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## Neural signaling metabolites may modulate energy use in hibernation

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The United States is experiencing an epidemic in obesity with an increase in childhood obesity and type 2 diabetes<sup>1</sup>. Understanding and promoting healthy lifestyles is a first step towards combating this epidemic, however, lifestyle changes are inherently difficult to achieve; highlighting the significance of understanding the neurochemistry of energy homeostasis; the balance between energy intake and expenditure. Several peptide neuromodulators modulate energy intake that in turn influence energy expenditure via downstream anabolic and catabolic responses.<sup>2</sup> Energy expended on thermogenesis through well-defined neural circuits also contributes to overall energy balance.<sup>3</sup> Despite basic understanding of pathways and modulators limited means exist to effect therapeutic changes in adiposity or metabolic rate other than life style changes. Here we review evidence that neural signaling metabolites may modulate the gain in thermoregulatory pathways during hibernation and torpor and offer novel insights into means to fine tune energy use. We extend prior reviews on mechanisms of metabolic suppression in hibernation<sup>4–10</sup> by focusing primarily on neural signaling metabolites.

Hibernation is an extreme example of energy conservation that offers a system in which to explore modulatory processes fundamental to the regulation of energy balance. Here we give a general overview of hibernation and discuss evidence that in hibernation the gain in thermoregulatory and possibly adiposity signaling is modulated, in part, by neural signaling metabolites. Specifically we discuss a role for metabolite signaling molecules, adenosine, 5'-AMP and glutamate in the onset and emergence from hibernation.

### Introduction to hibernation and torpor – A means of energy conservation

Hibernation is a means of energy conservation that is driven by a change in thermoregulation.<sup>11, 12</sup> Field and laboratory studies suggest that pre-hibernation energy conservation begins with a gradual decrease in body temperature ( $T_b$ )<sup>13–16</sup> presumably via a decrease in thermogenesis. In the arctic ground squirrel the pre-hibernation decrease in  $T_b$  begins prior to a decline in food intake<sup>14</sup> suggesting that decreased energy expenditure leads to a decrease in food intake. Hibernation conserves energy to such an extent that animals survive without food or water for 5–8 months despite ambient temperatures that can fall to  $-20^{\circ}\text{C}$ .<sup>17, 18</sup> Over the course of the hibernation season, the majority of energy is conserved during prolonged bouts of torpor where metabolic rate (MR) decreases to as low as 1–2% of basal metabolic rate (BMR),<sup>19, 20</sup> and body temperature falls to within 1–2°C above ambient

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temperature ( $T_a$ ).<sup>20</sup> Studies in arctic ground squirrels suggest that pre-hibernation remodeling involves a gain in the sensitivity of  $A_1$  adenosine receptors ( $A_1AR$ ) that eventually leads to the onset of prolonged torpor.<sup>14, 21</sup> Torpor onset is identified by an initial decrease in the rate of oxygen consumption ( $VO_2$ ) and a subsequent decrease in core body temperature ( $T_b$ ). A single bout of torpor consists of three phases; onset, maintenance and arousal.

Several diverse species of mammals hibernate and many other species show short (less than 24h) bouts of daily torpor. Daily torpor can be classified as shallow torpor in contrast to deep torpor or hibernation.<sup>23</sup> Daily torpor is not hibernation, but aspects of energy conservation are similar in daily torpor and hibernation and prolonged periods of lowered  $T_b$  and metabolic rate during hibernation may be called torpor, as is done in this review. Hibernation is distinguished from daily torpor by the prolonged length of the torpor bout which can last up to 2–3 weeks in some species of ground squirrels, or just a few days in hamsters. Brief (10–20h) interbout arousals interrupt prolonged torpor with predictable regularity and consume significant amounts of energy.<sup>22</sup> Hibernation can be described as obligate or facultative. Most typically, ground squirrels are obligate hibernators while hamsters are facultative hibernators. Obligate hibernators, hibernate according to an endogenous circannual rhythm. Facultative hibernators hibernate in response to shortening day length and decreases in food or water availability. Daily torpor occurs during acute periods of food or water deprivation. Daily torpor differs from hibernation in limited duration, by less extreme decreases in MR and  $T_b$ , and by more immediate expression in response to environmental challenge<sup>23</sup>. Both hibernation and daily torpor represent regulated shut down of energy consumption and are not pathological consequence of starvation<sup>24</sup>.

Several classes of molecules have been investigated as modulators or mediators of hibernation and torpor. These include neuropeptides, nutrients or molecules that block nutrient use such as 2-deoxyglucose, neurotransmitters such as 5HT and histamine and metabolite/neuromodulators such as 5'-AMP and adenosine (reviewed in Drew et al<sup>25</sup>). The orexigenic neuropeptide NPY and the metabolites AMP and adenosine have been studied more extensively than other signaling molecules. We review briefly evidence supporting a role for these molecules. We also acknowledge that comprehensive confirmation that these putative endogenous mediators are necessary, sufficient and present in the extracellular compartment in brain during onset of torpor is lacking.

## Putative endogenous mediators of torpor

### Neuropeptides

Although not a neural signaling metabolite amongst neuropeptides NPY shows the greatest promise as an endogenous regulator of torpor. Injection of NPY into the third ventricle induces torpor-like hypothermia which is reversed by a NPY Y1 receptor antagonist and, like torpor, depends on photoperiod.<sup>26</sup> Ablation of the arcuate nucleus abolishes torpor in Siberian hamsters and fasting-induced torpor in mice.<sup>27</sup> Ghrelin, a gut appetite hormone and neuropeptide activates NPY neurons in the arcuate nucleus and deepens torpor in mice.<sup>28</sup> These studies support the hypothesis that NPY Y1 receptor sites activated by NPY within the arcuate nucleus induce a torpor-like state through modulation of

thermoregulation.<sup>26, 27, 29</sup> The efficacious orexigenic effect of NPY, however, is difficult to reconcile within the context of hibernation because onset of hibernation is preceded by a hypophagic period.<sup>30</sup> Indeed, TRH is the only member of the group of neuropeptides, well-known to regulate food intake that is upregulated in the hypothalamus during pre-hibernation satiety.<sup>30</sup> TRH expression during the hypophagic period fits previous findings that TRH is anorexigenic<sup>31</sup>. Nonetheless, TRH reverses hibernation in Syrian hamsters<sup>32</sup> decreases sleep<sup>33, 34</sup> and increases body temperature.<sup>35</sup> Reversal of spontaneous torpor with a NPY Y1 receptor antagonist has, to our knowledge, not been tested or reported.

## Adenosine

Adenosine is unique amongst the putative mediators of torpor because it affects sleep, appetite and body temperature in a manner consistent with hibernation and torpor. Indeed, adenosine promotes sleep,<sup>36</sup> lowers body temperature<sup>37</sup>, and reduces food intake.<sup>38</sup> Moreover, stimulation of A<sub>1</sub>AR induces torpor in hibernating species most likely through an inhibition of thermogenesis and modulation of parasympathetic tone<sup>37, 39</sup>. Spontaneous torpor is reversed by A<sub>1</sub>AR antagonists in Syrian hamsters, arctic ground squirrels and fasting-induced torpor in mice.<sup>32, 40–42</sup>

In facultative hibernators, such as the Syrian hamster (*Mesocricetus auratus*), A<sub>1</sub>AR dependent signaling within the CNS is necessary for torpor onset. This claim is based on evidence that the A<sub>1</sub>AR antagonist cyclopentyltheophylline (CPT) reverses spontaneous torpor when administered into the lateral ventricle in Syrian hamsters during torpor onset.<sup>42</sup> Interestingly, CPT has no effect when delivered later in the torpor bout demonstrating that mechanisms controlling onset, maintenance and interbout arousal are mediated by distinct mechanisms. While blocking A<sub>1</sub>AR reverses torpor, CHA, an A<sub>1</sub>AR agonist, administered into the lateral ventricle of Syrian hamsters, induces a torpor-like decline in T<sub>b</sub> at a rate similar to the rate of fall in T<sub>b</sub> during spontaneous torpor.<sup>32, 42</sup>

In the hibernating arctic ground squirrel (AGS; *Urocitellus parryii*), like in hamsters, evidence suggests that torpor onset is regulated in part within the CNS by A<sub>1</sub>AR signaling. In these obligate hibernators the efficacy of the A<sub>1</sub>AR agonist <sup>6</sup>N cyclohexyladenosine (CHA) depends on a seasonal change in sensitivity (Jinka *et al.*, 2011). We observed that CHA injected ip or delivered via intracerebroventricular (icv) administration into the lateral ventricle induces torpor in AGS in winter, but not in summer. Moreover, we observed that CHA-induced torpor is similar to spontaneous torpor with regard to the magnitude and temporal profile of both the decrease in oxygen consumption and T<sub>b</sub>, and that the A<sub>1</sub>AR antagonist cyclopentyl theophylline (CPT) reverses naturally occurring torpor. We then found that A<sub>1</sub>, but not A<sub>2</sub> or A<sub>3</sub> adenosine receptor stimulation is necessary to support spontaneous torpor.<sup>40</sup> Circannual changes in sleep<sup>43</sup> and euthermic T<sub>b</sub><sup>14</sup> and sensitivity to CHA suggests that an increase in the gain in A<sub>1</sub>AR mediated signaling within sleep and thermoregulatory pathways underlies pre-hibernation remodeling of sleep and thermogenesis.<sup>14</sup> T<sub>b</sub> decreases by 1 to 2°C before animals hibernate and this gradual modification of thermogenesis and metabolism predicts onset of hibernation.<sup>14–16</sup> Remodeling of thermoregulation and metabolism is postulated to result from changes within the hypothalamus<sup>44</sup> as well as from altered sensitivity of A<sub>1</sub>AR.<sup>14</sup> Increased sleep drive and

lower  $T_b$  in winter ground squirrels that are not hibernating when compared to summer ground squirrels is consistent with increased sensitivity of  $A_1AR$ .<sup>43</sup>

Although the house mouse (*Mus Musculus*) does not hibernate many strains show daily torpor in response to fasting. C57BL mice display daily torpor within 6–7h when food is withheld during the dark period (when mice eat). During onset of this fasting-induced daily torpor MR falls prior to a fall in  $T_b$  in a manner similar to what is observed in hibernation. The slow rate of cooling during onset of hibernation and daily torpor compared to a faster rate of cooling during hypothermia suggests that the rate of cooling distinguishes torpor from hypothermia. Daily torpor in C57BL mice also depends on central  $A_1AR$  activation since, like hibernation in ground squirrels and hamsters, the onset of daily torpor is reversed by CNS administration of an  $A_1AR$  antagonist.<sup>41</sup>

The requirement of CNS  $A_1AR$  in daily torpor emphasizes a common mechanism despite several differences between daily (shallow) torpor and hibernation (deep torpor). During daily torpor  $T_b$  falls from around 37°C to between 15–25°C. By contrast, during hibernation  $T_b$  may decrease to as cold as -3°C<sup>23</sup> depending on the species and the ambient temperature.<sup>18</sup> In one study, metabolic rate (MR) during *daily torpor* in *Mus musculus*, weighing 45g, decreased from a basal MR (BMR) of about 1.5mLg<sup>-1</sup>h<sup>-1</sup> to a torpid MR (TMR) of 0.3mLg<sup>-1</sup>h<sup>-1</sup>. In these same animals  $T_b$  decreased from 37 to 19°C. By contrast MR during *hibernation* in a Western Jumping Mouse (*Zapus princeps*) weighing 33g decreased from a BMR of 1.5 mLg<sup>-1</sup>h<sup>-1</sup> to a TMR of 0.04 mLg<sup>-1</sup>h<sup>-1</sup> and  $T_b$  decreased from 37°C to 5°C<sup>23</sup> (Table 1). Some of the differences in torpid metabolic rates observed during daily (shallow) torpor and hibernation (deep torpor) may be due to colder ambient temperatures and colder torpid  $T_b$  in hibernating species. *Mus musculus* does not tolerate ambient temperatures below about 15°C. Consequently study of daily torpor in this species is usually at ambient temperatures of 20–21°C while study of hibernation is usually at ambient temperatures of 2–5°C.

The laboratory rat (*Rattus norvegicus*), a non-hibernating species, does not show spontaneous torpor and for this reason is an ideal rodent species to investigate if mechanisms involved in daily torpor or hibernation have potential to translate to humans. Adenosine agonists promote sleep in rats and other nonhibernating species, including humans.<sup>36, 45</sup> The  $A_1AR$  agonist CHA inhibits shivering and nonshivering thermogenesis in rats and increases parasympathetic tone to the heart, both physiological characteristics of hibernation.<sup>37</sup> A number of studies of the effects of  $A_1AR$  agonists and purine nucleotides in rats suggest that this mechanism will translate to non-hibernating species. Moreover, small seasonal fluctuations in metabolic rate have been observed in humans and it is tempting to speculate that  $A_1AR$  mechanisms are involved.

As discussed above, the onset of hibernation is anticipated by a decrease in basal metabolic rate and food intake.<sup>14, 46</sup> Gradual changes in metabolism are seasonally dependent and proposed to be controlled by the CNS via molecular mechanisms not fully understood<sup>14</sup>. These mechanisms appear to be present and operative in non-hibernating mammals. It has been shown that during the winter season humans acclimate to colder temperature, even in mild climates.<sup>47</sup> The acclimation is independent from body mass and body composition,

suggesting autonomic and endocrine changes as potential mechanisms (Ooijen et al. 2004).<sup>47</sup> Season is also known to affect weight and appetite as well as social and physical activity, which are associated with higher risk of obesity leading to metabolic syndrome.<sup>48</sup> Even if the effects of season are less dramatic in humans than in hibernating mammals, seasonality plays a role in physiological changes and in gene expression of non-hibernating mammals.<sup>49</sup> In obligate hibernators season drives remodeling of mechanisms that preserve energy balance. Similar pre-hibernation mechanisms are conserved in facultative hibernators<sup>16</sup>. Less is known about fasting-induced torpor. Understanding mechanisms of remodeling, which are proposed to underlie onset of hibernation, and potentially daily torpor, is likely to unveil novel mechanisms that affect energy expenditure via modulation of known thermoregulatory neural pathways. The ability to modulate these mechanisms may enable means to regulate metabolism in a manner that reduces morbidity associated with metabolic syndrome and type 2 diabetes.

### **Adenosine A<sub>1</sub>AR mediate onset of hibernation through an inhibition of thermogenesis**

Onset of torpor involves a suite of coordinated physiological processes; one of which is a decrease in  $T_b$  setpoint<sup>50</sup> that can be viewed mechanistically as an inhibition of thermogenesis. Because metabolic rate is influenced by an individual's thermoneutral zone, and thus energy expended for thermogenesis, study of hibernation presents an opportunity to reveal novel means to modulate the gain in thermoregulatory pathways. Understanding how thermogenesis is altered in hibernation may provide novel insights into treatments for obesity. By reversing mechanisms that downregulate thermogenesis and energy expenditure in hibernation we may be able to modulate the gain in thermogenesis to promote weight loss. Although large increases in thermogenesis would require large changes in heat dissipation, moderate increases in thermogenesis could be offset in cooler climates by lighter weight clothing and lower ambient temperatures.

A<sub>1</sub>AR modulates thermogenesis, one of the most energy demanding requirements of homeothermy, in part through effects on the nucleus of the solitary tract (nTS). Stimulating A<sub>1</sub>AR within the nTS<sup>37, 51</sup> inhibits shivering and nonshivering thermogenesis. Moreover evidence suggests that sensory processing is modified within the nTS in Syrian hamster as brain temperature declines<sup>52</sup>. Inhibition of thermogenesis during onset of hibernation decreases oxygen consumption and this immediate energy savings is followed by a subsequent decline in  $T_b$ . The preoptic area of the hypothalamus (POAH), the thermosensitive region of the CNS, is another potential site where A<sub>1</sub>AR may modulate thermogenesis.<sup>50, 53</sup> The POAH has been proposed as the main integrator of sleep and thermoregulatory information which is modulated by A<sub>1</sub>AR.<sup>53-56</sup> Alternative means of inhibiting thermogenesis is also sufficient to induce deep hypothermia, or suspended animation in rats<sup>57, 58</sup>, but these mechanisms have not been implicated as endogenous drivers of hibernation.

## Is adenosine the endogenous signaling molecule?

Adenosine is a likely candidate as an endogenous A<sub>1</sub>AR agonist that is capable of driving the onset of torpor based on the purine's role in sleep<sup>45</sup>, thermoregulation<sup>39, 53</sup> and energy homeostasis<sup>59</sup>. Adenosine is a neuroprotective neuromodulator and by-product of cellular metabolism that is positioned within neurons, astrocytes and peripheral tissues to modulate brain and systemic energy homeostasis through integrated biochemical and receptor mediated processes. Adenosine is an end-product and precursor of adenyly nucleotides (AMP, ADP and ATP) and has high affinity for a family of G-protein coupled receptors named; A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, A<sub>3</sub>. Through biochemical and receptor mediated processes adenosine functions as a bioenergetic network regulator.<sup>60</sup>

Extracellular adenosine mediates homeostatic sleep drive.<sup>61</sup> Sleep deprivation leads to an increase in adenosine in basal forebrain and other brain regions and declines once sleep ensues.<sup>62</sup> Adenosine contributes to homeostatic sleep by suppressing cortical arousal through inhibition of cell groups that promote arousal or disinhibition of cell groups that promote sleep. In this way, adenosine restores homeostasis through biochemical and hyperpolarizing influences; illustrating cross-talk between biochemical and neuromodulatory pools of adenosine. Adenosine also plays a role in thermoregulation<sup>63</sup> and A<sub>1</sub>AR agonists are potent inhibitors of shivering and nonshivering thermogenesis.<sup>37, 51</sup>

## Is AMP the endogenous signaling molecule?

AMP is well known as a biochemical regulator capable of modulating cellular energy homeostasis through biochemical mechanisms. Moreover, AMP induces a torpor-like state<sup>64, 65</sup>, identified as a decrease in the rate of oxygen consumption and T<sub>b</sub>. Like adenosine, AMP, inhibits thermogenesis via stimulation of A<sub>1</sub>AR even when conversion to adenosine is blocked.<sup>53</sup> This evidence as well as evidence that AMP inhibits firing of thermosensitive neurons within the hypothalamus<sup>53</sup> argues for AMP as a purinergic thermoregulatory neuromodulator with sufficient potency to orchestrate the onset of torpor through an inhibition of thermogenesis. AMP was also shown to induce, in mice, a torpor-like decrease in T<sub>b</sub>.<sup>65</sup> The same study showed that AMP increased during constant darkness, and suggested that AMP functioned as a circadian metabolic signal.<sup>65</sup> Circulating levels of AMP increased in mice when 24h darkness was used to mimic conditions of hibernation and fasting-induced daily torpor was associated with an increase in blood levels of AMP.<sup>65</sup> Moreover, AMP induced expression of murine procolipase, a gene upregulated in peripheral tissues during the 24h dark conditions.<sup>65</sup> The effects on T<sub>b</sub> originally attributed to activation of AMPK<sup>65</sup> was later found to depend on an A<sub>1</sub>AR dependent mechanism.<sup>66, 67</sup> Additional study spurred debate as to whether the AMP-induced hypothermia was torpor. Ablation of the arcuate nucleus eliminated fasting-induced torpor, but did not eliminate the torpor-like response to AMP.<sup>67</sup>

One interpretation is that activation of A<sub>1</sub>AR within the CNS by AMP and/or adenosine is sufficient to induce a torpor-like state through an inhibition of thermogenesis and that A<sub>1</sub>AR stimulation is the mechanism responsible for lowering the thermoregulatory set point during onset of hibernation. Fasting induced torpor in mice and hibernation in ground squirrels is



modulated by circadian and circannual signals. Importantly, as discussed above, the torpor-like effects of AMP in mice are enhanced under 24h darkness<sup>65</sup> and the torpor-like effects of the A<sub>1</sub>AR agonist CHA in ground squirrels are seen only in the hibernation season.<sup>40</sup> Indeed, the quality of response to CHA with regard to time course and magnitude of CHA-induced VO<sub>2</sub> and T<sub>b</sub> decline differs substantially between summer and winter ground squirrels.<sup>40</sup> The circadian and circannual characteristics of A<sub>1</sub>AR agonists and circulating levels of AMP argue for a role for adenosine and/or AMP as neural signaling metabolites in hibernation. Nonetheless, it remains to be determined if adenosine or AMP increase in brain according to a seasonal or circadian rhythm that predicts onset of torpor.

Given the poorly understood circadian and circannual modulation of neural control of torpor, or the extreme phenotype of deep and shallow torpor it may not be feasible to translate these mechanisms to humans for therapeutic applications. However, studies in rats suggest that the thermolytic and parasympathomimetic effects of CHA in rats mimics neuronal mechanisms governing hibernation.<sup>37</sup> Interestingly, the thermolytic potency of CHA in rats is increased by dietary restriction, although dietary restriction does not precede seasonal sensitivity to the thermolytic effects of CHA in ground squirrels.<sup>14, 39</sup> Nonetheless, the profound effect of CHA on thermogenesis suggests the presence of the same neuronal substrate involved in the regulation of energy balance irrespective of whether the animals actually hibernate. Further study of circadian and circannual influence on the response to CNS A<sub>1</sub>AR stimulation has potential to unveil mechanisms to fine-tune metabolism.

## Adenosine and 5' AMP in cellular energy homeostasis

Adenosine and 5' AMP (AMP) are reciprocal precursors of each other making them part of the same biochemical pathways involved in cellular energy homeostasis. Adenosine conversion to AMP is catalyzed by adenosine kinase. AMP conversion to adenosine is catalyzed by 5'-nucleotidases, termed ecto-5' nucleotidase (NT5E, a.k.a.CD73) to indicate that the enzyme is located outside the cell. Elevated adenosine levels are associated with local energy deficits reflected by increased levels of pyruvate and lactate and increased phosphorylation (activation) of AMPK.<sup>68</sup>

Cellular AMP is derived from ADP by the reaction catalyzed by adenylate kinase (2ADP ↔ ATP + AMP). Under normal circumstances, catabolism maintains the ATP:ADP ratio between 10:1<sup>69</sup> driving the adenylate kinase reaction towards ADP and maintaining AMP at low levels. However, under conditions of metabolic challenge the ATP:ADP ratio falls, and at steady state AMP is elevated relative to less metabolically stressful conditions. An increase in AMP: ATP activates AMPKK which phosphorylates and activates AMPK. AMPK stimulates ATP producing processes, and inhibits ATP consuming pathways.<sup>70</sup> Adenosine and AMP have integrated roles as biochemical modulators and share roles in activating A<sub>1</sub>AR in energy conservation and homeostasis. Such redundancy would make sense given the interdependency of these metabolites. Inosine and IMP are also products of adenosine and AMP deamination.<sup>71</sup> Although inosine's role in energy homeostasis in mammals is not well understood, inosine serves as a sink for adenine nucleotides under fermentative conditions and thus plays an essential role in energy homeostasis in yeast.<sup>72</sup>

If adenosine or AMP are endogenous mediators of torpor, where extracellular pools of these metabolites originate is another compelling question<sup>73</sup>. Two proposed mechanisms for increasing extracellular adenosine are 1. Release of ATP with subsequent extracellular conversion of ATP to adenosine via NT5E, and 2. Transport of intracellular adenosine to the extracellular space via equilibrative nucleoside transporters. Both equilibrative and concentrative nucleoside transporters (ENTs and CNTs) transport adenosine across the plasma membrane;<sup>73–75</sup> ENTs transport adenosine down its concentration gradient so when intracellular adenosine accumulates it is released and when extracellular adenosine accumulates it is taken up. Release via ENTs thus depends on intracellular adenosine concentrations which depend largely on expression of adenosine kinase (AdK), an enzyme compartmentalized within astrocytes in adult brain. AdK catalyzes phosphorylation of adenosine to make AMP and is regulated by cellular energy charge; AdK is stimulated by ADP/ATP as well as by AMP/adenosine<sup>76,76</sup>. Seizure after brain injury, and homeostatic sleep drive provide two examples that illustrate how adenosine kinase in astrocytes regulate intracellular and subsequently extracellular adenosine levels by release via ENTs. Astrocyte proliferation following brain injury increases AdK which then decreases intracellular and subsequently extracellular adenosine as the nucleoside flows down its concentration gradient. The decrease in extracellular adenosine promotes seizure activity<sup>77</sup>. Homeostatic sleep drive also depends on AdK and thus cellular metabolism. Mice deficient in glial AdK are unable to convert adenosine to AMP and consequently have higher intra and extracellular levels of adenosine and greater homeostatic sleep drive.<sup>36</sup> In both of these examples adenosine originates from astrocytes via ENTs and extracellular levels of adenosine are influenced by astrocyte density (eg. in seizure) or energy status (eg. in sleep).

The functional link proposed between cellular metabolism and sleep homeostasis<sup>36</sup> may also underlie onset of torpor. In arctic ground squirrel hypothalamus inhibition of adenosine kinase increases extracellular levels of adenosine while blockade of equilibrative nucleoside transporter has no effect (Drew et al., unpublished) suggesting that AdK regulates extracellular adenosine in these animals. Extracellular ATP is another potential source for extracellular adenosine and AMP.<sup>78</sup> Tanycytes are glial like cells that line the 3<sup>rd</sup> and 4<sup>th</sup> ventricle<sup>79</sup> and are emerging as key players in energy homeostasis.<sup>80–82</sup> Tanycytes release ATP<sup>44</sup> that is then converted to adenosine and AMP by extracellular enzymes.<sup>73</sup> Moreover, tanycytes are active during hibernation.<sup>83</sup>

## Limitations to Energy Conservation

Prolonged torpor is interrupted by regular, albeit brief, periods of euthermia when  $T_b$  returns to pre-torpid levels. Interbout arousals occur with precise regularity and depending on species and ambient temperature occur as frequently as every few days to as infrequently as every 2 to 3 weeks. During these interrupted periods of energy conservation animals remain at pre-torpid  $T_b$  for 10–20h before re-entering torpor. Hibernation in the fat tailed lemur, under protected conditions, and in hibernating bears are the only exceptions where prolonged torpor is not interrupted. In both of these cases hibernating  $T_b$  remains above 30°C.<sup>84, 85</sup> However, when hibernating  $T_b$  falls below 30°C, illustrated in the fat tailed lemur under unprotected conditions, torpor is interrupted by brief interbout arousals.<sup>85</sup>



Interbout arousals consume significant amounts of energy and thus represent a limit to energy conservation.<sup>22</sup> The energy expended for warming and maintaining high body temperature during these arousal episodes range from 23 to 86% of the total energy spent during the entire hibernation season depending on ambient temperature<sup>22</sup>. This high degree of seemingly uneconomical energy expenditure clearly indicates an essential function for these brief returns to euthermia. Many theories have been proposed for the benefit of these periodic arousals, some of which suggest that interbout arousals occur to eliminate metabolic waste products<sup>86</sup>, to replenish blood glucose levels<sup>87, 88</sup> or to restore renal function and electrolyte balance.<sup>89</sup> One of the most debated hypotheses is that interbout arousals allow for restorative sleep.<sup>90-92</sup> Elucidating the mechanisms that stimulate interbout arousal may reveal novel means to modulate thermogenesis and thus metabolism. These mechanisms could provide pathways to drug discovery to modulate metabolic rate for the treatment of obesity.

### **Proposed mechanism for cross-talk between neural signaling and metabolism in interbout arousal**

While the neurochemical mechanisms regulating interbout arousals are poorly understood,<sup>25, 93</sup> they are expected to be related to metabolic and/or circadian cycles<sup>94, 95</sup> and triggered by metabolic needs.<sup>87, 96</sup> Pioneering work of Mary McKenna and others illustrate the importance of astrocytes and glutamate oxidation in brain energy metabolism<sup>97-99</sup>. Cross-talk between metabolic and neural signaling pools is a principle that may be fundamental to the energy economy of hibernation and torpor. Interestingly NMDA receptors play a role in arousal and a decrease in glutamate availability is being investigated as a metabolic signal to arouse. Jinka et al., 2012 showed that inhibition of NMDA type glutamate receptors (NMDAR) induces arousal from torpor in AGS and that the receptor pool involved lies outside of the CNS or within a circumventricular organ (Fig. 2). Similar results have been found in other ground squirrel species.<sup>93</sup>

A metabolite and neurosignaling molecule such as glutamate illustrates how metabolic flux could influence neurally mediated interbout arousal. Metabolic flux influences glutamatergic signaling through effects on substrate availability and modulatory influence of metabolic end products. Glutamate is derived from glucose via the pyruvate carboxylase pathway. Recycling of glutamate from glutamine via the glu-gln cycle also maintains supplies of glutamate unless excess nitrogen drives equilibrium towards glutamine.<sup>100, 101</sup>

Both sources of glutamate, i.e., glucose and the glu-gln cycle, may become limiting during prolonged torpor. In AGS, blood glucose levels decrease from 10 to 5mM during torpor<sup>102</sup> until gluconeogenesis replenishes blood glucose levels during arousal.<sup>87, 103</sup> During prolonged torpor protein catabolism produces nitrogenous waste that would normally be eliminated as urea. However, urea blood levels and urea cycle activity decrease during torpor.<sup>87, 103, 104</sup> The subsequent buildup of nitrogen may shift the glu-gln cycle towards glutamine. Low levels of glucose in torpor would be expected to decrease glutamate supply and limit the capacity of the glu-gln cycle. An increase in glutamine and decrease in glucose are two of the most robust biomarkers of torpor.<sup>86, 103</sup> Another biomarker of torpor is an

increase in circulating ketone bodies such as  $\beta$ -hydroxybutyrate and acetoacetate.<sup>105</sup> Ketones influence glutamatergic transmission by inhibiting vesicular glutamate transporters.<sup>106</sup> These metabolic consequences of prolonged torpor offer intriguing possibilities for modulating neuronal signaling to induce interbout arousal. More research is needed to test these and alternative hypothesis regarding endogenous neural signaling in a process that is clearly essential to successful hibernation.<sup>22</sup>

In this review we have described the energy conserving phenomenon of hibernation and focused on three ubiquitous, metabolite signaling molecules, adenosine, AMP and glutamate. Much remains to be learned about cellular signaling in the energy conserving phenomenon of hibernation and readers are referred to other reviews for a more comprehensive discussion of the signaling molecules and processes known to influence hibernation and torpor,<sup>4–10</sup> Discovery of the signaling molecules that modulate thermogenesis during torpor onset and interbout arousal may suggest novel approaches to modulate metabolism and this knowledge may spur new ideas for treating obesity. Faced with an epidemic of obesity in the US with growing prevalence world-wide, the cross-talk between metabolites and neuronal signaling in energy expenditure is an important area for study.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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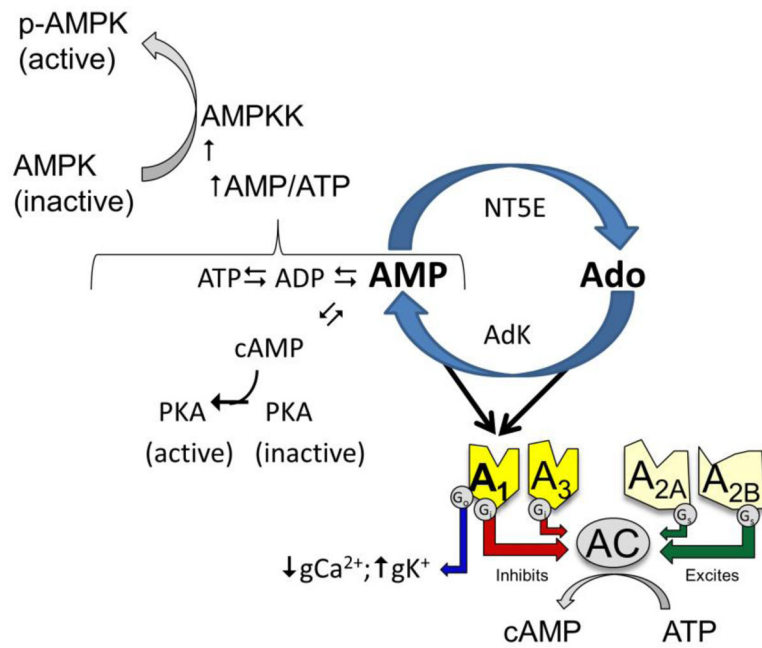
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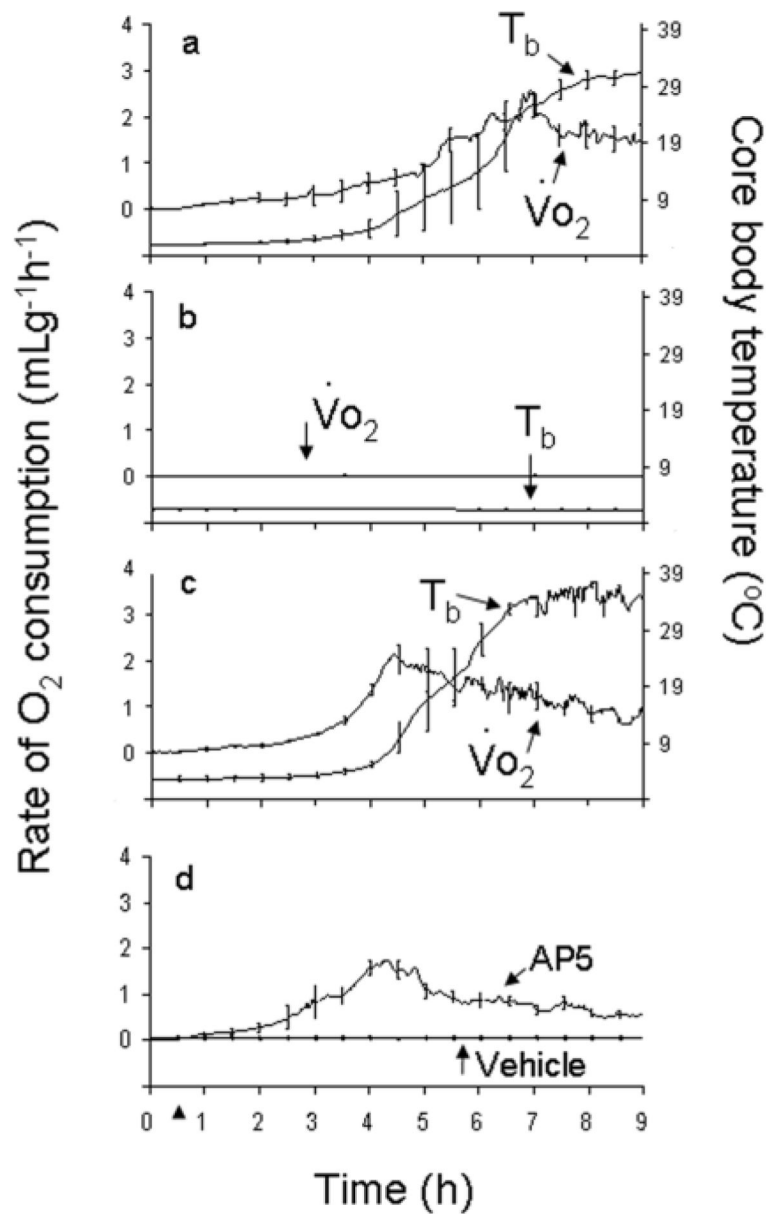


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**Figure 1.**

Adenosine is central to energy homeostasis where as a precursor to 5' AMP adenosine contributes to energy charge and activation of AMP kinase. Adenosine and AMP are reciprocal precursors of each other. Adenosine is converted to 5' AMP, a reaction catalyzed by adenosine kinase (AdK). 5' AMP is converted to adenosine, a reaction catalyzed by ecto-5' nucleotidase (NT5E), also known as CD73. At the receptor level both adenosine and 5' AMP stimulate the A<sub>1</sub> adenosine receptor.



**Figure 2.** Inhibiting NMDAR outside of the CNS is sufficient to induce arousal from hibernation: MK-801 (5 mg/kg; ip) induces arousal, indicated by an increase in the rate of O<sub>2</sub> consumption ( $\dot{V}O_2$ ) and core body temperature ( $T_b$ ) in a hibernating arctic ground squirrel (AGS) (a) while vehicle has no effect (b). Handling-induced arousal is shown for comparison (c). AP5 (5 mg/kg; ip), an NMDAR antagonist that does not cross the blood brain barrier, also induces arousal from hibernation (d). Arrowhead at 0.5h represents when drug (a, d) or vehicle (b, d) was delivered or arousal was initiated by handling (c). (From Jinka et al., 2012)

Table 1

Species	Metabolic Phenotype	Body Weight (g)	BMR (mL·g <sup>-1</sup> ·h <sup>-1</sup> )	Torpid MR (mL·g <sup>-1</sup> ·h <sup>-1</sup> )	Euthermic T <sub>b</sub> (°C)	Minimum T <sub>b</sub> (°C)
House Mouse ( <i>Mus musculus</i> )	Daily torpor	45	1.5	0.3	37	19
Western Jumping Mouse ( <i>Zapus princeps</i> )	Hibernation	33	1.5	0.04	37	5