

Melatonin Production Accompanies Arousal from Daily Torpor in Siberian Hamsters

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ABSTRACT

Arousal from deep hibernation is accompanied by a transient rise of melatonin (Mel) in circulation; there are no comparable analyses of Mel concentrations in species that undergo much shallower, shorter duration episodes of daily torpor. Serum Mel concentrations were determined during arousal from both natural daily torpor and torpor induced by 2-deoxy-D-glucose (2-DG) treatment (2,500 mg/kg, intraperitoneal [IP]); blood samples were drawn from the retro-orbital sinus of anesthetized Siberian hamsters. For animals kept in darkness during torpor, Mel concentrations were highest during early arousal when thermogenesis is maximal, and they decreased as body temperature increased during arousal and returned to baseline once euthermia was reestablished. In hamsters kept in the light during the torpor bout, Mel concentrations were elevated above basal values during arousal, but the response was significantly blunted in comparison with values recorded in darkness. Increased Mel concentrations were detected in hamsters only during arousal from torpor (either natural or 2-DG induced) and were not simply a result of the drug treatment; hamsters that remained euthermic or manifested mild hypothermia after drug treatment maintained basal Mel concentrations. We propose that increased Mel production may reflect enhanced sympathetic activation associated with intense thermogenesis during arousal from torpor rather than an adjustment of the circadian rhythm of Mel secretion.

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Introduction

Production of melatonin (Mel) by the pineal gland is controlled by the circadian system and entrained by the ambient photoperiod (Goldman 2001); typically, Mel is elevated only at night. In hibernating species, such as marmots, Syrian hamsters, and Turkish hamsters, Mel production is suppressed during the multiday bouts of hibernation (Florant et al. 1984; Vanacek et al. 1984, 1985; Darrow et al. 1986). Low blood concentrations of Mel during deep hibernation may reflect reduced sympathetic nervous system activity, an apparent prerequisite for the induction and maintenance of torpor (Lyman et al. 1982), or cessation of endocrine activity at tissue temperatures below 20°C (Barnes et al. 1987, 1988). During arousal from hibernation, the systemic increase in Mel (Florant et al. 1984; Vanacek et al. 1985) coincides with activation of the sympathetic nervous system and increased release of norepinephrine (Twente and Twente 1978; Lyman et al. 1982). Moreover, a phase reversal in the daily Mel rhythm in Syrian hamsters that aroused at noon relative to those that aroused at midnight led to the suggestion that circadian timekeeping was suspended during hibernation and reset on arousal (Vanacek et al. 1985). The latter conclusions are problematic given that the circadian clock in the suprachiasmatic nucleus (SCN) is buffered from the effects of low temperature. SCN neurons remain metabolically active during deep torpor at body temperatures (T_b 's) that eliminate or greatly reduce neural activity in other brain structures (Kilduff et al. 1990; Grahn et al. 1994; Miller et al. 1994; Ruby et al. 1996; Larkin et al. 2002). T_b rhythms are, however, eliminated in ground squirrels that have sustained SCN ablations (Ruby et al. 2002), further supporting the contention that the SCN circadian clock that governs Mel secretion remains functional during hibernation.

Unlike species that remain in their hibernacula and arouse infrequently from deep torpor during the hibernation season, many heterothermic rodents, including the Siberian hamster (*Phodopus sungorus*), remain behaviorally active and forage above ground in winter (Hudson 1978; Weiner 1982). Siberian hamsters undergo torpor two or three times per week during the early portions of the subjective day when euthermic animals are normally at rest or inactive (Ruby and Zucker 1992; Ruf et al. 1993; Deboer and Tobler 1994). Daily torpor is markedly shorter (2–8 h duration) and shallower (minimum $T_b \geq 15^\circ\text{C}$)

than the deep torpor bouts displayed by hibernators, which typically last several days, with minimum T_b 's of $\sim 5^\circ\text{C}$ (Lyman et al. 1982). Normal timing of daily torpor is contingent on an intact SCN; hamsters with SCN lesions enter torpor at any time of day and may display several torpor bouts in a single 24-h interval (Ruby and Zucker 1992).

The effect of low T_b on Mel secretion has not been studied in rodents that display shallow daily torpor. The impact of daily torpor on Mel secretion may be less profound in such species than in those that hibernate at much lower T_b 's. Many neurons characteristically cease firing during torpor at T_b 's $< 15^\circ\text{C}$ (Krilowicz et al. 1988; Miller et al. 1994; Ruby and Heller 1996), but neuronal activity may persist during torpor in Siberian hamsters because the minimum T_b during a typical bout exceeds 15°C . Moreover, arousal from torpor occurs almost exclusively during the light phase of the daily photocycle, which corresponds to periods of low or undetectable Mel concentrations in the circulation of Siberian hamsters (Goldman 2001). Decreases in Mel production below this baseline during torpor may not be possible, and increases in Mel secretion associated with the arousal process may be suppressed by the circadian timing mechanisms or ambient light. To address whether Mel production can be stimulated by arousal from torpor independent of the circadian system, serum Mel concentrations were measured during arousal and subsequent euthermia in Siberian hamsters treated with 2-deoxy-D-glucose (2-DG), a metabolic inhibitor of glycolysis that induces torpor at any time of day or night (Dark et al. 1994, 1996, 1999; Stamper et al. 1998), and in hamsters that displayed torpor naturally. We determined whether Mel release was associated with emergence from daily torpor and the impact of the environmental photocycle on Mel production during the arousal process.

Material and Methods

Adult male Siberian hamsters were born and maintained in long days (16 h light/day; lights on at 0800 hours, Pacific Daylight Time) at a room temperature of 23°C . Hamsters were transferred to an environmental chamber maintained on short days (8 h light/day; lights on at 0800 hours). They were housed singly with wood shavings for bedding material. Food (Purina Rodent Chow no. 5015) and water were provided ad lib. unless otherwise indicated. Four weeks after transfer to this chamber, a temperature-sensitive radio-transmitter (model VM-FM, Minimitter, Bend, Oreg.) was implanted in each animal under anesthesia induced by a ketamine mixture (21.0 mg of ketamine/2.4 mg of xylazine/0.3 mg of acepromazine/mL; 0.34 mL/100 g of body mass, IP). A midline incision was made in the abdomen, the transmitter was inserted into the peritoneal cavity, and the wound was closed with sterile sutures and treated with 0.1% nitrofurazone antibiotic ointment (Furacin). Hamsters were provided with acetaminophen plus codeine phosphate as a 1% solution in the drinking water for 2–3 d after

surgery. Throughout the experiment, T_b was recorded continuously at 10-min intervals by receiver boards beneath each animal's cage using the Dataquest Data Acquisition System (St. Paul, Minn.). Handling of animals in darkness was done under dim red light.

Experiment 1: Melatonin during Arousal from Torpor in the Subjective Day in Darkness

Hamsters ($N = 24$) were maintained at 5°C in short days. On experimental days, animals were transferred to an environmental chamber (short days at 5°C) 1 h after light onset (0900 hours) and injected IP with 2,500 mg/kg of 2-DG (Sigma, St. Louis) dissolved in sterile water. Lights were immediately turned off to permit torpor and arousal to occur in darkness during the hamsters' subjective day. Thus, torpor was induced at a time of day corresponding to onset of natural torpor. While hamsters remained in darkness, a single blood sample was drawn per individual animal per torpor bout either during torpor or after arousal from torpor. Blood samples were collected under anesthesia induced by methoxyflurane vapors (Metofane, Pitman-Moore, Worthington Crossing, N.J.); < 0.5 mL was drawn from the right retro-orbital sinus into nonheparinized tubes. Blood samples were centrifuged at 5,000 rpm for 20 min, and serum was stored at -70°C for subsequent assay of Mel. Successive 2-DG injections and blood samplings of individual animals were separated by 2 or more weeks; no animal received more than three 2-DG treatments.

Hamsters were considered torpid if T_b fell below 31°C for ≥ 20 min; depth of torpor was assessed by calculating the minimum T_b ($T_{b\text{min}}$) during the torpor bout. Blood was obtained at three time points after 2-DG-treated hamsters had attained $T_{b\text{min}}$: during (1) early arousal, when T_b was $< 28^\circ\text{C}$ ($n = 7$); (2) late arousal, when $28^\circ\text{C} \leq T_b < 34^\circ\text{C}$ ($n = 7$); and (3) post-arousal euthermia, 0–120 min after arousal from torpor ($T_b \geq 34^\circ\text{C}$, $n = 13$). Blood was also drawn 30–90 min after 2-DG treatment from hamsters that failed to display torpor ($T_{b\text{min}} \geq 34^\circ\text{C}$, $n = 12$). To determine whether rewarming from milder hypothermia triggers a Mel surge, blood was collected during bouts of hypothermia ($n = 11$) that were neither long enough nor deep enough ($T_{b\text{min}}$ of 31°C – 33.5°C) to qualify as torpor but in which T_b was still below euthermic values. Mel concentrations were determined from blood samples collected late in the dark period (0300 hours, 11 h after lights off; $n = 10$) and during the light phase (1400 hours, 2 h before lights off; $n = 9$).

Experiment 2: Melatonin during Arousal from Torpor in the Subjective Day in the Light

Adult hamsters ($n = 18$) were maintained in short days at an ambient temperature (T_a) of 5°C as in experiment 1. To induce torpor, animals were injected with 2-DG at 0900 hours during

the light phase as described in experiment 1, except that hamsters remained in their home cages in the light after treatment. Blood was drawn from torpid hamsters undergoing arousal at three time points after 2-DG injection: during the initial phase of arousal ($n = 9$, $T_b \leq 28^\circ\text{C}$), during the late phase of arousal ($n = 7$, $28^\circ\text{C} < T_b < 34^\circ\text{C}$), and 1 h after arousal ($n = 8$, $T_b > 34^\circ\text{C}$). As a control, time-matched samples were obtained from hamsters that did not enter torpor after injections of 2-DG ($n = 13$). Blood was also drawn from another group of hamsters during the dark phase (0400 hours, $n = 17$) to measure nighttime Mel concentrations.

Experiment 3: Melatonin during Natural Torpor in Darkness

After 3 mo in short days at 5°C , five of the 24 hamsters in experiment 2 had molted into white winter pelage and demonstrated short day-induced spontaneous torpor. The high incidence of hamsters that did not adopt the winter phenotype in short day lengths (nonresponders) was typical of our hamster colony at that time (Larkin et al. 2001). To increase the number of natural torpor bouts to compare with the 2-DG-induced torpor, we food-restricted 14 short day nonresponder hamsters to induce torpor (e.g., Bae et al. 2000). During 4 d of food restriction, hamsters that had failed to undergo testicular regression or molt to a white pelage were fed 75% of their previously determined food intake at 1000 hours each day; body mass and incidence of torpor were monitored daily during the food-restriction regimen. This protocol resulted in three additional hamsters entering torpor so that eight hamsters contributed to the natural torpor sample. Food-restriction-induced torpor was initiated between 0700 and 1000 hours, and arousal occurred between 1100 and 1400 hours. Beginning on the fifth day, all hamsters were fed ad lib. Hamsters were transferred to a dark, 5°C environmental chamber either at the time of light offset (1600 hours) the day preceding a torpor bout or after onset of torpor. Animals transferred during entrance into torpor maintained decreased T_b . Hamsters that did not enter torpor by 1100 hours were returned to the short day chamber. As described in experiment 1, blood samples were drawn during arousal from natural torpor ($n = 8$) or 0–120 min after arousal from natural torpor ($n = 4$) for assay of Mel. Individual hamsters did not contribute more than two blood samples, and these repeated samples were separated by more than 2 wk.

Melatonin Radioimmunoassay

Serum Mel concentrations were quantified in duplicate (Yellon and Truong 1998). Assay sensitivity ranged from 0.71 to 0.83 pg/tube (<12 pg/mL). Intraassay and interassay coefficients of variation were $<10\%$.

Statistics

Mel concentrations are presented as mean \pm SE. Two outliers were identified by stem-and-leaf analysis (Sokal and Rohlf 1981) outside the 95% confidence intervals of the dataset and were not included in subsequent analysis. Comparisons were made with ANOVA; when the overall ANOVA indicated a significant effect (criterion for significance was $P < 0.05$), post hoc pairwise comparisons were made with Fisher's Protected Least Significant Difference test (Statview 5.0, SAS, Cary, N.C.). The effect of T_b on Mel concentrations during arousal within each type of torpor and between natural and 2-DG-induced torpor was analyzed with regression and ANCOVA analyses, respectively. When the F -test revealed greater variability in Mel concentrations during arousal from natural torpor than did 2-DG-induced torpor, the two groups were compared using the nonparametric Mann-Whitney U -test. A nonparametric Kruskal-Wallis test was also applied to Mel concentrations during euthermia following arousal for the time intervals 0–10 min ($N = 9$), 20–30 min ($N = 9$), and 60+ min ($N = 5$) following arousal.

Results

Experiment 1: Melatonin during Arousal from Torpor in the Subjective Day in Darkness

In euthermic hamsters, T_b was $37.4^\circ \pm 0.3^\circ\text{C}$ (mean \pm SE, $n = 10$). T_b was significantly reduced in 80% of animals treated with 2-DG. Torpor was induced in 51% of hamsters within 40 min of 2-DG treatment ($T_{b\text{min}} = 27.2^\circ \pm 0.9^\circ\text{C}$, $n = 25$; $P < 0.01$ vs. euthermic controls). In 29% of 2-DG-treated hamsters, milder hypothermia was evident, with $T_{b\text{min}}$ at $32.4^\circ \pm 0.3^\circ\text{C}$ ($n = 14$). This hypothermia was neither long enough nor deep enough to qualify as torpor but represented a significant reduction in T_b below euthermic values ($P < 0.01$). No significant decrease in $T_{b\text{min}}$ occurred in the remaining 20% of 2-DG-treated hamsters ($T_{b\text{min}}$ of $36.4^\circ \pm 0.5^\circ\text{C}$, $n = 10$).

In control animals, serum Mel concentrations were higher in the dark phase than in the light phase (153 ± 47 vs. 19 ± 5 pg/mL; $P < 0.05$). Mel concentrations were significantly elevated in hamsters during arousal from 2-DG-induced torpor (Fig. 1A). Mel was highest during the first phase of arousal ($T_b < 28^\circ\text{C}$, T_b mean = $22.5^\circ \pm 2.0^\circ\text{C}$) and was still significantly elevated above baseline values during the late phase of arousal when T_b 's ranged between 28° and 34°C ($T_b > 28^\circ\text{C}$, T_b mean = $31.5^\circ \pm 0.3^\circ\text{C}$; Fig. 1A). Mel concentrations were at low daytime concentrations in animals sampled within 20 min of attaining euthermia (T_b mean = $34.7^\circ \pm 0.3^\circ\text{C}$; Fig. 1A). Hamsters that failed to undergo torpor after 2-DG treatment had low Mel concentrations at the limit of assay detectability (Fig. 1A; nontorpid).

Mel concentrations were correlated with T_b during arousal (Fig. 2). Mel concentrations remained low during rewarming

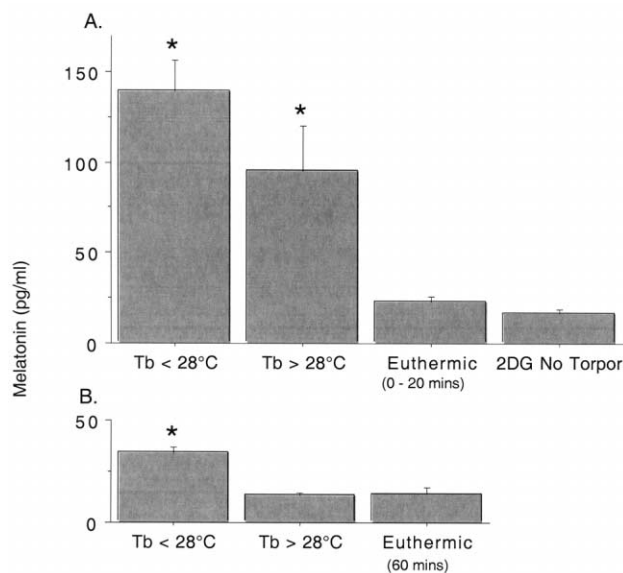


Figure 1. Serum melatonin (Mel) concentrations during arousal from torpor in hamsters injected with 2-DG 2 h after light onset and immediately transferred to darkness (A) or kept in the light (B). Mel values were elevated during arousal from torpor in darkness compared with hamsters that did not undergo torpor (A; $P < 0.01$, indicated by an asterisk) but were basal on resumption of euthermia. 2-DG treatments that failed to elicit torpor did not elevate Mel production relative to normal daytime values (A; 2-DG no torpor). The Mel surge accompanying arousal was significantly lower in hamsters kept in the light during arousal (B), with significant increases above basal values detected only during the earliest phase of arousal when T_b was $< 28^\circ\text{C}$.

in mildly hypothermic hamsters whose $T_{b\text{min}}$ was $> 32^\circ\text{C}$, but Mel concentrations were elevated when $T_{b\text{min}}$ fell below 32°C . In torpid hamsters, the highest Mel concentrations were measured at T_b 's ranging from 15° to 27°C ; these values were comparable with normal nighttime concentrations. At T_b 's between 20° and 36°C , Mel concentration decreased linearly in relation to T_b at the time of sampling (Fig. 2; $r^2 = 0.66$, $n = 24$). T_b 's $< 20^\circ\text{C}$ were excluded from the regression analysis because Mel concentrations were maximal at T_b 's from 15° to 20°C and were no longer temperature dependent.

Experiment 2: Mel during Arousal from Torpor in the Subjective Day in the Light

2-DG treatment induced torpor in 70% of hamsters, with a latency of < 40 min; $T_{b\text{min}}$ was $27.9^\circ \pm 0.6^\circ\text{C}$ and ranged from 21.1° to 30.8°C . $T_{b\text{min}}$ did not differ between 2-DG-treated euthermic and mildly hypothermic hamsters ($36.5^\circ \pm 0.1^\circ\text{C}$ and $33.8^\circ \pm 0.8^\circ\text{C}$, respectively; $P > 0.15$), and Mel concentrations did not differ between the latter groups (T_b was 36.2° – 36.3°C at the time of blood sampling).

As in experiment 1, Mel concentrations were significantly elevated (34.0 ± 3.0 pg/mL) during early stages of arousal from

torpor ($T_b < 28^\circ\text{C}$; Fig. 1B) but were well below normal nighttime values (153 ± 47 pg/mL) and were significantly lower than values for hamsters kept in darkness during the same stage of arousal from torpor ($P < 0.05$; Fig. 1A). Mel concentrations returned to low daytime values more rapidly in hamsters kept in light than darkness during arousal from torpor ($P < 0.05$); in the light, this occurred when T_b first exceeded 28°C , whereas in darkness, Mel values were still significantly elevated at this T_b (Fig. 1).

Experiment 3: Mel during Natural Torpor in Darkness

Neither T_b nor Mel patterns differed significantly between animals that displayed torpor spontaneously or during food restriction, so these two types of torpor were combined for analysis of "natural" torpor. During natural torpor, $T_{b\text{min}}$ was $19.6^\circ \pm 0.9^\circ\text{C}$ ($n = 12$); Mel concentrations were variable but elevated above typical daytime values during the arousal process, particularly at low T_b 's (mean Mel for $T_b < 28^\circ\text{C}$ was 102 ± 69 pg/mL vs. 19 ± 5 pg/mL in daytime controls; $P < 0.05$). Mel concentrations during early arousal ($T_b < 28^\circ\text{C}$) did not differ significantly between 2-DG-induced ($N = 7$) and natural torpor ($N = 8$, 139 ± 18 vs. 102 ± 69 pg/mL, respectively; Mann-Whitney U -test, $P > 0.5$), but there was significantly more variability in Mel concentrations during arousal from natural torpor (F -test, $P < 0.05$).

Mel Secretion during Euthermia after Arousal from Torpor

In hamsters that underwent torpor and subsequent arousal in darkness, Mel concentrations were basal when animals first regained euthermia but increased 1–2 h later ($r^2 = 0.38$,

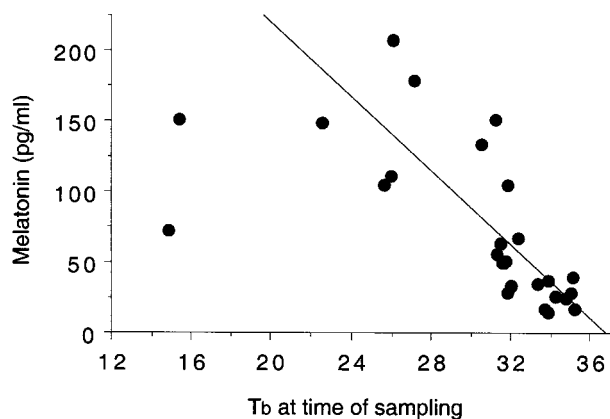


Figure 2. Relation between T_b at time of blood sampling during arousal from 2-DG-induced torpor ($^\circ\text{C}$) and serum melatonin (Mel) concentration for hamsters kept in darkness during torpor. The regression of T_b and Mel for T_b 's between 20° and 36°C was significant ($P < 0.001$, $r^2 = 0.66$, $N = 24$). Data below 20°C were excluded from the regression analysis because serum Mel concentrations were maximal at 20°C .

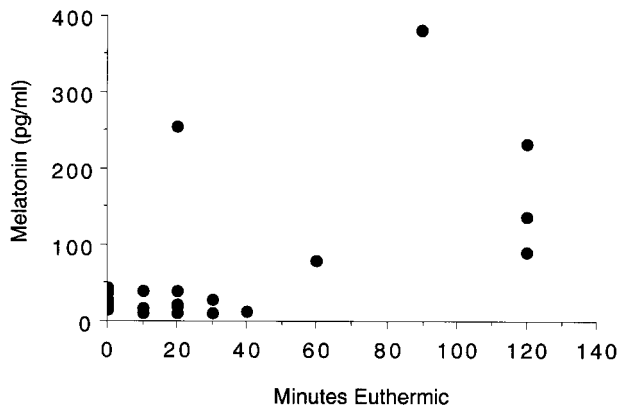


Figure 3. Variation in serum melatonin concentration as a function of time since arousal from torpor for hamsters kept in darkness during the torpor bout.

$N = 23$; see Fig. 3 for combined data from natural and 2-DG-induced torpor). The Kruskal-Wallis nonparametric test showed a significant ($P < 0.01$) increase in Mel concentrations only during the 60+ min after arousal group. This secondary increase in Mel was evident several hours before the beginning of the subjective night as judged on the basis of the entrained locomotor activity rhythm. The secondary surge of Mel production was absent in hamsters that aroused in the light (not illustrated).

Rate of Change in T_b during Entry and Emergence from Torpor

2-DG-induced and natural torpor were superficially similar, ranging in depth and duration from shorter, shallow bouts to longer, deeper bouts (Fig. 4). The rate of decline in T_b during entry into torpor was similar for the two types of torpor (Fig. 5; $0.18 \pm 0.01^\circ\text{C}/\text{min}$; $P > 0.50$), but T_b increased more rapidly during emergence from natural torpor bouts (Fig. 5; $P < 0.001$). Even though $T_{b\text{min}}$ was significantly lower for animals undergoing natural torpor than 2-DG-induced torpor ($19.6 \pm 0.9^\circ\text{C}$ vs. $27.9 \pm 0.6^\circ\text{C}$; $P < 0.01$), the former hamsters regained euthermia more rapidly ($P < 0.01$).

Discussion

Mel production increased during arousal from both natural and 2-DG-induced daily torpor. Serum Mel concentrations were highest during the early arousal phase (i.e., at T_b 's between 15° and 26°C , which is known to be a period of intense thermogenesis; Heldmaier et al. 1999). Mel concentrations declined as T_b rose during arousal and returned to basal values on achievement of euthermia when arousal-associated thermogenesis ceased. We suggest that an increase in sympathetic drive

associated with arousal from torpor is responsible for increased Mel production.

The Mel surge was blunted and curtailed in hamsters that were exposed to light during arousal from torpor. During the early phase of arousal ($T_b < 28^\circ\text{C}$) in the light, Mel concentrations were more than 60% lower than those in hamsters that aroused in darkness, whereas during the late phase of arousal, Mel secretion was completely inhibited in the light but still elevated in darkness. It is surprising that any increased serum Mel was observed during arousals in the light because rapid light-induced suppression of nocturnal Mel secretion is well documented in numerous mammals, including the Siberian hamster (Tamarkin et al. 1980). However, in Siberian hamsters, strong sympathetic stimulation has been shown to override the inhibitory effects of light; in euthermic Siberian hamsters, the adrenergic agonist isoproterenol counteracts the suppressive effect of light on Mel secretion (Steinlechner et al. 1984). Thus,

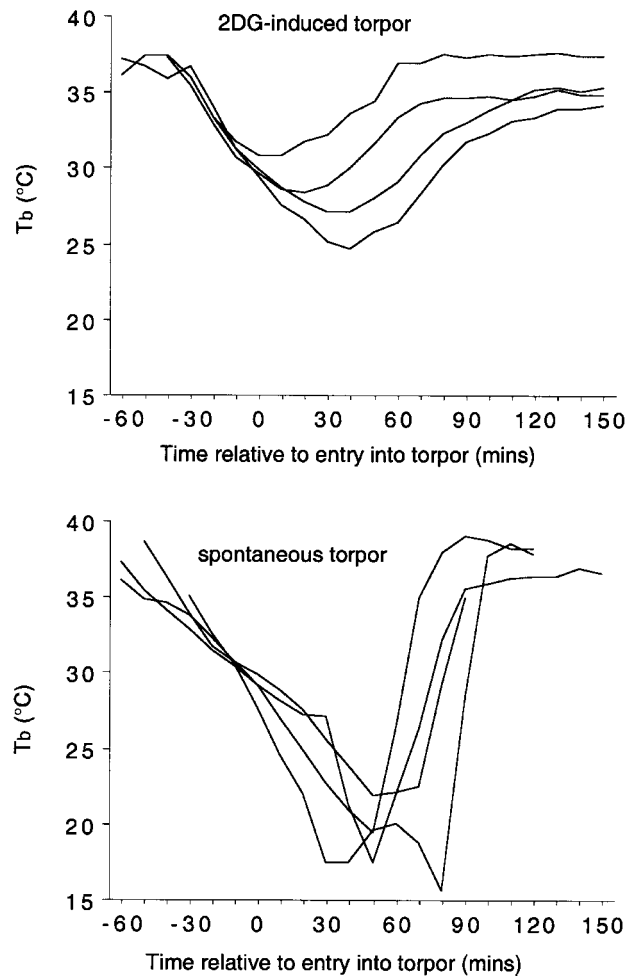


Figure 4. T_b during four typical 2-DG-induced torpor bouts (top) and four spontaneous torpor bouts (bottom), plotted relative to time of torpor entry. Time 0 is when T_b first declined to 31°C .

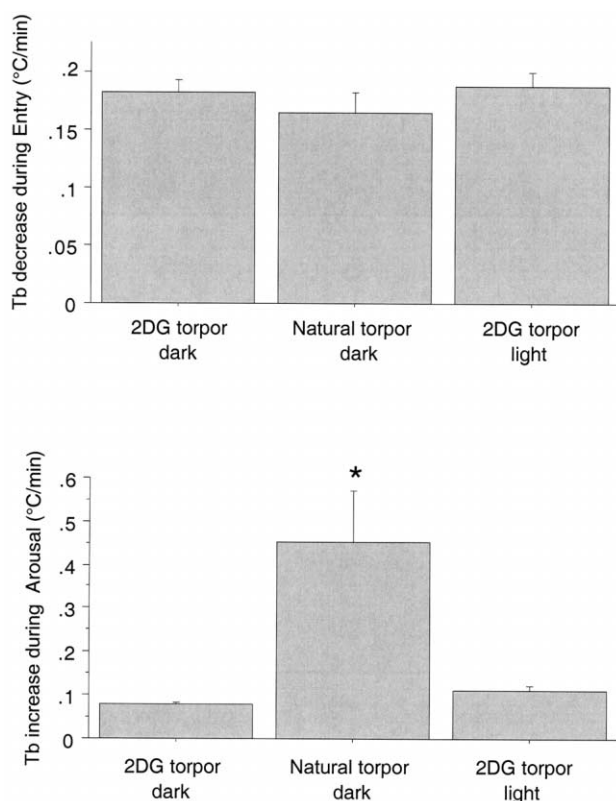


Figure 5. Rates of change in T_b during entry into (*top*) and arousal from torpor (*bottom*). An asterisk indicates $P < 0.05$ relative to other groups. Designation of light and dark refers to photic conditions during torpor and arousal. 2-DG torpor in dark $n = 5$; natural torpor in dark $n = 6$; 2-DG torpor in light $n = 6$.

we infer that the increase in sympathetic noradrenergic drive that mediates brown adipose tissue (BAT) thermogenesis in the early phase of arousal may partially override the inhibitory effect of light on Mel production, resulting in the blunted increase in serum Mel concentrations observed in arousal from torpor in the light. The attenuated increase in Mel in the light could reflect failure of light signal transduction to the SCN during arousal from torpor, thereby permissively facilitating Mel secretion in the light. There are conflicting results regarding maintenance of entrainment during torpor; loss of entrainment of circadian rhythms to the light-dark cycle has been reported during multiday torpor bouts in Syrian hamsters (Pohl 1987) but not in parallel experiments on golden-mantled ground squirrel (Ruby et al. 2002).

Mel production did not increase in hamsters that remained euthermic or exhibited only mild hypothermia after 2-DG administration. Serum concentrations of epinephrine and norepinephrine were increased after 2-DG treatment in rats (Scheurink and Ritter 1993); it is unknown whether similar increases occur in Siberian hamsters treated with 2-DG. In

either case, such changes are insufficient to provoke increased Mel production in the absence of torpor.

In the field, Siberian hamsters enter torpor in burrows that are shielded from light (Weiner 1982), so arousal-associated Mel surges most likely occur in darkness during the late subjective day; Mel amplitude and duration presumably are comparable to values recorded in the laboratory in darkness. The circadian clock is most responsive to Mel during late subjective day (Lewy et al. 1992; Benloucif et al. 1997; Van Reeth et al. 1997), which may explain why Siberian hamsters that regularly enter torpor have shorter circadian periods (τ) than did those that remained euthermic. This decrease in τ was attributed to direct effects of low T_b on circadian oscillators (Thomas et al. 1993). The present results suggest an alternative explanation: Mel pulses during arousal from torpor in late subjective day once every 2–3 d during the winter (Thomas et al. 1993; Heldmaier et al. 1999) may phase advance circadian rhythms (Lewy et al. 1992; Lockley et al. 2000), thereby accounting for the decrease in τ .

We are puzzled by the increased Mel concentrations 1–2 h after arousal from torpor in hamsters maintained in darkness. This secondary elevation was not an extension of the Mel secretion associated with arousal; serum Mel concentrations returned to basal values when animals attained euthermia only to increase subsequently. The secondary increase preceded the normal onset of darkness by 2–5 h and was therefore unlikely to be a component of the normal nocturnal increase in Mel secretion. It seems unlikely that this represents a phase advance of the circadian clock induced by the Mel surge that accompanies arousal; this would require a much larger phase shift than has previously been attributed to Mel in this species. Previous studies of the phase-shifting effects of Mel on circadian rhythms, however, were established in 12L : 12D or longer day lengths (Lewy et al. 1992; Benloucif et al. 1997; Van Reeth et al. 1997), so it remains possible that the phase-shifting response to Mel may be greater in short day lengths. Supporting this contention, the amplitude of the phase response curve is sensitive to day length, and photic phase shifts are more than doubled in Syrian hamsters entrained to short day lengths rather than long day lengths (Goldman and Elliott 1988).

Previous studies on 2-DG-induced torpor in this species have assumed that natural torpor and 2-DG-induced torpor were similar, but closer analysis did not support this assumption. One major difference was in the rate of T_b increase during arousal, which was four times slower in drug-induced torpor than natural torpor. Slower arousals may reflect enduring suppressive effects of 2-DG on BAT thermogenesis (Egawa et al. 1989a, 1989b). Although arousals in Siberian hamsters are mainly fueled by lipid rather than glucose metabolism (Heldmaier et al. 1999), 2-DG may have compromised the efficiency of thermogenesis by decreasing sympathetic nerve activity (Egawa et al. 1989b). The duration of torpor did not differ between natural torpor and 2-DG-induced torpor; the slower

arousals in 2-DG-induced torpor were offset by higher T_{bmin} , resulting in equivalent torpor duration.

Previous investigators speculated that the circadian oscillator that controls Mel production is arrested during hibernation and restarted on arousal (Vanecek et al. 1985); the Mel surge observed after arousal from multiday torpor bouts at any phase of the circadian cycle was attributed to reactivation of the circadian clock. A similar explanation could be advanced to account for increased Mel production after arousal from daily torpor. This hypothesis is unlikely on several grounds. First, circadian T_b rhythms are sustained throughout multiday torpor bouts in several species, which indicates that the circadian clock continues its time-keeping function throughout hibernation (Pohl 1987; Grahn et al. 1994; Ruby et al. 1996, 2002; Larkin et al. 2002). Second, the much higher T_b 's sustained during daily torpor are unlikely to interfere with normal SCN time-keeping activity (Miller et al. 1994). We suggest instead that the surge in Mel secretion associated with arousal from deep torpor in marmots and golden hamsters (Florant et al. 1984; Vanacek et al. 1985), and shallow torpor in Siberian hamsters (this study), is a direct consequence of increased sympathetic drive during arousal from torpor (Twente and Twente 1978; Himms-Hagen 1984; O'Shea 1993) unrelated to reactivation of a dormant circadian clock.

The sympathetic nervous system provides dense innervation to BAT in Siberian hamsters and innervates a variety of other peripheral tissues as well as the pineal gland (Bamshad et al. 1999). Selective control of sympathetic outflow to different targets has been demonstrated, but single neurons or clusters of neurons in the central nervous system also influence sympathetic activity in multiple target organs (Sved et al. 2001). The SCN is a major component of the neural system that controls firing rates of postganglionic noradrenergic fibers that terminate on pinealocytes and increase Mel secretion (Illnerova 1991). More recently, the SCN has been implicated as part of the sympathetic outflow pathway to BAT (Bamshad et al. 1999). As noted by Bartness et al. (2001, p. 196), "Individual neurons appear connected to more than one anatomic circuit" including sympathetic innervation of several peripheral tissues. On this basis, we speculate that SCN activation of BAT via the sympathetic nervous system, a sine qua non for arousal from torpor, concurrently increases sympathetic drive on the pineal gland. In this view, although nocturnal Mel production is regulated by the circadian system via the SCN, increased Mel secretion during arousal from torpor is an incidental byproduct of increased sympathetic tone and concomitant changes in SCN activity directed at increasing BAT thermogenesis. Although both entry and arousal from natural torpor are regulated by the circadian system, it is unlikely that arousal from 2-DG-induced torpor is gated by the circadian system because entry into torpor can be induced by 2-DG at any time of day or night. An intact circadian system is not necessary for torpor in this species because hamsters with SCN ablations can enter

and arouse from torpor (Ruby and Zucker 1992). Mel may also serve a protective role in mitigating possible oxidative stress (Reiter et al. 2001) associated with arousal from torpor.

In summary, arousal from bouts of daily torpor in Siberian hamsters is associated with marked increases in Mel production that parallel those manifested by species emerging from longer duration deeper hibernation bouts. We suggest that the increase in sympathetic drive, an omnipresent feature of the arousal process, is controlled by a neural system that includes the SCN. Increased SCN activity associated with BAT thermogenesis during arousal may concurrently depolarize postganglionic noradrenergic fibers that terminate in the vicinity of pinealocytes, thereby provoking increased Mel release.

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