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Circannual Rhythm in Body Temperature, Torpor, and Sensitivity to A₁ Adenosine Receptor Agonist in Arctic Ground Squirrels

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Abstract

A₁ adenosine receptor (A₁AR) activation within the central nervous system induces torpor, but in obligate hibernators such as the arctic ground squirrel (AGS; *Urocitellus parryii*), A₁AR stimulation induces torpor only during the hibernation season, suggesting a seasonal increase in sensitivity to A₁AR signaling. The purpose of this research was to investigate the relationship between body temperature (T_b) and sensitivity to an adenosine A₁ receptor agonist in AGS. We tested the hypothesis that increased sensitivity in A₁AR signaling would lead to lower T_b in euthermic animals during the hibernation season when compared with the summer season. We further predicted that if a decrease in euthermic T_b reflects increased sensitivity to A₁AR activation, then it should likewise predict spontaneous torpor. We used subcutaneous IPTT-300 transponders to monitor T_b in AGS housed under constant ambient conditions (12:12 L:D, 18 °C) for up to 16 months. These animals displayed an obvious rhythm in euthermic T_b that cycled with a period of approximately 8 months. Synchrony in the T_b rhythm within the group was lost after several months of constant L:D conditions; however, individual rhythms in T_b continued to show clear sine wave-like waxing and waning. AGS displayed spontaneous torpor only during troughs in euthermic T_b. To assess sensitivity to A₁AR activation, AGS were administered the A₁AR agonist N⁶-cyclohexyladenosine (CHA, 0.1 mg/kg, ip), and subcutaneous T_b was monitored. AGS administered CHA during a seasonal minimum in euthermic T_b showed a greater drug-induced decrease in T_b (1.6 ± 0.3 °C) than did AGS administered CHA during a peak in euthermic T_b (0.4 ± 0.3 °C). These results provide evidence for a circannual rhythm in T_b that is associated with increased sensitivity to A₁AR signaling and correlates with the onset of torpor.

Keywords

hibernation; ground squirrel; thermoregulation; seasonal rhythm; metabolic suppression; hypothermia

Hibernation is an adaptation of energy conservation that in the arctic ground squirrel (AGS, *Urocitellus parryii*) is expressed according to a robust circannual rhythm (Sheriff et al.,

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CONFLICT OF INTEREST STATEMENT

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2011). We and others have found that stimulation of A₁ adenosine receptors (A₁AR) within the central nervous system induces torpor onset (Tamura et al., 2005; Jinka et al., 2011; Iliff and Swoap, 2012). We also found that in AGS, A₁AR activation induces torpor only during the hibernation season. This finding suggests that a seasonal increase in sensitivity to A₁AR signaling underlies the transition into the hibernation phenotype (Jinka et al., 2011). Because stimulation of A₁AR decreases body temperature (T_b) in nonhibernating species (Anderson et al., 1994), we hypothesized that a seasonal increase in sensitivity to A₁AR would lead to lower T_b in nontorpid animals. A decrease in euthermic T_b precedes onset of torpor in free-ranging AGS (Sheriff et al., 2012), and torpor expression is preceded by a decrease in euthermic T_b and metabolic rate in other hibernating species (Arai et al., 2005; Levesque and Tattersall, 2010). Tonic A₁AR activation maintains T_b (Barros et al., 2006), and in rats fed a restricted diet a lower T_b predicts increased sensitivity to an A₁AR agonist (Jinka et al., 2010). Thus, T_b in euthermic AGS at rest may reflect sensitivity in A₁AR signaling and be functionally linked to seasonal expression of the hibernation phenotype.

Here we test the hypotheses that euthermic T_b in AGS decreases during the hibernation season and that a decrease in daily euthermic T_b reflects increased sensitivity to A₁AR activation and predicts torpor onset. We monitored T_b and spontaneous torpor in AGS housed at an ambient temperature of 18 °C throughout an 18-month period. Animals displayed an apparent circannual rhythm in nontorpid (euthermic) T_b where the hibernation season, indicated by spontaneous torpor in AGS housed, was noted by troughs in euthermic T_b. We tested A₁AR sensitivity by administering an A₁AR agonist during the highs and lows in euthermic T_b and found that animals in a T_b trough displayed greater A₁AR agonist sensitivity. We also monitored food intake.

METHODS

Body Temperature and Food Intake Monitoring

Procedures were approved by the UAF Institutional Animal Care and Use Committee. Arctic ground squirrels (AGS; *Urocitellus parryii*) were captured near 66°38'N, 149°38'W under permit from the Alaska Department of Fish and Game. AGS were housed individually at an ambient temperature of 18 °C on a 12:12 L:D cycle and fed rodent chow ad libitum. To monitor T_b, IPTT-300 transponders (Bio Medic Data Systems, Inc., Seaford DE; calibrated at 30 °C with an accuracy of ±0.5 °C between 24 and 40 °C) were inserted between the scapulae. Others have shown these transponders to provide reliable data (±0.6 °C) over subcutaneous temperature of 21.4 to 36.9 °C in small mammals that do not vary with ambient temperature and indicate when animals are torpid (Wacker et al., 2012).

Subcutaneous T_b was measured between 1030 and 1130 h daily in a total of 24 AGS (12 male and 12 female); 16 AGS were monitored for 16 months from 24 September 2008 until 1 February 2010, and 8 AGS were monitored for 9 to 13 months beginning on 24 September 2008. Animals were noted to be torpid if they were unresponsive to touch and displayed a T_b less than 30 °C. Daily mass of food intake was measured from 2 June 2009 to 18 December 2009 in 16 of the 24 AGS.

Experimental Design

Seasonal changes in T_b and food intake were monitored as described above. To determine whether seasonal lows in euthermic T_b predicted increased sensitivity to T_b -lowering effects of A_1AR activation, the A_1AR agonist N^6 -cyclohexyladenosine (CHA) or vehicle (phosphate buffered saline [PBS]) was administered to 22 AGS on 2 separate occasions.

AGS were handled and weighed the day before treatment. This handling induced arousal if animals were torpid. CHA was dissolved in PBS and filtered through 0.22- μ m syringe filters into a sterile BD vacutainer. Baseline T_b was collected for 1 h before the drug was administered. CHA (0.1 mg/kg, ip) or vehicle (PBS) was injected using 25-gauge, 1.5-inch needles. T_b was recorded every 30 min for 4 h following the injection and again at 24 h post injection.

Data Analysis

Seasonal fluctuation in T_b and food intake was assessed as present or absent from graphs of individual animals. In cases where a rhythm was evident during the 6-month observation period in both T_b and food intake, the temporal relationship between the waxing and waning of T_b and that of food intake varied. In some animals T_b decreased before food intake, and in others T_b and food intake decreased at approximately the same time. No animals showed a clear decrease in food intake before a decrease in T_b . We compared the frequency of animals that decreased T_b before or at approximately the same time as food intake to an expected random (50-50) distribution using a chi-square goodness-of-fit test.

Each individual animal's euthermic T_b cycle was assessed and the position assigned as a trough, a peak, or an intermediate position by a person unaware of the drug response. A 2-tailed t test was used to compare drug-induced change in T_b between AGS tested during a trough and those animals tested during a peak in T_b . Regression analysis was performed to assess the relationship between euthermic T_b and magnitude of drug response in all animals tested (MSEXcel). Significance was set at $p < 0.05$. Data shown are mean \pm SEM.

RESULTS

All 24 animals showed a waxing and waning in euthermic T_b that ranged between 30 and 40 $^{\circ}$ C and fluctuated with a circannual period between 7 and 9 months. Group synchrony was noted in the rhythm during the first 8 months but was lost in later months. T_b rhythms for individual animals persisted under constant L:D conditions (Fig. 1). Torpor, illustrated by a precipitous drop in T_b , was observed only during troughs in euthermic T_b (Fig. 1).

To test the hypothesis that a decrease in food intake produced the decrease in T_b , as described for rats fed a restricted diet (Jinka et al., 2010), we compared the temporal patterns of food intake and T_b . Food intake varied between 0 and 80 g/day, including days when animals were torpid. None of the AGS showed a decrease in food intake that preceded a decrease in T_b . Fourteen of the 16 AGS showed a clear waxing and waning in food intake and T_b during the 6-month monitoring period. In 6 of these 14 AGS, T_b began to decrease before food intake (Fig. 2B), and in 7 of these 14 AGS, T_b and food intake decreased at about the same time (Fig. 2A). The distribution of occasions when food intake decreased (1)

before T_b decreased, or (2) after or at the same time as T_b decreased, was different from an expected 50:50 distribution $\chi^2(1,14) = 0.0002$].

AGS tested with the A_1 AR agonist CHA during their trough in euthermic T_b showed a greater decrease in T_b (1.6 ± 0.3 °C) than AGS tested during their peak in euthermic T_b (0.4 ± 0.3 °C; *t* test; *p* < 0.05 trough vs. peak, *n* = 6 CHA tests during a trough and 6 tests during a peak in a total of 8 AGS; 4 males and 4 females). The magnitude of CHA-induced decline in euthermic T_b was not predicted by predrug T_b . Regression analysis of euthermic T_b and CHA-induced change in T_b in all animals tested with CHA (*n* = 22) was not statistically significant. By contrast, the position within an individual animal's rhythm in T_b predicted response to CHA (Fig. 3). Vehicle had no effect on euthermic T_b when administered during a trough or a peak (*n* = 4 vehicle tests during a trough and 5 tests during a peak in a total of 6 AGS, 3 males and 3 females).

DISCUSSION

Hibernation is a robust seasonal phenomenon, but mechanisms that regulate the transition into the hibernation phenotype displayed during the hibernation season are poorly understood. A_1 AR activation within the central nervous system is necessary and sufficient to induce torpor in AGS and other species (Tamura et al., 2005; Iliff and Swoap, 2012). However, in AGS, stimulating A_1 AR induces torpor only during the hibernation season, indicating that a higher order of regulation controls the ability of adenosine to induce torpor (Jinka et al., 2011). Our results support the hypothesis that this higher order of regulation involves increased sensitivity to A_1 AR-induced cooling. We show that a seasonal minimum in euthermic T_b reflects increased sensitivity to A_1 AR-induced cooling that waxes and wanes between the summer and the hibernation seasons. This seasonal rhythm in euthermic T_b predicts and is fundamental to the expression of spontaneous torpor.

Field data collected using data loggers implanted in the abdominal cavity in the fall and retrieved in the spring show a decrease in core T_b to 34 to 35 °C beginning 45 days prior to entrance into the first torpor bout. Body temperature during interbout arousals does not exceed this lowered basal temperature (Sheriff et al., 2012). A decrease in euthermic T_b precedes onset of torpor in other hibernating species as well (Arai et al., 2005; Levesque and Tattersall, 2010). Surprisingly, predrug body temperature in CHA-treated AGS in summer and winter was similar and did not predict CHA-induced torpor. This may be because baseline T_b prior to CHA administration did not accurately reflect T_b of animals at rest. Although animals were administered CHA at the same time each day, handling and movement from the home cage to the test cage likely resulted in an increase in activity and predrug T_b .

A seasonal increase in sensitivity to A_1 AR signaling may underlie seasonal rhythms in sleep drive as well as T_b . The seasonal decrease in euthermic T_b reported here coincides with a seasonal increase in sleep duration observed in ground squirrels housed under conditions similar to the present study (Walker et al., 1980). Hibernation is an extension of sleep (Walker et al., 1977, 1979), where homeostatic sleep drive is mediated in part through the activation of A_1 AR (Benington et al., 1995; Porkka-Heiskanen et al., 1997). Golden mantled

ground squirrels sleep more during the hibernation season (Walker et al., 1980), and sleep is associated with lower euthermic T_b (Berger and Phillips, 1988). Indeed, the lower euthermic T_b noted in the present study could be due to the animals sleeping.

The gradual transition into the hibernation season is consistent with a gradual decrease in the irritability of hibernating animals as the hibernation season progresses and a gradual increase in irritability as spring approaches (Twente and Twente, 1968). Waxing and waning in the sensitivity to A_1AR signaling were expected based on the observed gradual increase in the effectiveness of CHA to induce torpor in the midhibernation season compared with the early hibernation season (Jinka et al., 2011).

Although it was surprising to see the period in T_b rhythm decrease under constant L:D conditions, the results are consistent with shortening of circannual rhythm period in spontaneous torpor that we have noted previously (Jinka et al., 2011). Constant daylight is known to phase advance the circannual cycle of obligate hibernators (Pengelley et al., 1976). In the present study, group synchrony in euthermic T_b rhythm subsided while individual euthermic T_b began to free-run on a 7- to 9-month cycle. The rhythm was clearly associated with the hibernation season since animals displayed torpor only during troughs in T_b . These observations are consistent with other observations of circannual rhythms in captive ground squirrels housed under constant L:D conditions where the circannual cycle persists but begins to free-run, that is, oscillate on a cycle slightly shorter than 12 months (Lee and Zucker, 1991; Hiebert et al., 2000). Surprisingly, absolute euthermic T_b did not predict the magnitude of response to CHA except in animals expressing a maximum or minimum in euthermic T_b . If altered sensitivity in A_1AR signaling leads to a gradual decrease in euthermic T_b and seasonal torpor, we would expect to see a correlation between T_b and the magnitude of response to CHA. The lack of a statistically significant correlation in euthermic T_b and response to CHA could be due to the relatively small sample size studied and other factors that influence T_b .

Euthermic T_b is lower during interbout arousal than during summer euthermicity in free-living as well as captive AGS (Barnes, 1989; Karpovich et al., 2009; Sheriff et al., 2012). However, the gradual waxing and waning in T_b are not evident in AGS housed under natural conditions (Barnes, 1989). Rather, T_b during interbout arousal remains at about 35 °C throughout the hibernation season (Karpovich et al., 2009). The range of euthermic T_b in this study was greater than the range of euthermic T_b reported in free-living AGS during the summer season (Williams et al., 2012b). Although the T_b highs near 40 °C are similar to field data, lows near 30 °C are lower than T_b of summer, free-living AGS. We expect that in free-living animals, ambient temperatures near 0 °C promote onset of torpor so that circannual fluctuation in euthermic T_b is masked by torpid T_b .

Daily T_b readings did not allow for analysis of circadian variation in T_b , so it remains unclear whether circannual rhythm in euthermic T_b reflects a vertical shift in the entire circadian T_b curve and thus a decrease in maximum and minimum T_b or a change in the amplitude of the T_b rhythm. Daily T_b readings also cannot rule out the possibility that a circannual influence on the period of the circadian T_b rhythm explains the seasonal fluctuation in daily measures of T_b . In free-living AGS, body temperature rhythms persist

during the summer season but are not detected during hibernation at T_b near 0 °C (Williams et al., 2012a). Diurnal T_b rhythms in AGS entrain to approximately 24-h periods in fall and spring (Williams et al., 2012b) and a period of 24-h persists throughout the summer season (Williams et al., 2012a). Since it is not clear what AGS entrain to under the midnight sun and since AGS in the present study were housed at constant 12:12 L:D and ambient temperature, it is difficult to extrapolate between these observations and observations made in free-living AGS.

A decrease in T_b was found to precede or decline at the same time as the decrease in food intake. These results do not support our original hypothesis that voluntary dietary restriction prior to onset of torpor increases the response to CHA. Instead, they suggest that lower T_b may reflect a seasonal decrease in thermogenesis and thus metabolic rate that leads to, or is associated with, a decrease in food intake. Thus, it appears that the mechanism regulating seasonal fluctuation in T_b in AGS differs from the mechanism that sensitizes A_1AR during dietary restriction in rats (Jinka et al., 2010).

In conclusion, the present results suggest that an increase in sensitivity of A_1AR signaling is correlated with a decrease in euthermic T_b and predicts torpor onset. This finding is significant because a seasonal rhythm in A_1AR signaling represents a molecular mechanism that, in part, defines the hibernation season. More work will be required to determine the molecular basis of the seasonal fluctuation in A_1AR signaling to determine where in the brain changes occur, whether changes occur in extracellular levels of adenosine and/or at the receptor level, and what processes underlie these changes. Whether the decrease in euthermic T_b reflects an increase in sleep remains to be determined, but both sleep and T_b likely result from increased sensitivity in A_1AR signaling. The ability of a decrease in euthermic T_b to predict the onset of the hibernation season is also significant because secondary influences of torpor confound interpretation of seasonal differences in gene and protein expression.

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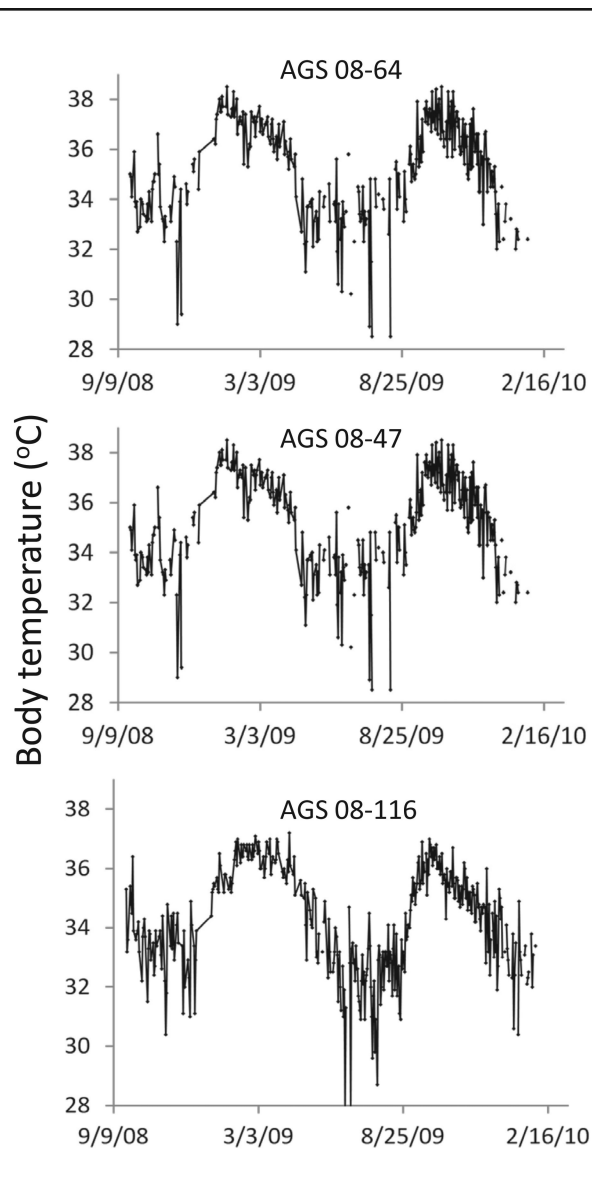


Figure 1. Spontaneous torpor, noted by a large drop in T_b , only occurs during a trough in the euthermic T_b cycle. Representative graphs show daily T_b in 3 AGS from September 2008 to February 2010. Graph titles indicate animal ID number.

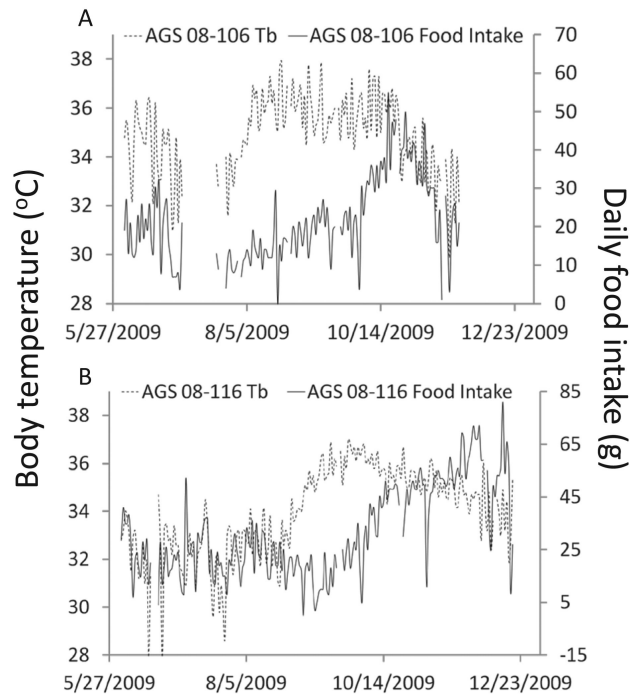


Figure 2. Representative examples illustrate T_b and food intake decreasing at approximately the same time (A) and T_b decreasing prior to food intake. (B). No animals showed a decrease in food intake prior to a decrease in T_b .

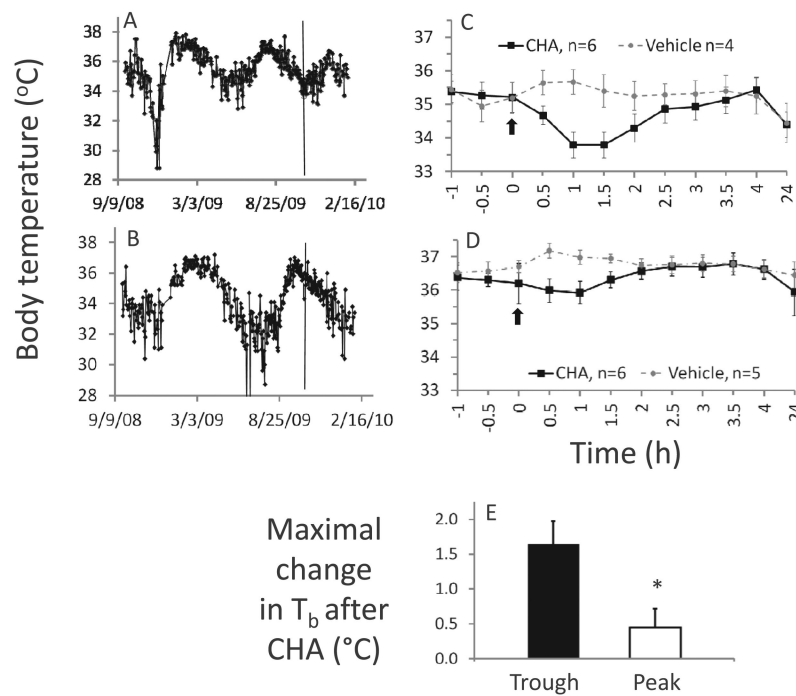


Figure 3. The drug-induced change in T_b depends on the position of AGS in their circannual T_b cycle. Timing of drug administration indicated by vertical lines was identified as a trough (A) or a peak (B). C and D show CHA (0.1 mg/kg)-induced effects on T_b for AGS tested in a T_b trough (C) or a peak (D); arrows indicate time of drug administration. The drug-induced change in T_b was greater in animals tested during a trough than in animals tested during a peak (E). Data shown are mean \pm SEM. * $p < 0.05$, n indicates the number of tests in a T_b peak or in a T_b trough.