Effects of Changing Ambient Temperature on Metabolic, Heart, and Ventilation Rates during Steady State Hibernation in Golden-Mantled Ground Squirrels (*Spermophilus lateralis*)

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ABSTRACT

To determine whether metabolic rate is suppressed in a temperature-independent fashion in the golden-mantled ground squirrel during steady state hibernation, we measured body temperature and metabolic rate in ground squirrels during hibernation at different \( T_a \)'s. In addition, we attempted to determine whether heart rate, ventilation rate, and breathing patterns changed as a function of body temperature or metabolic rate. We found that metabolic rate changed with \( T_a \) as it was raised from 5\(^\circ\)C to 14\(^\circ\)C, which supports the theory that different species sustain falls in metabolic rate during hibernation in different ways. Heart rate and breathing pattern also changed with changing \( T_a \), while breathing frequency did not. That the total breathing frequency did not correlate closely with oxygen consumption or body temperature, while the breathing pattern did, raises important questions regarding the mechanisms controlling ventilation during hibernation.

Introduction

Hibernation is a strategy used by some mammals as a means of energy conservation. It is characterized by physiological changes that include drastic reductions in metabolic rate, body temperature, ventilation, and cardiac output. During entrance into hibernation, the reductions in breathing frequency, heart rate, and metabolic rate occur rapidly and in parallel, while body temperature falls slowly (Lyman 1965). Thus, cardiac, respiratory, and metabolic rates reach minimum values long before body temperature does (12 h or more; Wang and Hudson 1971). Although these changes have been thoroughly documented, the underlying mechanisms that bring about these changes remain elusive. It is currently believed that the reduction in metabolic rate during entrance into hibernation is a consequence of lowering the hypothalamic set point for body temperature regulation (Heller et al. 1977), and differences in the time course of the falls in metabolism and body temperature argue strongly that active metabolic suppression must be involved in this process (Heldmaier et al. 1993). The role of active metabolic suppression (either through temperature-dependent and/or independent mechanisms) versus the Q\(_{10}\) effects of decreasing body temperature on biochemical reactions during steady state hibernation, however, is not so clear (Snapp and Heller 1981; Malan 1987; Snyder and Nestler 1990; Geiser 1993; Heldmaier et al. 1993; Storey 1997). It has been argued that levels of metabolism in steady state hibernation could be explained by Q\(_{10}\) effects alone once body temperature had fallen to minimum levels (Snapp and Heller 1981; Geiser 1988). In contrast, however, several investigators have shown that metabolic rates remain constant in steady state hibernation, although body temperatures may range from roughly 0\(^\circ\)C to 18\(^\circ\)C: arctic ground squirrels (*Spermophilus parryii*; Buck and Barnes 2000), alpine marmots (*Marmota marmota*; Ortmann and Heldmaier 2000), and the echidna (*Tachyglossus aculeatus*; Nicol et al. 1992). This argues that the apparent uncoupling of metabolic rate and body temperature observed during entrance into hibernation continues throughout deep, steady state hibernation at varying temperatures in these species and that metabolic rate is actively suppressed (to minimum values) throughout the entire hibernation bout. Others have shown that metabolism does change with body temperature, however (edible dormouse (*Glis glis*; Wilz and Heldmaier 2000) and bats (*Eptesicus fuscus*; Szewczak and Jackson 1992)), suggesting that different species may initiate and sustain falls in metabolic rate during hibernation using different mechanisms.

In most mammals, changes between steady state conditions such as quiet wakefulness, slow-wave sleep, exercise, and hypothermia produce parallel changes in metabolism and body temperature. Changes in either metabolism or body temperature may precede the other during the transition between states as a function of which is the dependent and which is the independent variable. For instance, changes in metabolism will precede changes in body temperature during exercise, but the opposite
will occur during hypothermia. The levels of ventilation and heart rate primarily tend to follow the changes in metabolism during these non–steady state transitions (Mortola and Gautier 1995). During entrance into hibernation, the changes in ventilation and heart rate also follow the fall in metabolism rather than the fall in body temperature (Strumwasser 1959). In addition to the reduction in the level of total ventilation during hibernation, the pattern of breathing also changes. In the golden-mantled ground squirrel, it changes from continuous to episodic breathing, where breaths are clustered into distinct episodes followed by long pauses (McArthur and Milsom 1990). This pattern is exhibited during steady state hibernation at all body temperatures between 5° and 20°C (M. B. Zimmer and W. K. Milsom, personal observations). There is evidence that suggests that the size and length of the breathing episodes change with temperature over this range during hibernation (Hammel et al. 1968; Pajunen 1984) and that during hypothermia, changes in breathing pattern are due to changes in body temperature rather than metabolic rate (Milsom et al. 1993). Given these limited observations, one would predict that, in species where metabolic rate remains constant while body temperature fluctuates during steady state hibernation, breathing pattern should change with temperature while the net cardiac and ventilatory frequencies remain constant but that, in species where metabolic rate changes with body temperature, all variables would change simultaneously. Accordingly, this study was designed to determine whether metabolic rate was actively suppressed in a temperature-independent fashion in the golden-mantled ground squirrel during steady state hibernation, as it appears to be in the arctic ground squirrel and alpine marmot, and, if so, whether heart rate, ventilation, and breathing pattern change as a function of body temperature or metabolic rate.

Material and Methods

Animals and Housing

Golden-mantled ground squirrels (Spermophilus lateralis) were obtained from a supplier in Redding, California, and were housed in an environmental chamber (21°C, 12L:12D) in individual cages with rodent chow, supplemented with sunflower seeds and fruit throughout the summer, and water provided ad lib. In October through November, the chamber temperature was reduced 3°C/d until the chamber reached 5°C, and the light cycle was changed to 2L:22D. Squirrels naturally entered hibernation within several weeks following these changes.

Surgical Procedures

The following experiments were performed under the guidelines established by the Canadian Council for Animal Care and approved by the University of British Columbia Animal Care Committee. Before surgery, squirrels were brought into the laboratory and allowed to arouse fully from hibernation (body temperature \( T_h = 37°C \)). They were then anaesthetized with sodium pentobarbital (Somnotol, 65 mg/kg intraperitoneal), and the top of the head and two small areas on each side of the body were shaved and cleaned. Small incisions were made on each side of the abdominal wall in the areas that had the greatest movement associated with breathing, and electrodes were sutured to the underlying muscle to record respiratory impedance. Two electrodes were also sutured to the muscles of the chest wall to record heart rate (electrocardiogram [ECG]). The electrode wires were fed under the skin to the top of the head where a midline incision was made extending over the skull to the base of the neck. Electrodes were sutured to the right and left trapezius muscles to record postural tone (electromyogram [EMG]), and four screws were placed in the skull to record cortical activity (electroencephalogram [EEG]). All electrode wires were attached to a pin strip using ammonium pins. A reentrant tube was placed stereotaxically into the thalamus in order to monitor brain temperature and, along with the pin strip, cemented to the top of the skull with Grip Cement (Dentsply International) and dental acrylic (Lang Dental). All incisions were sutured using 3-O silk and cleaned using a topical antiseptic solution (Pfizer Canada). The squirrel was placed in a humidified incubator (21°C) and carefully monitored until fully recovered. The squirrel was allowed to recover from surgery for 2–3 d at room temperature before being placed back in the environmental chamber at 5°C.

Experimental Protocol

Hibernating, instrumented squirrels were handled daily, and a face mask—made from the end of a 50-mL syringe, filled with plasticine, and covered with lanolin—was tied snugly over the snout. Doing this daily accustomed the animals to this procedure and could eventually be done without arousing them from hibernation. On the day of an experiment, a face mask was placed over the snout of a hibernating squirrel \((T_h = 5°C)\), and the animal was placed in a small Plexiglas box and put in a small environmental chamber in the laboratory in which \(T_h \) could be easily manipulated (between 5° and 14°C). A pneumotach was attached to the face mask and connected to a Validyne differential pressure transducer to measure breathing frequency. The electrodes were attached to Grass amplifiers, and a thermocouple was placed in the reentrant tube. The lid of the Plexiglas box was sealed with vacuum grease, and all leads exited through the top of the box through an opening that was sealed with plasticine. Ports in each end of the box were used to establish airflow (500 mL/min) through the box. The squirrel was allowed to acclimatize to these conditions for at least several hours (usually overnight), and measurements were taken after the animal’s \( T_h \) had stabilized to one of the three study temperatures \((T_h = 5^°, 10^°, \text{or } 14°C)\). The order in which the animals were exposed to the varying temperatures was random.
Once all measurements were recorded for one given temperature, the chamber temperature was changed to a new randomly selected temperature, the animal was allowed to again stabilize at the new temperature, and data was recorded for the new temperature. If the animal aroused at any time during this procedure, the experiment was terminated and the animal was placed back into the large environmental chamber. Respiratory impedance, EEG, ECG, and EMG signals were amplified and filtered using Grass AC amplifiers, and all data were recorded continuously on chart paper and on computer using a data acquisition system (DataQ Instruments 1998; DI-250, DataQ Instruments) sampling at a rate of 167 Hz/channel.

To measure metabolic rate, the airflow through the box was stopped, and the change in the gas composition in the box was measured over time. The inlet and outlet ports were connected to 50-mL glass syringes, one filled with air, one empty. After 10–20 breathing episodes, the air in the chamber was mixed using the two syringes, and a sample of chamber gas was dried and analyzed with a medical gas analyser (Beckman OM-11 oxygen analyzer) calibrated using room air. Metabolic rate was calculated by using the following equation:

$$\dot{V}_O_2 = \frac{[(F_{1,O_2} - F_{2,O_2})/\left[1 - (1 - RQ)F_{1,O_2}\right]]V_{sub}}{time},$$

where $F_{1,O_2}$ is the oxygen fraction in the chamber at time 1, $F_{2,O_2}$ is the oxygen fraction in the chamber at time 2, RQ is assumed to be 0.7 during hibernation (see Lyman and Chatfield 1955 for review), and $V_{sub}$ is the volume of the chamber minus the volume of the squirrel. All values are reported in milliliters O$_2$ per minute stpd.

Breathing frequency was measured by respiratory impedance from chest electrodes and confirmed by monitoring inspiratory and expiratory airflow through a face mask mounted on the snout of the squirrel. Because the face mask could easily become dislodged at high temperatures, this signal was not used to quantify tidal volume but was used only to confirm measures of breathing frequency. During hibernation, the golden-mantled ground squirrels breathed episodically with breaths clustered into distinct episodes separated by long apneas. Therefore, total breathing frequency (breaths/min) was calculated by counting the number of breaths over at least six consecutive episodes. The episodic breathing pattern was quantified by examining the number of breaths within each episode (breaths/episode), the frequency of the breaths within the episode (instantaneous frequency [breaths/min]), the average length of the apnea between episodes (min) and the frequency of the episodes (episodes/h). ECG signals were used to calculate heart rate (beats/min) by counting the number of heart beats during at least six breathing episodes and during 2-min segments of six consecutive apneas.

Hibernation is generally accompanied by increased cortical (EEG) activity. We have termed these events “central neural arousals” and have analyzed our data to quantify their occurrence and the extent to which breathing episodes were associated with these events. To this end, we analyzed 100 min of data in each animal at each temperature and recorded the percentage of time a breathing episode occurred with these events and the percentage of time that these events occurred with a breathing episode. We also determined the resting, background levels of EEG and EMG activity at each temperature. We averaged the integrated EEG and EMG activity over 15 min during periods of apnea and expressed this in units of volts · minutes.

**Statistical Analysis**

Statistical differences between groups were analysed using a one-way ANOVA followed by a Student-Newman-Keuls all pairwise multiple comparison procedure or, alternatively, a Kruskal-Wallis ANOVA followed by the Dunn’s method of all pairwise multiple comparison procedure. Results were considered significant if $P<0.05$. All results are presented as means ± SE.

**Results**

Figure 1 illustrates recordings of the raw EEG, EMG, and the respiration (Resp) trace of a golden-mantled ground squirrel hibernating at two different $T_a$’s, 7°C (Fig. 1A, 1B) and 14°C (Fig. 1C, 1D). In steady state hibernation, squirrels exhibited an episodic breathing pattern where breaths were clustered into distinct episodes (Fig. 1B, 1D) separated by long apneas. In most animals, the spacing between episodes became quite regular (Fig. 1A, 1C). During hibernation, neural activity and muscle tone were greatly reduced, and at lower temperatures, the majority of the electrical signal detected in the EEG and EMG was from contraction of cardiac muscle. A ventilatory tachycardia was associated with most breathing episodes, and this is clearly evident in this animal at 14°C (Fig. 1D).

$T_a$ in hibernating golden-mantled ground squirrels increased significantly as $T_a$ was raised from 5°C to 14°C (6.98 ± 0.466°C, n = 9 at 5°C; 10.97 ± 0.172°C, n = 9 at 10°C; and 13.96 ± 0.163°C, n = 10 at 14°C, Fig. 2A), and metabolic rate increased proportionately. Calculations of the $Q_{10}$ for metabolism over this temperature range (5°C–14°C) averaged 3.38, which was not dissimilar from the $Q_{10}$ calculated over a $T_a$ range that included values for euthermic (5°C–37°C) animals, 3.41 (37°C resting-metabolic-rate data taken from results in golden-mantled ground squirrels during periodic arousal from hibernation at thermoneutrality; S. Bradley and W. K. Milsom, unpublished data; Fig. 3). Thus, although absolute values for $\dot{V}_O_2$ were not significantly different between 5°C and 10°C (5°C = 0.068 ± 0.0106, n = 7; 10°C = 0.120 ± 0.0172, n = 7) or between 10°C and 14°C (14°C = 0.203 ± 0.0324, n = 9; Fig. 2B), they increased in a manner that was predictable based on $Q_{10}$ effects. Because these
values are on the asymptote of the relationship between metabolic rate and $T_b$ (Fig. 3A), differences only became significant when comparing data between 5°C and 14°C, although the proportionality of the changes can be clearly seen when the data are expressed in semilogarithmic format (Fig. 3B).

Heart rate also increased significantly in a temperature-dependent fashion in these animals (Fig. 2D). The ground squirrels displayed a respiratory sinus arrhythmia that was significantly greater at the higher temperatures, and thus heart rate increased significantly both during periods of apnea (5°C = 6.85 ± 0.402 beats/min, $n = 7$; 10°C = 8.61 ± 0.348 beats/min, $n = 7$; 14°C = 13.81 ± 1.087 beats/min, $n = 4$) and during breathing episodes (5°C = 9.53 ± 1.36 beats/min, $n = 7$; 10°C = 15.66 ± 1.18 beats/min, $n = 7$; 14°C = 26.49 ± 2.60 beats/min, $n = 4$).

Total breathing frequency, however, did not increase significantly with increasing ambient and $T_b$'s (Fig. 2C; 5°C = 2.12 ± 0.227, $n = 12$; 10°C = 2.79 ± 0.168, $n = 14$; 14°C = 2.85 ± 0.568, $n = 4$). There was a trend for frequency to increase, but it did not attain significance. Examination of the breathing pattern at the three study temperatures revealed that the number of breaths within each episode tended to decrease as temperature was raised (5°C = 26.40 ± 2.31, $n = 12$; 10°C = 27.73 ± 2.83, $n = 14$; 14°C = 21.92 ± 4.36, $n = 4$), while the frequency of the breaths within each episode increased significantly as temperature was raised (5°C = 24.63 ± 1.09 breaths/min, $n = 12$; 10°C = 31.84 ± 1.89 breaths/min, $n = 14$; 14°C = 38.32 ± 9.03 breaths/min, $n = 4$). The length of the apnea between episodes tended to be shorter (5°C = 12.19 ± 1.44 min, $n = 12$; 10°C = 8.71 ± 0.63 min, $n = 14$; 14°C = 7.19 ± 0.56 min, $n = 4$), which caused a slight increase in the number of episodes observed per hour (5°C = 5.19 ± 0.69, $n = 12$; 10°C = 6.40 ± 0.40, $n = 14$; 14°C = 7.74 ± 0.56, $n = 4$; Fig. 4). Thus, there was a trend toward an increase in overall breathing associated with more frequent breathing episodes with fewer, more rapid breaths as temperature increased (see Fig. 1), but this was a trend only.

We describe central neural arousals as periods with distinct,
large, transient bursts of EMG activity coincident with a distinct burst of EEG activity (Fig. 5) and return of muscle tone to the body wall. These arousals were always associated with a breathing episode (Fig. 6, left graph), although breathing episodes were not always associated with such arousals. At lower temperatures, only 15.16% (± 5.88%, n = 11) of the breathing episodes were associated with periods of arousal, but at higher \( T_a \) breathing episodes were more likely to be associated with a central neural arousal (10°C = 35.69% ± 6.48%, n = 11; 14°C = 55.67% ± 9.57%, n = 6; Fig. 6, middle graph). At higher \( T_a \) there was also a significant increase in the resting background level of EEG activity as measured by the mean integrated EEG activity (5°C = 1.18 ± 0.226, n = 4; 10°C = 1.72 ± 0.245, n = 4; 14°C = 2.44 ± 0.226, n = 4) and a trend toward an increase in the mean integrated EMG activity (5°C = 1.50 ± 0.288, n = 4; 10°C = 2.17 ± 0.72, n = 4; 14°C = 5.30 ± 2.98, n = 4).

**Discussion**

*Does Metabolic Rate Change with \( T_c \) in Steady State Hibernation in the Golden-Mantled Ground Squirrel?*

Our data for the golden-mantled ground squirrel (Spermophilus lateralis) are consistent with data from studies on bats (Eptesicus fuscus; Szewczak and Jackson 1992) and the edible dormouse (Glis glis; Wilz and Heldmaier 2000) as well as from earlier studies on the golden-mantled ground squirrel (Hammel et al. 1968) showing that metabolism changes proportionately with \( T_c \) during steady state hibernation. This is in contrast to other recent investigations in arctic ground squirrels (Spermophilus parryii; Buck and Barnes 2000), alpine marmots (Marmota marmota; Ortmann and Heldmaier 2000), and the echidna (Tachyglossus aculeatus; Nicol et al. 1992) that have shown that metabolic rates remain constant in steady state hibernation even though \( T_c \)’s may range from roughly 0°C to 18°C. These data argue that while metabolic rate may be actively suppressed to some specific minimum value throughout the entire hibernation bout in some species, this is not the case for all species. The mechanistic reasons for this and the biological significance remain unknown, although it is interesting to note that those species in which metabolic rate has been shown to be temperature independent are larger than those in which metabolism continues to fall with \( T_c \) in steady state hibernation (Geiser 1988).

*Is Metabolic Rate Actively Suppressed during Steady State Hibernation in the Golden-Mantled Ground Squirrel?*

While maintenance of a constant, low, temperature-independent metabolic rate throughout steady state hibernation in some species argues strongly that metabolism is actively suppressed to minimum values, the fact that metabolic rate in the golden-mantled ground squirrel continues to change in a temperature-dependent fashion during steady state hibernation does not necessarily indicate that metabolism is not actively suppressed during this state in this species. Paradoxically, it could indicate that this species retains a higher degree of metabolic suppression at lower hibernation temperatures. In species for which a Q10 of 1 (i.e., a constant metabolic rate) has been recorded across a wide range of hibernation temperatures (Buck and Barnes 2000; Ortmann and Heldmaier 2000), the degree of metabolic suppression must be reduced as \( T_c \) falls for metabolic rate to remain constant. That is, a temperature-independent metabolic rate is indicative of a temperature-dependent metabolic suppression. If it were not, metabolic rate would fall as \( T_c \) was reduced, as is seen in the golden-mantled ground squirrel. The question then arises, is the reduction in metabolism with decreasing \( T_c \) in the golden-mantled ground squirrel indicative of a constant level of metabolic suppression at all hibernating temperatures, a temperature-
Temperature Effects in Hibernation

Figure 3. Metabolic rate drops in a negative exponential decay as $T_a$ is decreased from 37°C to 5°C (A). The $Q_{10}$ for metabolism over this range averaged 3.41 (solid line). Since the values for $V_o_2$ at 5°C and 10°C are on the asymptote of that relationship, they appear linear (dotted line). The manner in which they increase, however, can be clearly seen when the data are expressed in semilogarithmic format (B).

dependent metabolic suppression, or no metabolic suppression in steady state hibernation?

It has been argued that levels of metabolism in steady state hibernation could be maintained by the $Q_{10}$ effects of decreased $T_b$ on biochemical reactions alone once $T_b$ had fallen to minimum levels (Snapp and Heller 1981; Geiser 1988). However, Wang and Lee (2000) have recently reiterated the fact that while we depend heavily on the use of $Q_{10}$ values to assess the extent to which metabolic rate is temperature dependent during hibernation, there are inherent problems in using $Q_{10}$ values to assess cause and effect. This is clear in this study. This study shows that metabolic rate in the golden-mantled ground squirrel changes with $T_b$ during steady state hibernation with a $Q_{10}$ of 3.41. Some would argue that a $Q_{10}$ value of 3.41 is too high to be explained by the effects of decreased $T_b$ on biochemical reactions alone (a $Q_{10}$ value of 2–3), implying that active metabolic suppression must be involved throughout steady state hibernation. However, when calculating our $Q_{10}$ value of 3.41, we averaged all of the individual $Q_{10}$ values for each temperature range that we studied, 5°C–10°C, 5°C–14°C, 5°C–37°C, and so forth. The range of $Q_{10}$ values varied from 2.47 between 5°C and 10°C to 4.46 between 10°C and 14°C. This would suggest that perhaps active metabolic suppression is present at higher temperatures but not at lower temperatures during steady state hibernation. When calculating our $Q_{10}$ value of 3.41, we also used metabolic rates from resting winter squirrels at room temperature ($T_b = 37°C$). If we had used metabolic rates from sleeping winter squirrels (taken at room temperature versus the cold to avoid the effects of additional thermoregulatory demands), as Snapp and Heller (1981) suggest, the average $Q_{10}$ value would have been 3.02, similar to the accepted values of 2–3 for temperature-dependent phenomenon. These calculations demonstrate that by altering the way in which we express our data, we could argue for metabolic suppression at all temperatures, metabolic suppression only at higher hibernating temperatures, or no metabolic suppression at any temperature in steady state hibernation.

Thus, this study shows only that metabolic rate remains temperature dependent in the hibernating golden-mantled ground squirrel. The data do not allow us to draw conclusions about the extent to which there is any active metabolic suppression during steady state hibernation. At this point, the data allow us to speculate that different species may initiate and sustain falls in metabolic rate during hibernation using different mechanisms, but clearly, more thorough investigations are needed to address this question.

Do Heart Rate and Breathing Frequency Change with $T_a$ in Steady State Hibernation in the Golden-Mantled Ground Squirrel?

Under most steady state conditions, metabolism, $T_b$, heart rate, and breathing frequency are all tightly coupled due to complex interactions (Mortola and Gautier 1995). During the transitional periods of entrance into and arousal from hibernation, however, metabolic, cardiac, and ventilation rates all change in
parallel but well in advance of the changes in \( T_s \) (Lyman 1965). Under these conditions, heart and breathing frequencies are more dependent on metabolism than temperature. Given that some degree of metabolic suppression is believed to remain in steady state hibernation in some or all species, questions then arise concerning the relative roles of \( T_s \) and metabolism in establishing the resting cardiac and breathing frequencies. In this study, metabolic rate and \( T_s \) changed simultaneously, making it difficult to distinguish between any possible differences in the relative roles of each in setting the level of resting heart rate. Similar measurements in a species that exhibits a constant metabolic rate with changing \( T_s \), such as the arctic ground squirrel (Buck and Barnes 2000), might prove useful in dissecting out these effects.

The changes in heart rate observed during early entrance into hibernation have been attributed to a large parasympathetic activation (Lyman 1958). As entrance progresses and \( T_s \) begins to fall, this parasympathetic activation is slowly withdrawn. This causes heart rate to slow through asystoles and skipped beats, which eventually gives rise to a slow, even beat during deep hibernation (Lyman 1965). Harris and Milsom (1995) found that a level of resting parasympathetic tone remains in the golden-mantled ground squirrel during steady state hibernation during the breathing apneas and that it is reduced proportionately with reductions in \( T_s \). During the breathing episodes, however, the parasympathetic influence on the heart is removed, and cardiac frequency increases. This alternating parasympathetic tone acting on the heart results in a respiratory-related sinus arrhythmia or ventilatory tachycardia, such that heart rate speeds up during the breathing episode and slows down during the apnea. We also found that a ventilatory tachycardia was present at all hibernation temperatures during steady state hibernation and that it was more pronounced at higher temperatures (Fig. 2D), which reflects a larger parasympathetic tone that is present at high hibernation temperatures. The proportionate reductions in heart rate with decreasing \( T_s \) during both breathing episodes (when no parasympathetic tone is present) and during periods of apnea (when parasympathetic tone is present) indicate that both intrinsic and extrinsic mechanisms are acting on cardiac frequency and change with temperature and metabolism in steady state hibernation in this species.

It is interesting to note that the manner in which parasympathetic tone is expressed during entrance and steady state hibernation is similar to the manner in which metabolism is hypothesized to be actively reduced, that is, a large activation that is slowly withdrawn as \( T_s \) falls. It will be intriguing to discover whether the parasympathetic tone leads to a metabolic...
suppression or whether both are manifestations of a common central mechanism.

As with heart rate, breathing frequency falls in parallel with metabolic rate during entrance into hibernation in advance of any fall in $T_b$ (Lyman 1965). Once the animals were in steady state hibernation, however, breathing frequency did not change significantly with changes in $T_b$ or metabolic rate. There was a trend for the breathing frequency to decrease with decreases in temperature (and metabolic rate), but it was not significant. C. L. Webb and W. K. Milsom (unpublished results; see Milsom 1992 for review) have previously shown that the tidal volume of golden-mantled ground squirrels in hibernation ($T_b = 7^\circ-4^\circ$C) does not change significantly from euthermic values. In addition, tidal volumes from hypothermic, golden-mantled ground squirrels at similarly low $T_b$'s ($5^\circ$ and $10^\circ$C) were not significantly different from euthermic values (M. B. Zimmer and W. K. Milsom, unpublished results). If tidal volume remained constant during hibernation at $5^\circ$, $10^\circ$, and $14^\circ$C in this study, as these other studies suggest it should, then total ventilation also would not change significantly as $T_a$ was changed.

It has also been shown that animals tend to hyperventilate in hibernation relative to euthermia; that is, the ratio of ventilation to oxygen consumption increases and the oxygen extraction from respired air must decrease accordingly (Malan 1982). Our data would suggest that this trend is greater at lower hibernating temperatures. The reasons for this are not clear but may be related to requirements linked to CO$_2$ excretion. During hibernation, wide fluctuations in blood gases occur as a result of the episodic breathing pattern. During the periods of apnea, O$_2$ is depleted from lung stores while CO$_2$ accumulates in body fluids and tissues. During the breathing episode, O$_2$ stores are replenished rapidly, while CO$_2$ elimination is a slow and gradual process, such that during the last couple of breaths of an episode, O$_2$ extraction becomes constant while CO$_2$ elimination continues to fall (Garland and Milsom 1994). This reflects the effects of temperature on CO$_2$ solubility in blood and tissues and the slow mobilization of CO$_2$ stores at very low temperatures. Thus, the relative constancy of breathing frequency (and, if tidal volume remains constant, overall ventilation) with reductions in $T_b$ during steady state hibernation may reflect an interplay between reductions in CO$_2$ production coupled with increased CO$_2$ solubility and decreased CO$_2$ mobilization. This also remains an intriguing hypothesis for further testing.

Does Breathing Pattern Change with $T_a$ in Steady State Hibernation in the Golden-Mantled Ground Squirrel?

As $T_b$ begins to fall in the golden-mantled ground squirrel, a waxing and waning in the breathing frequency gradually appears and gives rise to periods of breathing separated by pauses, that is, an episodic breathing pattern (Hammel et al. 1968; McArthur and Milsom 1990). We found that this episodic pattern was exhibited at all temperatures examined during deep and steady state hibernation, ($5^\circ$–$14^\circ$C) and was even observed in squirrels hibernating as high as $20^\circ$C (M. B. Zimmer and W. K. Milsom, personal observations). Similarly, Wilz et al. (2000) have shown that the edible dormouse breathes episodically during hibernation over a wide range of temperatures ($2^\circ$–$15^\circ$C). In this study, we further found that some variables of the episodic breathing pattern changed significantly with $T_a$ during steady state hibernation (Fig. 4), with a trend toward faster, shorter, more frequent breathing episodes at higher hibernation temperatures.

Since $T_a$ and metabolic rate changed together throughout this study, our data do not allow us to distinguish between the effects of changes in metabolism versus temperature on breathing pattern. In studies in which hypothermia has been
experimentally induced, however, an episodic breathing pattern was manifested at low \( T_h \)’s even though metabolic rate remained high due to shivering (Milsom et al. 1993; M. B. Zimmer and W. K. Milsom, unpublished results), but when \( T_h \) was high and metabolism was experimentally reduced to low levels, the breathing pattern was continuous (Osborne and Milsom 1993). This evidence suggested that the episodic pattern of breathing was dependent on \( T_h \) regardless of the resting metabolic rate. The breathing episodes were not as distinct and the apneas were much shorter in hypothermia compared with hibernation, however, suggesting that, while hibernation itself was not necessary for the production of episodic breathing, either the fall in metabolism, the change in state, or both did contribute to the shaping of the breathing episodes.

In addition, Hammel et al. (1968) and C. L. Webb and W. K. Milsom (unpublished data; see Milsom 1992 for review) have shown that the breathing pattern was converted from an episodic pattern to one of evenly spaced single breaths as hibernation temperature was reduced below 5°C. Webb and Milsom showed that, during this time, the tidal volume, breathing frequency, and thus overall ventilation did not change significantly, while the breathing pattern did. While metabolism may continue to influence ventilation, this example of an extreme pattern change along with these results lend support to the hypothesis that changes in the total level of resting ventilation and changes in the breathing pattern may arise by different mechanisms.

The basic breathing rhythm in mammals is believed to be generated by a small complex of neurons found within the brain stem (see Bianchi et al. 1995 and Smith et al. 2000 for reviews). Inputs acting on this rhythm generator, from central and peripheral sites, change ventilation by changing the shape and frequency of individual breaths. In the golden-mantled ground squirrel, once hibernation is initiated, control of ventilation is no longer centred on the production and shaping of individual breaths but is now centred on the production and shaping of individual episodes of breathing (Milsom 1992). It is currently believed that this is the result of alternating influences of excitation and inhibition from central sites acting on the rhythm generator, with excitation producing the breathing episode and inhibition producing the apnea.

In this context, it is interesting to note that large, transient bursts of EEG and EMG activity have been reported to occur in this species in association with breathing episodes, and these have been termed “central neural arousals” (Milsom 1992; Fig. 5). We found that such events occurred more frequently at higher hibernation temperatures and were always associated with a breathing episode. We also found, however, that not all breathing episodes were associated with such central neural arousals, although the coincidence was higher at higher hibernation temperatures (Fig. 6). The significance of these observations is not clear, although they do suggest that changes in central neural activity, which often occur in a rhythmic fashion, may play a substantial role in the regulation of the breathing pattern at higher hibernating temperatures.

Conclusions

This study shows that metabolism remains temperature dependent during steady state hibernation in the golden-mantled ground squirrel. In addition, heart rate, breathing pattern, and the incidence and correlation of central neural arousals with breathing episodes changed significantly with changing \( T_h \), while the total breathing frequency did not. If tidal volume remains constant regardless of the temperature during hibernation, as other studies indicate, this would suggest that ventilation does not change significantly with changing \( T_h \) during hibernation. This could suggest that there is an increase in the air convection requirement \((V_e/V_o_2)\) and ventilation perfusion ratio at decreasing hibernation temperatures that may reflect differential changes in the ability to excrete \( O_2 \) versus take up \( O_2 \). It also supports previous findings that suggest that different mechanisms may be involved in determining the total level of ventilation versus the pattern of breathing employed to produce this level of total ventilation (Milsom et al. 1993).

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Literature Cited


