Ambient temperature modulates hypoxic-induced changes in rat body temperature and activity differentially

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Received 5 June 2000; accepted in final form 5 December 2000

Bishop, B., G. Silva, J. Krasney, H. Nakano, A. Roberts, G. Farkas, D. Rifkin, and D. Shucard. Ambient temperature modulates hypoxic-induced changes in rat body temperature and activity differentially. Am J Physiol Regulatory Integrative Comp Physiol 280: R1190–R1196, 2001.—When rats, acclimated to an ambient temperature (T a) of 29°C, are exposed to 10% O2 for 63 h, the circadian rhythms of body temperature (T b) and level of activity (L a) are abolished, T b falls to a hypothermic nadir followed by a climb to a hyperthermic peak, L a remains depressed (Bishop B, Silva G, Krasney J, Salloum A, Roberts A, Nakano H, Shucard D, Rifkin D, and Farkas G. Am J Physiol Regulatory Integrative Comp Physiol 279: R1378–R1389, 2000), and overt brain pathology is detected (Krasney JA, Farkas G, Shucard DW, Salloum AC, Silva G, Roberts A, Rifkin D, Bishop B, and Rubio A. Soc Neurosci Abstr 25: 581, 1999). To determine the role of T a in these hypoxic-induced responses, T a and L a data were detected by telemetry every 15 min for 48 h on air, followed by 63 h on 10% O2 from rats acclimated to 25 or 21°C. Magnitudes and rates of decline in T a after onset of hypoxia were inversely proportional to T a, whereas magnitudes and rates of T b climb after the hypothermic nadir were directly proportional to T a. No hyperthermia, so prominent at 29°C, occurred at 25 or 21°C. The hypoxic depression of L a was least at 21°C and persisted throughout the hypoxia. In contrast, T a was a strong determinant of the magnitudes and time courses of the initial fall and subsequent rise in T b. We propose that the absence of hyperthermia at 21 and 25°C as well as a persisting hypothermia may protect the brain from overt pathology.

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hypothermic and hyperthermic components of the hypoxic-induced responses might be influenced by the Ta. For example, if the hypothermic component was smaller and the hyperthermic component was larger at 29°C than at 25 or 21°C, these differences could well contribute to the greater brain damage at 29°C. Thus the objective of the present study was to determine the effect of Ta on the magnitude and time course of the changes in Tb and La during 63 h of 10% O2.

**METHODS**

**Animal Preparation and Protocols**

Our methods have been described in detail previously (2). Mini Mitter probes with diameters of 10 mm were calibrated for temperature, sterilized, and surgically implanted aseptically in the abdomen of 32 adult Sprague-Dawley rats for continuous detection of Tb and La. Upon recovery from surgical anesthesia, each rat was placed in its own cage in a sound-proof, all-weather room. The temperature and lighting of the room were tightly controlled. For the two protocols used in the present experiments, the Ta was maintained at either 21 or 25°C. The automated lighting system switched from dark to light every 12 h (at 0600 and 1800) throughout the 159 h of each experimental protocol.

After acclimation to the dark-light cycles and the selected Ta, the body weight was obtained from each animal before and after the 63-h exposure to hypoxia.

**Instrumentation**

A receiver for detecting the Tb and La signals from the implanted probes was placed under the cage of each rat. Hypoxic rats were placed in cages within an airtight large plastic chamber through which either room air or a 10% O2 in N2 gas mixture was delivered at a flow rate of 5 l/min, a rate at which the percentage of O2 in the chamber was maintained at 10% by opening a nitrogen flood valve. When the percentage of O2 within the chamber approached the 10% level, preset valves were opened, and a continuous flow of the hypoxic mixture was delivered to the chamber for 63 h. This reduction in chamber gas to 10% O2 took ~35-40 min. Hypoxia was always initiated at 1800, when both Tb and La were rising toward the nocturnal peaks of their circadian cycles. At termination of the 63-h exposure to hypoxia, the gas valves were closed and the chamber was opened to room air. Hence, return to air breathing was essentially instantaneous.

**Data Acquisition**

The temperature-specific frequency and the instantaneous position of each animal were acquired continuously throughout the 159 h of an experiment from the Mini Mitter probes. These data were relayed to a computer in an adjacent room through a multiplexer for reduction by a Windows-based software program (Vital View; Mini Mitter). This program was converted to “counts per 15 min” as a measure of La.

Data acquired for rats maintained at 29°C in an earlier study (2) are included in this study for comparison purposes.

**RESULTS**

**Effects of Ta on Body Weight**

Before hypoxia, the mean weight of all the rats was 392.2 ± 32.4 g. Each control rat gained weight over the 159 h of the experiment. Both the magnitude and the percent of the gain were inversely related to Ta as shown by data in Table 1. In contrast, over the same period of time, the hypoxic-exposed rats lost 10% of their body weight from that of the control rats. Before hypoxia, the mean weight of all the rats was 392.2 ± 32.4 g. Each control rat gained weight over the 159 h of the experiment. Both the magnitude and the percent of the gain were inversely related to Ta as shown by data in Table 1. In contrast, over the same period of time, the hypoxic-exposed rats lost 10% of their body weight from that of the control rats. Before hypoxia, the mean weight of all the rats was 392.2 ± 32.4 g. Each control rat gained weight over the 159 h of the experiment. Both the magnitude and the percent of the gain were inversely related to Ta as shown by data in Table 1. In contrast, over the same period of time, the hypoxic-exposed rats lost 10% of their body weight from that of the control rats.

**Table 1. Body weights, changes in body weight, and %change in body weight of 8 control rats acclimated to 12:12-h dark-light cycles at ambient temperatures of 29, 25, and 21°C and of 6 rats before and after 63 h of exposure to 10% hypoxia**

<table>
<thead>
<tr>
<th>Group</th>
<th>Prehypoxia</th>
<th>Posthypoxia</th>
<th>Change in Body Wt</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>29°C</td>
<td>381.2 ± 38.4</td>
<td>390.8 ± 32.4</td>
<td>9.7</td>
<td>+3</td>
</tr>
<tr>
<td></td>
<td>403.1 ± 32.4</td>
<td>361.6 ± 31.6</td>
<td>-41.5*</td>
<td>-10*</td>
</tr>
<tr>
<td>25°C</td>
<td>359.4 ± 68.0</td>
<td>375.0 ± 69.0</td>
<td>15.6</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>360.4 ± 82.4</td>
<td>322.7 ± 83.3</td>
<td>-37.7*</td>
<td>-10*</td>
</tr>
<tr>
<td>21°C</td>
<td>309.6 ± 32.3</td>
<td>330.0 ± 28.4</td>
<td>20.4</td>
<td>+7</td>
</tr>
<tr>
<td></td>
<td>328.7 ± 20.9</td>
<td>294.4 ± 18.5</td>
<td>-34.3*</td>
<td>-10*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Units are g. *Difference in the mean body weight of the hypoxic rats from that of the control rats was statistically significant at P < 0.001 (P values for Student’s t-test between groups).
their body weight regardless of the $T_a$. The weight loss of the hypoxic group in absolute grams was greatest at 29°C and least at 21°C (Table 1).

**Effects of $T_a$ on $T_b$**

**Phase I of the $T_b$ response to hypoxia.** Before hypoxia, the differences among the means of $T_b$ at $T_a$ values of 21, 25, and 29°C were not significantly different. The 10% $O_2$ was introduced in the rat’s chamber at a time when $T_b$ was rising toward its nocturnal peak. $T_b$ immediately ceased climbing and instead declined over the next hour or two toward a marked hypothermia (Fig. 1). This decline in $T_b$ to the nadir constituted phase I of the hypoxic-induced response and occurred whether $T_a$ was 21, 25, or 29°C (Fig. 1, top, and Fig. 2). The $T_b$ at the nadir was significantly lower than the prehypoxic $T_b$, regardless of the $T_a$. However, the magnitude of the decline in phase I (i.e., the difference between $T_b$ at the onset of hypoxia and $T_b$ at the nadir) was dependent upon $T_a$. At 21°C the decline was more than two times that at 25°C and more than fivefold that at 29°C. The duration of the hypoxic-induced hypothermia also depended on $T_a$. At 21°C it was 1.1 h longer than at 25°C and 2.2 h longer than at 29°C (Table 2 and Figs. 1 and 2). During phase I, the rate of decline in $T_b$ ($dT_b/dt$ in °C/h) was fastest at 21°C and slowest at 29°C. Of all the parameters of phase I, the times required to reach the nadir (i.e., the duration of phase I in Table 2) were least influenced by $T_a$ (Fig. 2). Nonetheless, except for the absolute $T_b$ at the nadir, all
other phase I parameters of the hypoxic-induced response were significantly less at 29°C than at 25 or at 21°C. At the latter T_a, the hypothermia was most severe, the fall in T_b was fastest, and the duration of the hypothermia was longest when compared with the two higher T_a values (Table 2 and Figs. 1 and 2).

**Effects of T_a on Mean T_b**

The mean T_b of control rats at 21 and 29°C was 37.5°C and 37.6°C at 25°C (Fig. 3). The mean T_b in the hypoxic rats during the 63 h of exposure to hypoxia was very dependent on T_a. Compared with the controls at the same T_a, the means for T_b of the hypoxic rats at 21 and 25°C were significantly reduced (P < 0.001). At 29°C, the mean T_b of the hypoxic rats was actually 0.1°C above that of the controls, a difference with a P value <0.05.

**Phase II of the Hypoxic-Induced Response**

The onset of phase II was marked by a progressive climb of T_b from its hypothermic nadir toward its hypoxia and the mean T_b over the full 63 h of hypoxia at T_a values of 21, 25, or 29°C. *Significant differences among the T_b values at the 29°C, the mean T_b of the hypoxic rats was actually 37.5°C and 37.6°C at 25°C (Fig. 3). The mean T_b in the two higher T_a values (Table 2 and Figs. 1 and 2).

**Effects of T_a on the Mean L_a and on the Hypoxic-Induced Responses of L_a**

L_a means at the three T_a values. The mean L_a for control rats and the hypoxic rats at each T_a is shown in Fig. 4. The mean L_a values for both groups show wide variability at each T_a. The mean L_a values for the control rats at 21 and 25°C were not different. The mean L_a at 29°C was significantly lower than at the other T_a values. The mean L_a values for the hypoxic groups decreased with each increase in T_a. For example, the mean L_a decreased from 39.5 to 30.5 and to 16.9 counts/15 min with T_a increases from 21 to 25 and to 29°C, respectively.

**Phase III of the Hypoxic-Induced Response**

In phase III, the interval between the change in the rising slope of T_b and the termination of the hypoxia, T_b continued to climb throughout the remaining 36 h of phase III at a T_a of 21 or 25°C, but this rise was at a slower and more variable rate compared with that during phase II (Fig. 1). In contrast, as reported previously (2) when T_a was 29°C, T_b declined from its hyperthermic peak during phase III. At the end of phase III, when hypoxia was terminated, T_b was not different across the three T_a values and circadian rhythm of T_b was reestablished.

**Table 2. Phase I parameters of the hypoxic-induced response of T_b at the three T_a**

<table>
<thead>
<tr>
<th>T_a, °C</th>
<th>n</th>
<th>Mean Prehypoxic T_b, °C</th>
<th>T_b at Nadir, °C</th>
<th>Magnitude of Decline, °C</th>
<th>Duration of Phase I, h</th>
<th>Rate of T_b Fall (dT_b/dt), °C/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>5</td>
<td>37.5 ± 0.3</td>
<td>36.4 ± 0.5*</td>
<td>−1.1 ± 0.3</td>
<td>3.5 ± 0.7</td>
<td>−0.50 ± 0.34</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>37.5 ± 0.2</td>
<td>35.3 ± 0.5*</td>
<td>−2.3 ± 0.7</td>
<td>4.6 ± 0.5</td>
<td>−0.91 ± 0.13</td>
</tr>
<tr>
<td>21</td>
<td>6</td>
<td>38.8 ± 0.3</td>
<td>32.6 ± 0.7*</td>
<td>−5.6 ± 0.8</td>
<td>5.7 ± 0.9</td>
<td>−1.86 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. T_b, body temperature; T_a, ambient temperature. *T_b at the nadir is significantly lower (P < 0.01) than during the prehypoxic period.

**Table 3. T_b phase II parameters of the hypoxic-induced responses at the three T_a**

<table>
<thead>
<tr>
<th>T_a, °C</th>
<th>Peak T_b in Phase II, °C</th>
<th>Rate of Rise in T_b, °C/h</th>
<th>Duration of Phase II, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>38.7 ± 0.6*</td>
<td>+0.10 ± 0.04</td>
<td>−26.0</td>
</tr>
<tr>
<td>25</td>
<td>37.1 ± 0.4*</td>
<td>+0.09 ± 0.02</td>
<td>−22.5</td>
</tr>
<tr>
<td>21</td>
<td>35.1 ± 0.4*</td>
<td>+0.14 ± 0.03</td>
<td>−22.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Difference between T_b at the nadir and at the end of phase II is significant (P < 0.01).
and remained at a low level compared with its prehypoxic level throughout the hypoxia. During the hypothermia of the T_b response (i.e., phase I), the differences among L_a values across the three T_a values were not statistically significant. In other words, the initial hypoxic depression of L_a was independent of T_a. 

21°C. At 21°C during phase I of T_b, L_a fell from a mean prehypoxic level of ~50 counts/15 min (100%) to <25 counts/15 min (i.e., a 40–50% decrease in counts) at its most depressed period. Toward the end of phase II, L_a gradually climbed toward its prehypoxic mean level. During phase III, L_a values were not different from their prehypoxic values, and the circadian rhythm of L_a had reappeared. In other words, at 21°C the effect of hypoxia on L_a was considerably less than when the rats were at 25 or 29°C.

25°C. At 25°C, with the onset of hypoxia, L_a declined over the first 6 h from 50 to ~15 counts/15 min. Although L_a remained depressed throughout phase II of the T_b response, low-level activity and low-level circadian oscillations in phase with the dark-light cycles gradually reappeared during phase III of the T_b response (Fig. 1). The magnitude of the cyclic activity (i.e., the full excursion of the cycle) was only 50% or less compared with that recorded in the prehypoxic period (Fig. 1).

The hypoxic-induced response of T_b at 29°C. As reported previously (2), within 7 h of the onset of hypoxia, activity counts decreased from ~50 to 7 counts/15 min (i.e., close to quiescence). During phases II and III of the hypoxic-induced response to T_b, L_a counts remained significantly lower than those during the prehypoxic period. After ~25 h (i.e., close to the onset of phase III of the T_b response), L_a increased somewhat but still remained suppressed compared with its prehypoxic level (Fig. 1). Throughout the entire 63 h of hypoxia at 29°C, L_a was essentially devoid of circadian rhythm.

**DISCUSSION**

**Previous and New Findings Regarding Hypoxic Responses**

The results of this study are an extension of our previous study (2) in which we reported for the first time that rats, acclimated to 29°C, had a delayed hyperthermia in response to a prolonged hypoxia. The early hyperthermia at the onset of hypoxia confirmed previous findings reported by numerous investigators (4, 13–15, 20), but the delayed hyperthermia was a new finding, missed by others because their animals were exposed to bouts of hypoxia too short to reveal the full expression of the T_b hypoxic-induced response. The hypothermic component of the hypoxic response of T_b is thought to reflect a suppression of the thermal regulatory system (7), to be neuroprotective (3), and to override the thermogenesis generated by the hypoxia-induced increase in ventilation (20). The results of the current study have revealed that T_a is an important determinant of the magnitude and time course of the hypoxic-induced T_b response to a prolonged (63 h) hypoxia. The major new findings of this study are that rats, acclimated to 25 or 21°C, responded immediately to hypoxia with the expected decline in T_b that was followed by a progressive climb in T_b. However, unlike rats acclimated to 29°C, none of the rats acclimated to 25 or 21°C exhibited hyperthermia. The progressive rise in T_b never reached the prehypoxic nocturnal peak at any time during the exposure to hypoxia. Nonetheless, the fact that T_b climbed from its hypothermic nadir suggests that the thermoregulatory events that caused the hypothermia waned or were counteracted by competing events, or both. Another key finding was that T_a exerted differential affects on the T_b and L_a responses to hypoxia.

**Thermoregulation During Hypoxia**

Adult rats are excellent thermoregulators, whereas newborn rats lack both well-developed thermoregulatory and locomotor abilities. For the hypoxic-induced hypothermia to occur, heat loss must exceed heat production. The major heat-loss mechanisms in rats are vasodilation of their feet and furless tails and an increase in evaporative water loss produced by licking and grooming after applying saliva to their pelts (7). Behavioral thermoregulation is a major component of the rat’s thermoregulatory armamentarium (8). When the environment provides a thermal gradient, rats select a higher T_a when their T_b is low and vice versa, as if their thermal regulation were geared to reduce the circadian oscillations in T_b (18). Behavioral regulatory responses were not an option in the present study since the T_a was preselected and tightly controlled.

For T_b to rise during phase II, heat conservation and heat production must have overcome or replaced heat-loss mechanisms. Heat production is qualitatively similar in animals acclimated to temperatures from 23 to 6°C (7). Among the major heat-production mechanisms are vasoconstriction and shivering. Hypoxia is a potent
stimulus for the release of catecholamines, which provoke thermogenesis (11).

Role of Ta in the Hypoxic-Induced Response

A Ta of 29°C falls within a rat’s thermal neutral zone (7). Hence, minimal values of heat production and heat loss are required to maintain a constant Tb. The thermal gradient between a Ta of 29°C and Tb is smaller than that at a Ta of 25 or 21°C and may be a limiting factor in the animal’s ability to lose heat (5, 7, 9). Nonetheless, a Ta of 29°C is reputed to be optimal for the ventilatory, circulatory, thermoregulatory, metabolic, and other vital control centers. However, in comparison with 25 or 21°C, a Ta of 29°C may be devastating for homeostasis during hypoxia, as suggested by the characteristics of the hypoxic-induced Tb response. It has been claimed that the hypothermia in phase I of the hypoxic response is protective in that it overrides the thermogenesis generated by the hypoxic-induced increase in ventilation (20, 22). At 29°C the magnitude and duration of the phase I hypothermia are significantly less than at 25 or 21°C (Table 2 and Fig. 2). Furthermore, heat dissipation to the environment, whether by way of conductive, radiative, or convective heat exchange, is considerably less than would be the case at 25 or 21°C (21).

In phase II of the 29°C response, Tb transiently climbed above the nocturnal peak Tb. This hyperthermic component of the hypoxic response is likely maladaptive and selectively deleterious to some neuronal populations. Subdural hematomas, intraventricular hemorrhages, and other signs of overt brain pathology were seen in posthypoxic 29°C brains but not in the 25 or 21°C brains that experienced no hyperthermia (12). The differences among the Tb, responses to hypoxia and the existence of brain pathology seen at different Ta values revealed, for the first time, that Ta plays a critical role in determining 1) the profile of the hypoxic-induced Tb response, 2) the output of the thermoregulatory system, and 3) the pathological effects hypoxia exert on the brain.

Hypoxia Disrupts Circadian Control

The circadian rhythms of both Tb and La were totally disrupted at the onset of hypoxia in every rat at each Ta as if the circadian control system were totally inhibited. At 25 and 21°C, but not at 29°C, a very low level of Tb circadian cycling reappeared in phase III. Failure of the rhythm to reappear at 29°C suggests that at the warmer temperature some mechanisms were in play to sustain the circadian disruption.

The circadian clock’s location, neural composition, and molecular mechanisms underlying its rhythmicity have been elucidated at the molecular level in recent years (18, 19). Nonetheless, an understanding of the circadian clock’s intracircuitry and the interconnections it makes with the thermoregulatory, ventilatory, cardiovascular, and physiological control systems remain to be elucidated. Perhaps then the Tb and La responses to hypoxia will be understood. Feedback circuits between the brain regions involved in thermal regulation and the suprachiasmatic nucleus, the site of the biological clock’s control center, remain to be identified.

Ta Modification of Hypoxic-Induced Responses of La

Onset of hypoxia, at a time when La was rising toward its normal nocturnal peak, caused La to reverse and undergo a progressive decline. This change in La did not just interrupt the circadian rhythm of La but abolished it (Fig. 1, bottom). The reduction in La was not secondary to the decline in Tb. It more likely was due to a specific centrally driven suppression of motor activity. By the time Tb had reached its hypothermic nadir (i.e., the onset of phase II), La was also at its lowest level regardless of the Ta. The depression of La was less severe and less enduring at 25 and 21°C than at 29°C. Before the termination of the prolonged hypoxia, the La circadian rhythm gradually reappeared at a low level when rats were at Ta values of 25 or 21°C but not when at 29°C.

Other Potential Consequences of Hypoxia

Poncet et al. (17) have demonstrated that sustained hypoxia disrupts the circadian rhythms for central neurotransmitters. It remains to be determined whether the impact of hypoxia on the circadian rhythmicity of central neurotransmitters or hormones, such as melatonin, are as dependent on Ta as are the Tb and La rhythms.

Summary and Conclusions

Regardless of Ta, the responses of Tb and motor activity to hypoxia were dramatic (2, 15, 16) and independent of one another. Hypoxic exposure completely disrupted the circadian rhythms of both Tb and La whether Ta was 21, 25, or 29°C.

This study demonstrated that the depth to which Tb plunges upon exposure to hypoxia and the level to which it “recovers” or reverts toward its prehypoxic mean is critically dependent on Ta. It remains to be determined to what lower limit Tb could be driven during phase I of the hypoxic-induced response by lowering Ta below 21°C. The transitory nature of the hypoxic-induced hypothermia may maintain homeostasis until more slowly recruited adaptive mechanisms of ventilatory acclimation and increased arterial O2 capacity come into play to counter the continuing stresses imposed by hypoxia. The delayed reversal of Tb may signal a “recovery” of the circadian and thermal regulatory systems from the initial suppression or disruption imposed by the hypoxia.

The daily rhythms of Tb and La are independent of one another during normoxia (18). As revealed by this study, the hypoxic-induced responses of La and Tb are also independent of one another. Despite prolongation of hypoxia, La partially recovers at 21 and 25°C but remains depressed at 29°C when the animals are hyperthermic. It seems unlikely that hyperthermia contributes to the La depression.
Newborn infants display hypothermia during hypoxia, a putative cerebroprotective response (12). The observations reported in the present study are relevant to the question of defining the optimal T na for management of infants in the setting of clinical hypoxia. Because the hypothermic response to hypoxia is inversely related to body mass, it seems less likely that exposure to hypoxia would elicit major alterations in the T b of adult humans. Indeed, which circadian rhythms are affected by hypoxia in humans remains to be determined. Depression of L a clearly occurs during hypoxia in adult humans (11). If hypoxia depresses the circadian rhythms of T a and L a and the metabolic rate then these responses may serve as early adaptive mechanisms to cope with hypoxia in adult humans and in rats.

**Perspectives**

Regardless of the T a, T b, and L a responses to hypoxia are dramatic (2, 15). The circadian rhythms of both T b and L a are completely disrupted for the duration of the hypoxia, and both T b and L a decrease to minimums below normal nadirs. It is unknown if the depth of the decline and the peak of the climb are potentially destructive. The circadian rhythms of both T b and L a and the metabolic rate then decline and the peak of the climb are potentially destructive (2, 15). The circadian rhythms of both T b and L a are dramatically (2, 15). The circadian rhythms of both T b and L a are completely disrupted for the duration of the hypoxia, and both T b and L a decrease to minimums below normal nadirs. It is unknown if the depth of the decline and the peak of the climb are potentially destructive.

We thank Dr. David Megirian for enthusiastic encouragement and constructive criticisms throughout these experiments. We also thank Alex Salloum’s organizational skills and contributions to the implementation of the experiments and the acquisition and reduction of the data. Last, we thank the reviewers who provided constructive criticisms and excellent guidance that helped us to improve the manuscript.

**REFERENCES**