Behavioral/Systems/Cognitive

Season Primes the Brain in an Arctic Hibernator to Facilitate Entrance into Torpor Mediated by Adenosine A_1 Receptors

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Torpor in hibernating mammals defines the nadir in mammalian metabolic demand and body temperature that accommodates seasonal periods of reduced energy availability. The mechanism of metabolic suppression during torpor onset is unknown, although the CNS is a key regulator of torpor. Seasonal hibernators, such as the arctic ground squirrel (AGS), display torpor only during the winter, hibernation season. The seasonal character of hibernation thus provides a clue to its regulation. In the present study, we delivered adenosine receptor agonists and antagonists into the lateral ventricle of AGSs at different times of the year while monitoring the rate of O_2 consumption and core body temperature as indicators of torpor. The A_1 antagonist cyclopentyltheophylline reversed spontaneous entrance into torpor. The adenosine A_1 receptor agonist N^6-cyclohexyladenosine (CHA) induced torpor in six of six AGSs tested during the mid-hibernation season, two of six AGSs tested early in the hibernation season, and none of the six AGSs tested during the summer, off-season. CHA-induced torpor within the hibernation season was specific to A_1AR activation; the A_3AR agonist 2-Cl-IB MECA failed to induce torpor, and the A_{2a}R antagonist MSX-3 failed to reverse spontaneous onset of torpor. CHA-induced torpor was similar to spontaneous entrance into torpor. These results show that metabolic suppression during torpor onset is regulated within the CNS via A_1AR activation and requires a seasonal switch in the sensitivity of purinergic signaling.

Introduction

Hibernation is essential for survival during seasonal deficiencies in food supply in several diverse lineages of mammals (Carey et al., 2003; Dausmann et al., 2004; Heldmaier et al., 2004). Survival is achieved by severe metabolic suppression, termed torpor, where rates of O_2 consumption fall to as low as 1% of resting metabolic rate and core body temperature (T_b) falls to as low as −3°C (Barnes, 1989; Geiser, 2004; Heldmaier et al., 2004). Torpor in hibernating mammals thus defines the nadir of mammalian metabolism and T_b, but mechanisms regulating initiation of torpor have been poorly understood (Heldmaier et al., 2004; Drew et al., 2007). Transition into the torpid state has been postulated to include three processes: (1) altered CNS control of thermoregulatory processes (Heller et al., 1977) and an extension of sleep (Walker et al., 1977, 1980), (2) active inhibition of metabolism such as inhibition of mitochondrial oxidative phosphorylation (Muleme et al., 2006), and (3) temperature-dependent effects on metabolic rate, or "Q_{10} effects" (Geiser, 2004). For larger mammals, the debate has centered more extensively on CNS control versus active suppression of metabolism via modulation of biochemical processes within metabolically active tissues.

In seasonal (obligate) hibernators, such as the arctic ground squirrel (AGS; *Urocitellus parryii*), torpor depends on a circannual cycle. The circannual cycle persists under constant photoperiods with food provided *ad libitum* (Heller and Poulson, 1970; Pengelley et al., 1976; Lee and Zucker, 1991). The seasonal character of hibernation thus provides a clue to its regulation. Once torpor ensues animals rewarm spontaneously every 2–3 weeks for brief (12–24 h) periods of normal body temperature (termed euthermy). This cycle continues until torpor ceases to occur in the spring. A two-switch model suggests that one physiological switch initiates the onset of the hibernation season and another switch initiates the onset of torpor (Serkova et al., 2007). The role of the CNS or the nature of the signaling events involved in either of these two switches have been unknown. Central purinergic signaling via A_1 adenosine receptors (A_1ARs) mediates sleep drive (Benington et al., 1995; Porkka-Heiskanen and Kalinchuk, 2011) and decreases body temperature (Miller and Hsu, 1992; Barros et al., 2006), and more recently, endogenous adenosine within the CNS has been found to decrease body temperature at presumed torpor onset in hamsters (*Mesocricetus auratus*) (Shiomi and Tamura, 2000; Tamura et al., 2005). Here we used intracerebroventricular drug administration to test the hypothesis that a seasonal change in purinergic signaling within the CNS is necessary for the onset of spontaneous torpor in the AGS, a

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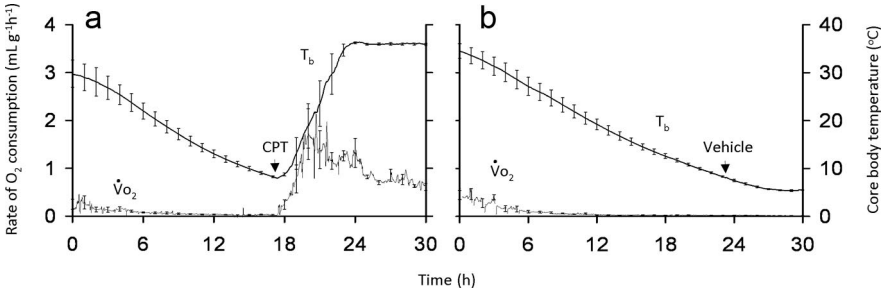

Figure 1. Onset of torpor requires A_1AR activation. ***a***, An increase in the rate of O_2 consumption ($\dot{V}_{O_2}$) and an increase in T_b to euthermic levels occurred in all animals tested following administration of CPT (3 nmol, i.c.v.) during onset of spontaneous torpor. This indicates that A_1AR activation is necessary for torpor onset. ***b***, Vehicle had no effect in any of the animals tested. Results are shown as means and SEM; $n = 6$ AGSs.

seasonal hibernator. We show for the first time that activation of A_1AR within the CNS is necessary and sufficient to induce torpor in AGS during the hibernation season, but not during the off-season, when AGSs do not spontaneously hibernate. The CNS and purinergic signaling are therefore key regulators of torpor onset, and sensitization to the effects of endogenous adenosine serves as a seasonally regulated switch that facilitates A_1AR-mediated torpor.

Materials and Methods

Animals. Procedures were approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee and Department of Defense Animal Care and Use Review Office. AGSs were captured near 66°38′N, 149°38′W under permit from the Alaska Department of Fish and Game. Animals were fed rodent chow and housed at 20°C with natural lighting for their wild-trapped latitude until mid-August, when they were moved to environmental chambers set to 2°C and 4:20 h light: dark (L:D). AGSs remained in these conditions until the end of the study. The hibernation season was defined by the presence of spontaneous torpor. The off-hibernation season (off-season) was defined by an absence of spontaneous torpor. Torpor was monitored daily by placing shavings on the animals' backs.

Surgery. Under sterile conditions, telemetry transmitters (model VM-FH, MiniMitter, or model CTA-F40, Data Sciences International) were implanted under isoflurane anesthesia. The head was leveled in a rat stereotaxic frame (Stoelting). Copalite (Cooley & Cooley) was applied to the skull. A target was marked at AP_{EBZ} +8.5 mm, L_{EBZ} +3.0 mm, the arm was tilted 15°, and the cannula tip was repositioned on the target. An internal cannula extending 1.0 mm beyond the guide cannula was connected to a syringe primed with sterile saline. The cannula assembly was lowered 5.5 mm from the brain surface and retracted until CSF was withdrawn. The guide cannula was secured to anchoring screws (Stoelting) and plugged with a dummy cannula (Plastics One). Animals received enrofloxacin (Bayer Health Care) (5 mg/kg, s.c., BID for 3 d), and ketoprofen (Fort Dodge Animal Health, 1 mg/kg, QD, s.c., for 3 d total). When CTA-F40 transmitters were used, animals received buprenorphine (Hospira, 0.03 mg/kg, QD, i.m., for 3 d) and 2 weeks separated transmitter surgery and intracerebroventricular cannula surgery. Following surgery, animals were housed at 20°C 4:20 h L:D and wounds were cleaned for 10 d before returning to environmental chambers at 2°C. Surgery was performed at least 1 month before drug testing.

O_2 consumption and body temperature. A cylindrical Plexiglas metabolic chamber (diameter 28 cm, height 23 cm) on a rat-turn (Bioanalytical Systems) was positioned over a telemetric receiver and T_b was acquired using DataQuest software A.R.T.2.3 (Data Sciences International). Air was drawn from a gas-tight swivel at the bottom of the chamber, filtered, and passed through a mass flow controller at 3 L/min (Model 840, 0–5 L/min, Sierra Instruments), and a subsample was passed through a multiplexing valve system and dried by a Nafion drier used in reflux mode (model PD-50T-24-PP, Perma Pure) before passing through the O_2 and CO_2 analyzers (Model FC-1B and CA-2A, Sable Systems International). The automated data acquisition and analysis software (LabGraph, developed by Tøien) interpolated between calibrations. O_2 consumption was corrected for respiratory volume change according to the principles of the Haldane transformation (Wagner et al., 1973; Karpovich et al., 2009). The integrity of the system was tested during and after the study period by burning 100% ethanol. Measured O_2 consumption was within 4% of that calculated from the weight loss of the lamp.

For monitoring subcutaneous temperature following intraperitoneal CHA injections, animals were implanted with IPTT-300 transponders (Bio Medic Data Systems), subcutaneously between the scapula. T_b was monitored using a telemetry system (DAS-6000; Bio Medic Data Systems) in the home cage every 30–60 min for at least 1 h before drug injection and every 1 h after injection for 4 h and again 30 h after intraperitoneal injection of CHA. Because the IPTT transponders are not reliable below ~30°C, to confirm minimal T_b at 30 h, rectal temperature was monitored with a thermocouple (Model H H21 Microprocessor Thermometer, Type J-K-T Thermocouple, OMEGA Engineering) in animals that were torpid after 30 h.

Drug administration. Animals tested for drug-induced torpor were aroused on day 3 or 4 of a torpor bout and moved from the environmental chamber (2°C, 4:20 L:D) to a warmer room (20°C, 4:20 L:D), where they remained overnight. On the following day, they were handled as described below and placed in the metabolic chamber for baseline recordings for at least 1 h before drug administration. For intracerebroventricular administration of CHA, injection cannulae primed with CHA or vehicle by an observer unaware of treatment were connected to a perfusion pump (Harvard Apparatus). Euthermic animals were lightly anesthetized with isoflurane as described for surgery and fit with a harness and injection cannula in a way that allowed animals to move freely within the metabolic chamber. After recovery from anesthesia, baseline O_2 consumption and T_b were collected for 1 h before delivering the drug (0.5 nmol CHA/10 μl, delivered over 1 min) or vehicle (10 μl, delivered over 1 min). The cannula was left in place and O_2 consumption and T_b were monitored for at least 24 h or until T_b was stable. Solutions used to dissolve the drugs (vehicle) were administered in a balanced cross-over design by an observer unaware of treatment. In this way, half of the animals received drug on the first test and vehicle on the second test and the other half received vehicle on the first test and drug on the second. Drug and vehicle tests were separated by at least 1 week. In a separate group of animals, a Y-injection cannula (Plastics One) was primed with 2-Cl-IB-MECA (3 nmol/10 μl), and the secondary line was primed with CHA (0.5 nmol/10 μl). Animals were treated as above except that the injection of 2-Cl-IB MECA (10 μl, delivered over 1 min) was followed by a second injection of CHA (3.3 μl to clear the cannula of 2-Cl-IB MECA, then 10 μl of CHA at 10 μl, delivered over 1 min).

Additional animals received pentobarbital (20 mg/kg, i.p.) during the midseason or off- (nonhibernating) season and T_b and O_2 consumption were monitored as described above. To ensure that the stress of intraperitoneal injections did not interfere with drug-induced torpor, a separate group of AGSs was administered CHA intraperitoneally during midseason.

For antagonist studies, torpid AGSs were habituated to handling before drug testing. During habituation, AGSs were handled daily to mimic handling necessary for the experiment until handling failed to induce arousal. At the next signs of torpor when T_b dipped to ~34°C, AGSs were fit with a harness and an injection cannula primed with antagonist (CPT, 3.0 nmol/10 μl) or vehicle by an experimenter unaware of treatment and onset of torpor proceeded without interruption. When T_b reached 10°C, 10 μl was delivered over 1 min and the cannula was left in place for an additional 24 h. MSX-3 (3.0 nmol/10 μl) was administered in the same way to another group of animals. The 3 nmol dose of MSX-3 was considered to be equipotent to the 3 nmol dose of CPT since MSX-2 has

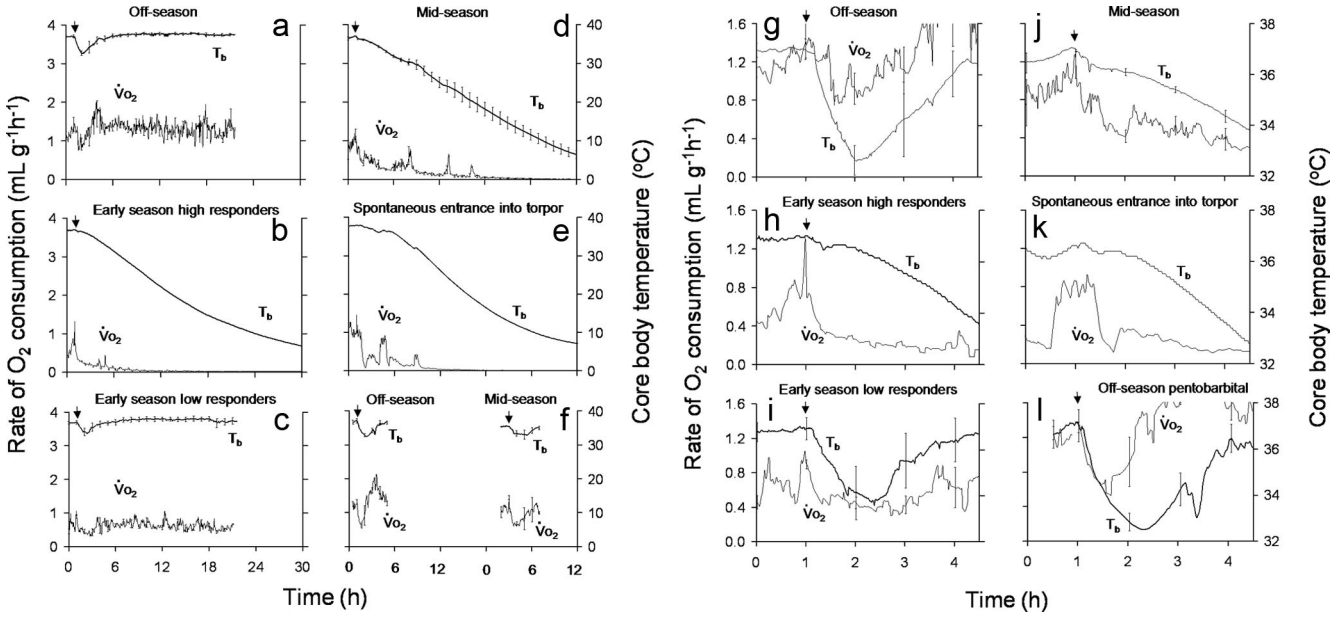

Figure 2. Sensitivity to the torpor-inducing effects of the A_1AR agonist CHA increases as the hibernation season progresses. ***a***, CHA during the off-season, when animals were not displaying spontaneous torpor, induced a slight decrease in $\dot{V}_{O_2}$ and T_b in all six AGSs tested. ***b***, Early in the hibernation season after all animals showed evidence of spontaneous torpor, CHA induced a torpor-like response in two of six animals tested. ***c***, In the remaining four animals, the same dose of the drug did not induce torpor. ***d***, By the middle of the hibernation season (midseason), the same dose of CHA induced torpor in all six AGSs tested. ***e***, Spontaneous torpor in one AGS. ***f***, Pentobarbital, regardless of season, induced a response similar to CHA during the off-season ($n = 3$). (The time scale on the *x*-axis in ***c*** applies to ***d*** and ***e*** and is a continuous 30 h.) ***g–l***, Detail of the first 4.5 h of ***a–f*** illustrates that CHA-induced torpor resembles spontaneous torpor where a rapid drop in metabolism is followed by a slow gradual decrease in T_b. ***g***, During the off-season CHA induces a rapid drop in T_b that begins before and at the same rate as the decline in O_2 consumption. ***h***, ***j***, ***k***, When CHA induces torpor (***h***, ***j***) and when animals spontaneously enter torpor (***k***), T_b declines more slowly than O_2 consumption. ***g***, ***i***, ***l***, When CHA fails to induce torpor (***g***, ***i***) and after pentobarbital (***l***), T_b and O_2 consumption decline at similar rates. Data shown are means ± SEM.

similar affinity for the A_{2a}AR as CPT has for the A_1AR. The 3 nmol dose of 2-Cl-IB MECA was considered to be higher than an equipotent dose of 0.5 nmol of CHA since 2-Cl-IB MECA has a slighter higher affinity for A_3AR than CHA has for A_1AR (Bruns et al., 1986; Klotz, 2000; Sauer et al., 2000; Solinas et al., 2005).

Drugs. N^6-Cyclohexyladenosine (CHA), 8-cyclopentyltheophylline (CPT), and phosphoric acid mono-(3-{8-[2-(3-methoxyphenyl) vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl}propyl) ester disodium salt (MSX-3) hydrate were purchased from Sigma-Aldrich, and 2-chloro-N^6-(3-iodobenzyl) adenosine-5′-*N*-methyluronamide (2-Cl-IB MECA) was purchased from Tocris Bioscience. CHA was dissolved in 0.01 M phosphate buffer, CPT and 2-Cl-IB-MECA were dissolved in 1% DMSO, and MSX-3 hydrate was dissolved in water. All solutions were sterilized by 0.2 μm filtration before use except for pentobarbital sodium, which was obtained as an injectable solution (50 mg/ml) (Abbott Laboratories).

Results

Onset of torpor requires A_1AR activation

To investigate whether A_1AR activation by endogenous adenosine within the CNS is necessary for the onset of spontaneous torpor in AGS, the A_1AR antagonist CPT (3 nmol/10 μl) was administered into the lateral ventricle during onset of spontaneous torpor, via an indwelling intracerebroventricular cannula. CPT, delivered by an investigator unaware of treatment, reversed torpor onset in all animals tested, while vehicle had no effect (Fig. 1).

Sensitivity to the torpor-inducing effects of the A_1AR agonist CHA increases as the hibernation season progresses

We next asked whether A_1AR activation within the CNS was sufficient to induce a state of torpor that mimicked spontaneous torpor both in temporal profile and in magnitude of decline in the rate of O_2 consumption and T_b. We also investigated whether the sensitivity to torpor-inducing effects of CHA, an A_1AR agonist, would increase as the hibernation season progressed. Six AGSs instrumented with intracerebroventricular cannula open to the lateral ventricle were administered CHA (0.5 nmol/10 μl) or vehicle in a blinded, balanced, cross-over fashion at three times of the year. These three tests commenced during the off-season when AGSs were not displaying spontaneous torpor, during the early hibernation season after all AGSs had begun to display spontaneous torpor, and during the middle of the hibernation season.

CHA administered during the off-season, induced a slight, temporary, reduction in O_2 consumption and T_b in all AGSs tested (Fig. 2*a*,*g*). Early in the hibernation season the same dose of

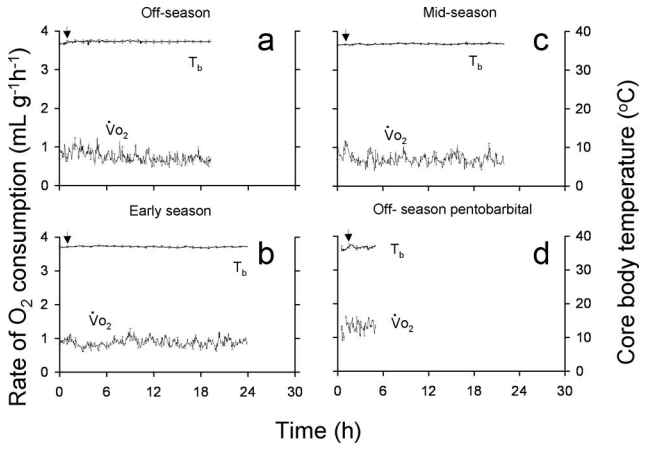

Figure 3. None of the vehicles tested produced a notable effect on T_b or $\dot{V}_{O_2}$. ***a–d***, Vehicle (0.01 M phosphate buffer, i.c.v., for CHA; ***a–c***); and saline (i.p., for pentobarbital; ***d***) failed to produce any notable change in T_b or $\dot{V}_{O_2}$. Data shown are means and SEM; $n = 6$ AGSs.

Table 1. Characteristics of AGS treated with CHA (0.5 nmol, i.c.v) during the three test seasons

	05-25	04-86	04-58	05-03	05-13	05-06
Age	Adult	Adult	Adult	Adult	Adult	Adult
Sex	Male	Male	Male	Male	Male	Male
Last day of torpor during previous season	18-Apr-07	4-Feb-07	19-Feb-07	3-Feb-07	17-Jan-07	15-May-07
First day of spontaneous torpor	26-Aug-07	1-Jul-07	25-Jul-07	22-Jul-07	1-Jul-07	1-Jul-07
Body weight (g)						
Off-season	601	551	820	680	630	681
Early season	860	881	801	940	700	801
Midseason	906	800	860	808	700	751
No. of spontaneous torpor bouts prior to CHA test						
Off-season	0	0	0	0	0	0
Early season	3	5	4	4	8	7
Midseason	9	9	8	9	13	13
Minimum T_b induced by CHA (°C)						
Early season	31.9	32.0	32.8	30.9	4.3	3.9
Midseason	4.6	3.9	5.2	5.3	4.3	4.7
Minimum $\dot{V}_{O_2}$ induced by CHA ($ml \cdot g^{-1} \cdot h^{-1}$)						
Early season	0.22	0.17	0.26	0.3	<0.02	<0.02
Midseason	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02

Column heads are AGS ID numbers.

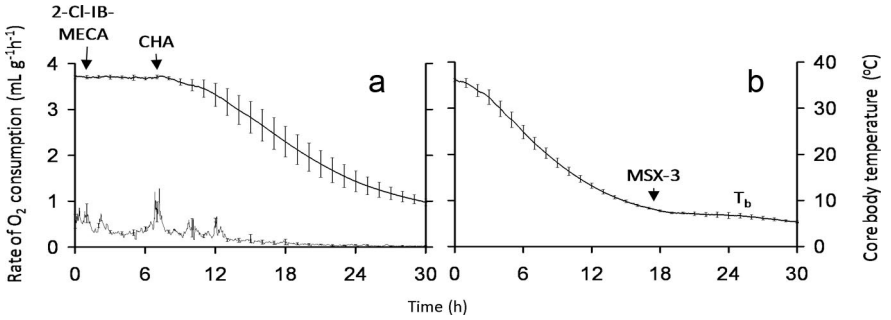

Figure 4. CHA-induced and spontaneous torpor is specific to A_1AR. ***a***, The selective A_3AR agonist 2-Cl-IB-MECA (3 nmol, i.c.v.) failed to induce torpor in any of the animals tested, while a subsequent injection of CHA (0.5 nmol, i.c.v.) induced torpor ($n = 3$). Top trace is T_b; bottom trace is $\dot{V}_{O_2}$. ***b***, MSX-3 (3 nmol, i.c.v.), a water-soluble prodrug of the A_2AR antagonist MSX-2, failed to reverse onset of spontaneous torpor ($n = 3$). Data shown are means and SEM.

CHA delivered to the same six AGSs induced a torpor-like decline in O_2 consumption and T_b in two of six animals tested (Fig. 2*b*,*h*) and an off-season-like response in the remaining four animals (Fig. 2*c*,*i*). By mid-hibernation season (midseason), the same dose of CHA induced a torpor-like response in all of these same six animals (Fig. 2*d*,*j*). The torpor-like response to CHA resembled spontaneous entry into torpor (Fig. 2*e*,*k*). Pentobarbital (20 mg/kg, i.p.) produced an off-season-like response regardless of season (Fig. 2*f*,*l*). Vehicle (phosphate buffer, i.c.v. for CHA or saline, i.p. for pentobarbital) did not produce a notable effect on T_b or rate of O_2 consumption at any season tested (Fig. 3).

We asked whether characteristics such as body weight, sex, age, and timing or evidence of prior torpor bouts predicted the magnitude or quality of the CHA-induced response during the early hibernation season. The two animals that displayed CHA-induced torpor when tested early in the season (Early season) had exhibited slightly more bouts of spontaneous torpor before this CHA test than the other four animals (Table 1). Other variables did not predict the larger response to CHA in these animals. Data shown in Table 1 illustrate that the hibernation season was defined by the presence of spontaneous torpor. In these animals, progression of the hibernation season was evident from the number of torpor bouts noted since the onset of spontaneous torpor. The circannual cycle of obligate hibernators, such as AGS, will free run when animals are housed under constant L:D conditions (Pengelley et al., 1976; Lee and Zucker, 1991). This free-running circannual cycle explains why the first day of spontaneous torpor occurred in July in many of the animals in this study.

CHA-induced and spontaneous torpor is specific to A_1AR

Although CHA is fairly selective for A_1AR, it has some affinity for A_3AR (Gao et al., 2003), leading us to ask whether A_3AR activation could account for CHA-induced torpor. The A_3AR agonist 2-Cl-IB-MECA (3 nmol/10 μl, i.c.v.), delivered during midseason, failed to induce torpor in any of the animals tested, although a subsequent injection of CHA (0.5 nmol/10 μl, i.c.v.) induced torpor as observed previously (Fig. 4*a*), indicating that A_3AR activation is not sufficient to induce torpor. Both A_1AR and A_{2a}AR play a role in sleep (Porkka-Heiskanen et al., 1997; Huang et al., 2005; Oishi et al., 2008); and torpor is in part an extension of sleep (Walker et al., 1977). We therefore asked whether A_{2a}AR receptors contribute to the onset of torpor. MSX-3, a water-soluble prodrug of the selective, high-affinity A_{2a}AR antagonist MSX-2 (Solinas et al., 2005), failed to reverse onset of spontaneous torpor in any of the animals tested (Fig. 4*b*). These results indicate that A_{2a}AR activation is not necessary for torpor onset.

Pentobarbital is a positive allosteric modulator of $GABA_A$ receptors, promotes sleep, and shows seasonal-dependent changes in efficacy across the hibernation season in thirteen-lined ground squirrels (Hengen et al., 2011). To investigate whether the seasonal change in response to CHA-induced torpor was specific to an adenosine agonist, pentobarbital was administered during the midseason and off-season. Pentobarbital was administered, intraperitoneally, to two groups of animals. One group was tested during the off-season, when animals failed to demonstrate spontaneous torpor. Another group was tested during the mid-hibernation season, when the total number of bouts of spontaneous torpor ranged between 12 and 16 bouts. Figure 2, *f* and *l*, shows that pentobarbital failed to induce torpor at any

Table 2. Characteristics of AGSs treated with pentobarbital (20 mg/kg, i.p.) during the off-season and during the middle of the hibernation season

	Off-season			Midseason		
	04-25	04-48	04-73	08-61	08-46	07-74
Age	Adult	Adult	Adult	Adult	Adult	Adult
Sex	Male	Male	Male	Female	Female	Male
Body weight (g)	714	707	689	826	606	911
No. of spontaneous torpor bouts prior to pentobarbital test	0	0	0	12	16	13
Minimum T_b induced by pentobarbital (°C)	30.0	32.6	32.0	33.2	30.0	33.1
Minimum $\dot{V}_{O_2}$ induced by pentobarbital ($ml \cdot g^{-1} \cdot h^{-1}$)	0.56	0.85	0.52	0.62	0.23	0.52

Column heads are AGS ID numbers.

Table 3. Characteristics of AGSs treated with CHA (0.5 mg/kg, i.p.)

	Off-season					Midseason			
	05-27	07-106	05-21	07-54	07-98	08-82	08-98	08-86	08-77
Age	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
Sex	Male	Male	Male	Male	Male	Female	Female	Female	Female
Body weight (g)	929	769	722	951	684	713	711	700	722
No. of spontaneous torpor bouts prior to CHA	0	0	0	0	0	9	7	7	8
Minimum T_b induced by CHA (°C)	30.8	35.2	35.9	34.6	32.7	3.8	4.4	5.1	4.4

Column heads are AGS ID numbers.

season tested. Table 2 shows that the characteristics of AGSs treated with pentobarbital during these two seasons are similar to the off-season and midseason groups of AGSs treated with CHA. Injections of pentobarbital, i.p., were noted to produce a brief, but detectible, increase in $\dot{V}_{O_2}$ that was not noted with intracerebroventricular administration of CHA (Fig. 2*f*). To ensure that intraperitoneal injections did not interfere with drug-induced torpor, separate groups of AGSs were treated with CHA (0.5 mg/kg, i.p.) during the off-season and during the midseason. Data shown in Table 3 show that intraperitoneal injections of CHA induced torpor during the midseason, but not during the off-season as seen for intracerebroventricular administration. Characteristics of AGSs were similar to other groups of animals tested during these two seasons.

Discussion

These results indicate that the CNS regulates the onset of torpor via activation of A_1AR. Sensitivity to the torpor-inducing effects of the A_1AR agonist CHA increases as the hibernation season progresses, and the torpor-inducing efficacy of CHA is specific to A_1AR activation. It is unlikely that the seasonal response to CHA was due to differences in cold adaptation, since animals were housed at 2°C throughout the study period.

Torpor in hibernating animals represents an extreme example of decreased metabolism, but the central or peripheral sites or signaling mechanisms involved in torpor onset have been unknown (Heldmaier et al., 2004). By administering purinergic ligands specific for the adenosine A_1, A_{2a}, or A_3 receptors (Table 4) into the lateral ventricle of AGSs at various times across the annual hibernation cycle, we find that adenosine within the CNS meets all of the necessary requirements for an endogenous mediator of torpor in AGS. A progressive increase in the sensitivity of AGS to A_1AR-mediated signaling within the CNS parallels the seasonal transition into the hibernation phenotype and provides

Table 4. Binding affinities for adenosine ligands

		K_i (nM)			
Drug	Dose (nmol)	A_1AR	A_{2a}AR	A_{2b}AR	A_3AR
CPT	3.0	10.9[a]	3170[b]	902_h[b]	>10,000[b]
MSX-2	3.0	900[c]	9.1[b]	$2{,}900_h$[b]	>10,000[b]
CHA	0.5	0.9[d]	514[e]	n.a.	167[e]
2-Cl-IB-MECA	3.0	820[f]	470[f]	n.a.	0.33[f]

[a]Bruns et al. (1986); [b]Solinas et al. (2005); [c]Sauer et al. (2000); [d]Gao et al. (2003); [e]van Galen et al. (1994); [f]Kim et al. (1994); h, human recombinant receptors; n.a., not available.

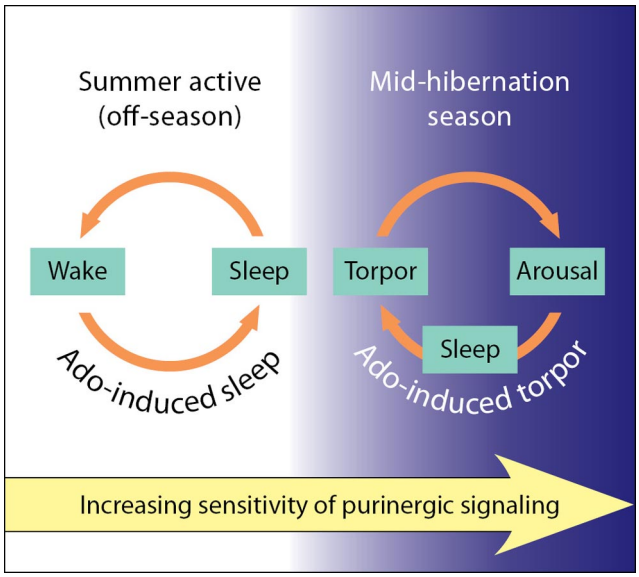

Figure 5. Enhanced purinergic signaling turns on the seasonal switch to hibernate in arctic ground squirrels. Schematic diagram modified from the two-switch model of Serkova et al. (2007) illustrates how seasonal sensitization of purinergic signaling primes the brain for adenosine-induced torpor during the hibernation season. The off-season, commonly referred to as the "summer-active" season, is indicated by a white background. During the off-season, overflow of adenosine that occurs as part of normal purinergic signaling fails to induce torpor. Here we use homeostatic sleep drive as an example of normal purinergic signaling (Porkka-Heiskanen et al., 1997; Basheer et al., 2004). The present report shows that an increase in the gain in purinergic signaling occurs during the hibernation season. The hibernation season is indicated by a dark background and the shading from light to dark illustrates an increase in gain in purinergic signaling as the season progresses. This increased gain in purinergic signaling during the hibernation season primes the brain such that overflow of endogenous adenosine with subsequent activation of A_1AR now induces torpor. The effect of endogenous adenosine is demonstrated by the ability of an A_1AR antagonist (CPT) to reverse onset of spontaneous torpor.

an example of a seasonal switch proposed in the two-switch model for obligate hibernation (Serkova et al., 2007). We show that in the context of this model, increased gain in central purinergic signaling serves as the first switch, and stimulation of central A_1AR by endogenous adenosine serves as a second switch, that induces torpor (Fig. 5).

Because A_1AR activation is necessary for the homeostatic sleep response (Bjorness et al., 2009), an increased gain in purinergic signaling predicts an increase in sleep drive during the hibernation season. Although sleep drive has not been monitored in AGS, golden-mantled ground squirrels sleep more during the hibernation season (Walker et al., 1980).

Prolonged torpor in hibernating mammals is distinguished by at least three distinct processes that include onset of torpor, maintenance of torpor, and arousal from torpor (Drew et al., 2007). In hamsters (*M. auratus*), A_1AR activation is necessary for torpor onset, as shown here for AGS, but is not necessary to maintain prolonged torpor (Tamura et al., 2005). Seasonal alter-

ations in signaling events involved in torpor maintenance or interbout arousal are as yet unclear. Moreover, while the present results demonstrate that A_1AR activation is necessary and sufficient to induce torpor in AGS, it is unlikely that adenosine is the only neuromodulator involved with torpor onset.

The present results clearly show that A_1AR stimulation is sufficient to initiate torpor onset that results in a decrease in metabolic rate to levels that are below basal metabolic rate, a value determined to be within 0.40 and 0.61 $ml \cdot g^{-1} \cdot h^{-1}$ for AGS (Scholander et al., 1950; Withers et al., 1979). Geiser (2004) describes a scenario in which decreased thermogenesis leads to cooling, which then via thermodynamic effects decreases oxygen consumption to torpid metabolic rates. This scenario may account for how central A_1AR-induced inhibition of thermogenesis in AGS could lead to a consequent lowering of T_b that is then sufficient to account for torpid metabolic rates reported here. This explanation incorporates two of three proposed mechanisms of metabolic suppression in hibernating animals. One mechanism includes the central inhibition of thermogenesis associated with a lowering of brain temperature necessary to induce thermogenesis (Heller et al., 1977). A second mechanism involves thermodynamic effects of cooling on metabolic rate described by the Van't Hoff equation (Atkins and De Paula, 2006). In biological systems, temperature effects are often referred to as Q_{10} effects, where reaction rates generally double or triple with every 10°C increase in temperature (Schmidt-Nielsen, 1997). A third set of mechanisms involves "active" suppression of cellular processes such as ion channel arrest (Hochachka, 1986) or inhibition of cellular respiration (Muleme et al., 2006). It is difficult to explain how central A_1AR activation could directly cause global, "active" suppression of cellular processes; however, global effects could occur downstream to torpor initiation.

Given that cooling contributes to metabolic rate reduction, cooling during onset of torpor will influence the subsequent decrease in metabolic rate. Unexpectedly, a rapid rate of cooling induced by CHA during the off-season contrasted with a significantly slower rate of cooling induced by CHA during the hibernation season. All animals had been housed at 2°C for several months, so cold adaptation is unlikely to account for differences in the rate of cooling. O_2 consumption and T_b were measured in the present study as physiological parameters used to clearly distinguish torpor onset from hypothermia. Since thermoregulatory systems are independently regulated, further study of multiple thermoeffector activities is warranted to achieve a more complete understanding of torpor as a thermoregulatory response (Romanovsky, 2007; Nakamura et al., 2009).

The mechanism that increases the gain in purinergic signaling may involve changes in receptor expression or function, changes in extracellular levels of adenosine, or changes in neural circuits regulating sleep, metabolism, or body temperature. Seasonal changes in sensitivity to allosteric modulation of $GABA_A$ receptors by pentobarbital in thirteen-lined ground squirrels have been observed. These changes are restricted to cardiorespiratory neurons and are associated with altered expression of ε and δ $GABA_A$ receptor subunits (Hengen et al., 2009, 2011). In the present study, pentobarbital did not induce torpor; however, a role for altered allosteric modulation of $GABA_A$R in adenosine-mediated torpor induction cannot be ruled out.

The capacity of an A_1AR agonist to induce a torpid state may confer some of the neuroprotective aspects of hibernation noted previously (Zhou et al., 2001). Central A_1AR stimulation prevents cardiac arrhythmias during cooling in hamsters (Miyazawa et al., 2008) and may offer a means to avoid cardiac arrhythmias or other side effects encountered during therapeutic hypothermia (Polderman and Herold, 2009). H_2S-induced suspended animation has led to investigation of H_2S as a therapeutic agent (Blackstone et al., 2005). Likewise, understanding how hibernating mammals regulate metabolic suppression has potential to translate to improved therapies for conditions in which oxygen and energy supply fail to meet demand. Such conditions include stroke, cardiac arrest, hemorrhagic shock, and trauma.

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