) by standard telemetric techniques and stored on a computer as previously described (23).

\( \dot{V}_{O_2} \). \( \dot{V}_{O_2} \) was measured by the open-flow method (11). The inflowing and outflowing gas concentrations were monitored by a calibrated polarographic O₂ analyzer (OM-11, Beckman) and by an infrared CO₂ analyzer (CD-3A, Applied Electrochemistry or LB-2, Beckman). In the chronic experiments, a programmable solenoid valve switched the sampling port of the \( \dot{O}_2 \) and CO₂ analyzers from the outflow to the inflow pathway of the chamber for 1 min every 30 min to check for drifts in the recording system. \( \dot{V}_{O_2} \) was computed as the product of the flow and the inflow-outflow concentration difference of O₂ and calculated in milliliters STPD (1 ml O₂ STPD = 0.0446 mmol O₂) normalized to the animal’s body weight in kilograms. The small error introduced by a respiratory quotient less than unity (10) was neglected.

\( \dot{V}_E \). The barometric technique was used for measurements of breathing pattern. Tidal volume (\( \dot{V}_T \); at STPD, normalized to the animal’s body weight in kg) was computed using the equation proposed by Drorbaugh and Fenn (7).

In the acute experiments, once \( \dot{V}_{O_2} \) samples were obtained, the inlet and outlet of the respirometer were sealed for ≈1 min, and oscillations in chamber pressure were monitored by a sensitive pressure transducer (±25 cmH₂O; DP45, Valdyne) and recorded on paper at 10 mm/s. The pressure signal was then integrated for volume fractions of CO₂ in the outflow to the inflow. Each sample gas was sucked into the chamber using a syringe. Chamber temperature was monitored by two tungsten-constantan thermocouples (DP30, Omega) positioned at opposite ends of the chamber. Relative humidity was taken as 100%, as condensation was often observed on the chamber walls.

The barometric chamber used for the chronic experiments permitted continuous monitoring of \( \dot{V}_E \) for several days without investigator intervention (32). A double pump system, with one pump pushing air into the chamber and the other sucking air out through very tiny openings, enabled the chamber to be flushed continuously (at ∼2 l/min) despite behaving as a functionally closed system. The average time constant for a step increase in pressure to dissipate through the openings was 2.1 s, or ∼10 times longer than the inspiratory time of the rat. Chamber pressure was measured by a sensitive transducer (±2.5 cmH₂O; #DUXL30D, Data Instruments), and temperature and relative humidity were measured by a thermistor (55033, Yellow Springs) and a humidity sensor (HHH-3602-C, Honeywell). Data were acquired by computer (sampling rate 100 Hz). The chamber pressure root mean square (RMS) was also continuously recorded. Data filtering criteria included an empirically derived RMS threshold, used to eliminate gross body movements, breathing rate >300 breaths/min to eliminate high-frequency periods such as during sniffing, and pressure oscillations <0.001 mmHg, as these were within the noise level of the setup.

Data analysis. In the acute experiments, ventilatory data are based on 100 consecutive breaths per condition. The records were analyzed with the help of a graphics table connected to a minicomputer to obtain inspiratory and expiratory time (TI and TE, respectively, in s) and \( \dot{V}_T \) (ml at STPD). Total breath duration (\( T_{tot} \) in s) was calculated as the sum of \( T_I \) and \( T_E \) (breathing rate (\( f_b \), breaths/min) as 60/\( T_{tot} \) and \( \dot{V}_E \) (ml/min) as the product of \( f_b \) and \( \dot{V}_T \). Data measured during CO₂ breathing were analyzed as the percent change (\( \dot{V}_E \), \( \dot{V}_{O_2} \), \( \dot{V}_E/\dot{V}_{O_2} \) or the difference (\( T_b \)) from the corresponding normocapnic value.

In the chronic experiments, \( \dot{V}_{O_2} \) was measured at half-hour intervals and ventilatory, \( T_b \), and activity data were averaged over 30 min. In each rat, data at corresponding times were averaged over the 3 days in air to obtain mean daily air patterns. Over the course of the 3-day hypercapnic exposure, no significant changes were found in the response to 2% CO₂ (see RESULTS, Fig 2). Hence, as for the air values, data at corresponding times were averaged over the 3 days in
CO2. The light and dark phase values in each condition (air and hypercapnia) were defined in each rat as the average value for the middle 8 h of each phase (light: 9:00 AM-5 PM; dark: 9:00 PM-5:00 AM), and analysis was done on these mean light and dark values. Although rats are nocturnal animals, they do have periods of activity during the light phase. Hence, for each variable, it was also possible to compare Ve and VO2 between the light and dark phases for 15-min epochs of the same level of high activity (see RESULTS, Table 2). At least four such epochs, each comprising >500 breaths, were analyzed during the light and dark phases in all rats.

Values are presented as means ± SE. Data were compared by two-tailed paired t-test or two-way repeated-measures ANOVA followed by post hoc limitations (Bonferroni's) to compare values in CO2 to the corresponding air values and, for each FiCO2, to compare variables at different times of the day, namely 11:00 AM vs. 11:00 PM in the acute experiments and light vs. dark in the chronic experiments. In all cases, a significant difference was defined at P < 0.05.

RESULTS

Acute measurements. Table 1 provides the values of all variables measured in air at 11:00 AM and 11:00 PM. Ve, VO2, and Tb were all significantly higher at 11:00 PM compared with the corresponding morning value. Although breathing rate was similar at both times, there was a small but significant decrease in Tr/TTot at 11:00 PM compared with 11:00 AM (0.39 vs. 0.36, P < 0.05). Ve and VO2 rose proportionately from 11:00 AM to 11:00 PM (~14%), and Ve/VO2 at 11:00 PM was, therefore, unchanged from the 11:00 AM value.

Increased FiCO2, whether 2 or 5%, had similar effects at 11:00 AM and 11:00 PM on each of the variables considered relative to its normocapnic value (Fig. 1). Tb dropped significantly (~0.5°C) with the 5% but not the 2% CO2 exposure. In 2% CO2, the hyperventilatory response (increase in Ve/VO2, Fig. 1) was achieved solely by hyperpnea (~30% increase in Ve), whereas in 5% CO2 a slight hypometabolism (~8% drop in VO2) in addition to the hyperpnea (approximately +95%) contributed to the Ve/VO2 response. These response patterns occurred at both 11:00 AM and 11:00 PM, and the degree of hyperventilation, expressed as the percentage of the normocapnic Ve/VO2, was similar between 11:00 AM and 11:00 PM for 2 and 5% CO2. Both concentrations of CO2 increased Tr/TTot, and the extent of the increase was similar at 11:00 AM and 11:00 PM (~6 and 17% for 2 and 5% CO2, respectively).

Chronic measurements. The daily patterns of all variables during the days in air and in hypercapnia are presented in Fig. 2. In air, the level of each variable was lower during the light phase but started to climb toward the higher dark phase level before the lights were switched off (Fig. 3, open symbols). The average normocapnic values for the central 8 h of the light and dark phases are indicated in Table 2, top. Ve was higher in the dark phase (+27 ± 2%) due to increases in both Vt (+17 ± 1%) and f (+9 ± 1%). Also, Vo2 was increased in the dark, but disproportionately less so than Ve (+15 ± 0.5%), such that their ratio, Ve/VO2, was significantly higher in the dark compared with the light phase by ~11%. The same patterns were apparent when the light-dark difference in activity was excluded as a variable by comparing the light and dark phases at the same level of very high activity (Table 2, bottom).

During chronic hypercapnia, the daily patterns resembled those in air, with significantly higher values in the dark phase (Figs. 2 and 3, closed symbols). In

Fig. 1. Responses to acute hypercapnic exposure. Symbols indicate means from 16 conscious rats studied both at 11:00 AM (○) and at 11:00 PM (●), bars represent ± SE. Responses to increased fractional concentration of inspired CO2 (FiCO2; 2 and 5% CO2 in normoxia) are represented as the difference from normocapnia (body temperature) or as the percent of the corresponding normocapnic value (all other variables). #Significant difference between air and hypercapnia (P < 0.05, repeated-measurements ANOVA with 6 post hoc Bonferroni limitations). None of the values differed significantly between 11:00 AM and 11:00 PM.

Table 1. Normocapnic ventilatory, metabolic, and body temperature data for the 16 rats of the acute experiments

<table>
<thead>
<tr>
<th>Variable</th>
<th>11 AM</th>
<th>11 PM</th>
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<tbody>
<tr>
<td>Ve, ml/kg</td>
<td>680 ± 27</td>
<td>761 ± 21*</td>
</tr>
<tr>
<td>Vt, ml/kg</td>
<td>5.4 ± 0.2</td>
<td>6.0 ± 0.2*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>126 ± 3</td>
<td>130 ± 4</td>
</tr>
<tr>
<td>Ve/VO2</td>
<td>32 ± 1.4</td>
<td>31.1</td>
</tr>
<tr>
<td>VO2, ml/kg⁻¹·min⁻¹</td>
<td>21 ± 0.4</td>
<td>24 ± 0.6*</td>
</tr>
<tr>
<td>Tb, °C</td>
<td>37.1 ± 0.2</td>
<td>37.8 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ve, pulmonary ventilation; Vt, tidal volume; f, breathing frequency; VO2, oxygen consumption; Tb, body temperature. *Significant difference between 11 AM and 11 PM (P < 0.05, 2-tailed paired t-test).
CO2, activity levels during both phases were as in air. The light phase values for T\textsubscript{b} were similar in air and hypercapnia, whereas the dark phase values were slightly but significantly higher (\sim -0.1°C) in CO2. During both the light and dark phases, V\textsubscript{E} in hypercapnia was significantly higher than during the corresponding phase in air (+107 ± 16 and +203 ± 21 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} in light and dark, respectively), and this was contributed to by increases in both V\textsubscript{T} and f. During the light phase, the hyperpnea was accompanied by a significantly reduced V\textsubscript{O2} (\sim -3 ± 1 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}). The absolute increase in V\textsubscript{E}/V\textsubscript{O2} was significant for both phases (+9 ± 1 and +9.5 ± 1 in light and dark, respectively; Fig. 4A), and the degree of hyperventilation (\sim -32% increase in V\textsubscript{E}/V\textsubscript{O2}) was essentially the same at all times of the day (Fig. 4B).

When expressed as the percent change from the corresponding air value, the hypercapnic hyperpnea was higher in the dark (+16 ± 2% in light vs. +23 ± 2% in dark; Fig. 5A). However, the lower hyperpneic response in the light was accompanied by a drop in V\textsubscript{O2} such that the hyperventilatory response, expressed as the percent increase in V\textsubscript{E}/V\textsubscript{O2} from the normocapnic value, was the same during the light and dark phases (Fig. 5A).

When the analysis was restricted to the same level of high activity in the light and dark phases (Fig. 5B), significant changes in V\textsubscript{O2} with hypercapnia were no
longer apparent in the light phase. The hyperpneic responses became similar between the light and dark phases (H11001 24% H11006 5 in light vs. H11001 25 H11006 5% in dark), due primarily to a rise in the response during the light phase from that observed when activity was low. Consequently, the degree of hyperventilation (% increase in V˙E/V˙O2) remained similar between the light and dark phases.

DISCUSSION

The major finding of this study was that the hypercapnic hyperventilatory response, defined as the percentage increase in V˙E/V˙O2, was similar throughout the day, whether the CO2 exposure was acute or prolonged and whether activity varied between the light and dark phases.

Considerations of methodology. The barometric method is ideally suited for circadian studies because of its noninvasive nature. However, the technique has potential errors, mostly related to the assumption that the heat and humidity added to the inspired air are fully recovered during expiration. In fact, expired air more closely approaches nasal rather than chamber conditions, depending on nasal temperature, the temperature and humidity of the chamber, and Tl/TTot. Overlooking these factors could lead to a substantial underestimation of VT that worsens as Tl/TTot and/or the expired temperature at the nares increases (9, 14). The question is whether these factors could have affected the daily pattern of hypercapnic hyperventilation. Assuming that nasal temperature maintains its proportionality to Tb throughout the day and using the values of Tl/TTot measured in the acute experiments, VT may be underestimated by as much as ~20% in the light and ~23% in the dark phase; that is, the error remains similar throughout the day. This argument applies equally to the air and hypercapnic conditions, because the Tb oscillation was similar, and Tl/TTot, although slightly higher in CO2, retained the light-dark relationship that it had in air. Hence, it seems unlikely that technicalities related to the barometric method need to be considered when interpreting the daily pattern of the hypercapnic V˙E response.

Circadian pattern of chemosensitivity. In normocapnia, the daily fluctuations in Tb, V˙O2, and V˙E were similar to what we observed previously in adult rats under synchronized conditions (23, 32). Throughout the prolonged CO2 exposure, V˙E continued to oscillate,
Table 2. Normocapnic data for the 7 rats of the chronic experiments

<table>
<thead>
<tr>
<th></th>
<th>Light Phase</th>
<th>Dark Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity, counts/2 min</td>
<td>3.72 ± 0.2</td>
<td>8.76 ± 0.3*</td>
</tr>
<tr>
<td>V̇E, ml·kg⁻¹·min⁻¹</td>
<td>698 ± 12</td>
<td>889 ± 13*</td>
</tr>
<tr>
<td>V̇R, ml/kg</td>
<td>5.5 ± 0.09</td>
<td>6.5 ± 0.1*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>126 ± 3</td>
<td>137 ± 3*</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>24.7 ± 0.6</td>
<td>28.5 ± 0.8*</td>
</tr>
<tr>
<td>V̇E/V̇O₂</td>
<td>28.6 ± 0.8</td>
<td>31.8 ± 1.2*</td>
</tr>
<tr>
<td>Tb, °C</td>
<td>37.2 ± 0.06</td>
<td>37.9 ± 0.05*</td>
</tr>
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</table>

Iso-Activity

<table>
<thead>
<tr>
<th></th>
<th>Light Phase</th>
<th>Dark Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity, counts/2 min</td>
<td>12.4 ± 0.6</td>
<td>12.4 ± 0.7</td>
</tr>
<tr>
<td>V̇E, ml·kg⁻¹·min⁻¹</td>
<td>753 ± 17</td>
<td>921 ± 26*</td>
</tr>
<tr>
<td>V̇R, ml/kg</td>
<td>6.0 ± 0.2</td>
<td>6.6 ± 0.3*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>127 ± 4</td>
<td>140 ± 3*</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>26.6 ± 1.0</td>
<td>29.3 ± 1.1*</td>
</tr>
<tr>
<td>V̇E/V̇O₂</td>
<td>28.5 ± 1.5</td>
<td>31.3 ± 2.2</td>
</tr>
<tr>
<td>Tb, °C</td>
<td>37.4 ± 0.06</td>
<td>37.8 ± 0.08*</td>
</tr>
</tbody>
</table>

Values are means ± SE of the middle 8 h in each phase, light and dark phase, 7 AM to 7 PM and 7 PM to 7 AM, respectively. Isoactivity, data were compared between the light and dark phases for 15-min epochs of the same level of activity. *Significant difference between light and dark phases (P < 0.05, repeated-measures ANOVA with 4 post hoc Bonferroni limitations).

but at a level exceeding that in normocapnia. Although the absolute increase in V̇E was greater in the dark phase, there was a small drop in V̇O₂ during the light phase such that the V̇E/V̇O₂ response, expressed as the percent increase from the normocapnic level, showed no systematic variation throughout the day. After constraining the analysis to epochs of the same level of activity during the light and dark phases, the light-dark differences in V̇O₂ and V̇E were no longer apparent, and the V̇E/V̇O₂ response remained similar between the two phases. Finally, the V̇E/V̇O₂ response to acute hypercapnia was similar between 11:00 AM and 11:00 PM, whether it was achieved by hyperpnea alone during the 2% exposure or in combination with hypometabolism during 5% CO₂. These results support the notion that the gain of the hypercapnic V̇E response was adjusted to the metabolic level, as has been found under other conditions of changed metabolism such as exercise and cold-induced thermogenesis (20). The mechanisms that control the coupling between metabolism and V̇E are unknown, although evidence points to a role for the metabolically produced CO₂ (22, 27).

Unlike hypoxia, where V̇O₂ either drops or changes little depending on the normoxic thermogenic status, the metabolic effects of moderate concentrations of hypercapnia (2–5%) are mixed and unpredictable (20). The latter may be related to the relative degree of sympathoadrenal activation and acidemia elicited by hypercapnia, as these effects would have opposing actions on V̇O₂ (24). We found a drop in V̇O₂ with hypercapnia in the light phase of the chronic exposure, when activity was low and, similar to Peever and Stephenson (25), during the acute experiments when rats were quiet. Hence, during hypercapnia, relatively low levels of stress may favor a drop in V̇O₂, whereas more stressful situations, such as when animals were active in the present study or restrained (15, 31), could mask a hypometabolic effect or lead to an increase in V̇O₂.

In humans studied under 40 h of constant ambient conditions and wakefulness, V̇E per unit change in end-tidal CO₂, assessed every few hours by rebreathing, followed a circadian pattern (33, 34). Different
from rats, this occurred either in the absence of any circadian variation in metabolic rate (34) or was poorly correlated to the metabolism oscillation (33). Differences in the frequency and method of testing the acute CO2 response are considerations when comparing the results in rats and humans. Because circadian rhythms may be irregularly shaped and superimposed by ultradian oscillations, the brief intermittent measurements used in both species to evaluate the acute response may give misleading results concerning the circadian pattern. The rebreathing method carries with it the possibility that brain tissue and end-tidal PCO2 do not change together. However, theoretical considerations suggest that their relationship is linear and insensitive to changes in cerebral blood flow when rebreathing is initiated with 7% inspired CO2 (28), as was done in the human studies. Hence, it seems unlikely that circadian alterations in cerebral blood flow (4, 8) are at the basis of the circadian pattern in acute hypercapnic Ve response in humans. Another consideration is related to a possible time-of-day effect of the acidemia accompanying the hypercapnia; acidemia is likely to have been little compensated during the rebreathing trials in humans, but it is likely to have been better compensated in the rats after 30 min of CO2 breathing. This view would predict that the circadian pattern in Ve chemosensitivity would no longer be apparent in humans with prolonged hypercapnia, such as in patients who retain CO2.

Factors affecting the hypercapnic hyperventilatory response. Hypercapnia causes a multitude of changes that can secondarily affect the Ve response, such as acidosis, a reduction in Tb, and enhanced catecholamine release. The magnitude of these effects depends on the severity of the hypercapnia (15, 16, 31), and their recovery during a prolonged exposure can occur with different time courses (15). Therefore, it was of interest to compare the Ve/Vo2 response at different times of the day for different CO2 concentrations and durations of exposure. We found a similar time of day Ve/Vo2 response irrespective of both the CO2 concentration and the duration of the exposure. This suggests that the acidemia, which would have been greater with the 5% than with the 2% acute CO2 exposure, and the compensatory mechanisms operating to return arterial pH toward the normal value during prolonged CO2 breathing did not alter the daily pattern of the hyperventilatory response.

Sleep is known to be accompanied by a reduced Ve sensitivity to CO2, at least in humans, dogs, and cats (26). Hence, it would not have been surprising to find a lower Ve/Vo2 response during the light phase, when most of the sleep in rats occurs. In fact, we did not find a difference in the hypercapnic hyperventilatory response between the light and dark phases, suggesting that sleep did not blunt the response. The Ve/Vo2 response was also similar between the light and dark phases when compared at the same level of high activity, further supporting the notion that the hypercapnic Ve sensitivity was not influenced by the state of arousal. Why rats should differ in this regard from humans and other larger species is not clear. One possibility relates to differences in the daily organization of sleep-wake behavior; the rest phase in rats, and presumably in other small species, is characterized by alternating bouts of sleep and activity, whereas humans typically sleep in one continuous episode.

A blunting effect of low Tb on CO2 chemosensitivity has been previously suggested, with a central, via the brain stem or hypothalamus (2, 5, 18), rather than a peripheral site of action (13). A reduction in Tb has been associated with a decrease in not only the hyperpneic response (17) but also in the Ve/Vo2 response (21, 31). The similarity in the Ve/Vo2 response throughout the day in the present study therefore suggests that circadian variations in Tb played an irrelevant role in determining the daily pattern of Ve sensitivity to hypercapnia. The same conclusion was reached in human subjects, based on a substantial (~6 h) phase difference between the circadian rhythms of hypercapnic Ve sensitivity and Tb (33). It is possible that the circadian changes in Tb were too small to have an appreciable effect on the hypercapnic response. Yet, it is noteworthy that, in rats, a modest (~1°C) fall in Tb was associated with an ~30% decrease in the Ve/Vo2 response to 4% CO2 (21). The mechanisms governing the Tb change may be a factor. Circadian Tb oscillations involve changes in the thermoregulatory set point (29), whereas the Tb changes in the above-mentioned studies likely represent deviations from the set point. To better evaluate a potential influence of the circadian Tb changes on hypercapnic Ve responsiveness, the daily Ve/Vo2 pattern during CO2 breathing could be compared among animals with very different Tb oscillations.

In conclusion, the hyperventilatory response to CO2 in rats was similar throughout the day, whether the hypercapnic exposure was acute or chronic. This result was achieved through a degree of hyperpnea that was perfectly adjusted to the metabolic level at a given time of the day. Hence, the advantage of a biological clock and the oscillations of numerous physiological variables do not appear to pose a limit on the rat’s ability to respond to hypercapnic challenges.

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