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The pharmacology and molecular mechanisms underlying temperature regulation and torpor

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Abstract

The ability to enter a hypometabolic state upon restriction of caloric intake is pivotal for animal survival: balancing the energy budget in endotherms can be a real struggle when food is not available and/or the demand for heat production to maintain homeothermy becomes excessive. Bouts of torpor, characterized by metabolic rates well below a basal metabolic rate and core body temperatures that may be just a few degrees above the ambient temperature, are utilized among many organisms across the animal kingdom, including those that could be described as typical laboratory animals, like the mouse or hamster. Daily heterotherms, which are the focus of this commentary, enter shallow torpor bouts and do so usually under acute food shortage conditions and a relatively cool environment. Due to their small size, the body temperature of these animals is very responsive to food deprivation, pharmacological inhibition of metabolic rate, and cardiovascular depressants. This commentary examines recent developments concerning the neuroendocrine mechanisms in place that may mediate fasting-induced torpor in daily heterotherms. Further this commentary highlights pharmacological induction of hypothermia in small mammals.

1. Introduction

The idea that all small mammals, including mice, are not true endothermic homeotherms is not well appreciated by either the lay public or among many life scientists. Small mammals such as ground squirrels, marmots, chipmunks, and mice can spend a significant portion of their day, week, or even season at body temperatures well below 37 °C, although rats are not known to enter these torpor bouts [1–6]. Some of these animals obtain cues to enter bouts of torpor from day length and ambient temperature. Many excellent reviews on these obligate hibernators have been published [5,7–10], and are not the focus of this review. Rather, this review will examine those animals that enter a hypometabolic and hypothermic state in response to cool ambient temperature and calorie restriction. These animals undergo a relatively shallow torpor bout, with a minimum body temperature closer to 22 °C than 2 °C as seen in obligate hibernators [9]. Daily torpor usually lasts less than 24 hours, instead of days or weeks, where it is interrupted with bouts of food-seeking behavior and/or circadian rhythms within the organism. These animals that are daily heterotherms tend to be small, typically 5 – 50 grams, and include hamsters, mice, shrews, gerbils, hummingbirds, numerous marsupials and many others (see Table 1 for an abbreviated list and reference [9] for a complete list of animals that enter torpor).

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Upon an acute shortage of food intake, daily heterotherms utilize torpor to help balance their energy budget as most of their fuel comes from ingested food and not stored fat. The metabolic savings approach 70% in animals that undergo shallow torpor bouts. At the onset of the bout of torpor, the metabolic rate drops precipitously before the fall in body temperature (see Figure 1), suggesting an active inhibition of metabolism at least during the entrance into torpor. Energy savings by metabolic reduction as the torpor bout proceeds may stem more from passive processes of the temperature dependence of enzymatic activities [9].

Studies on torpor and exogenous compounds used to induce hypothermia may have great therapeutic potential. Hypothermia has been shown to have major protective benefits for bouts of ischemia and exposure to toxic chemicals [11]. Most studies of hypothermia have utilized small mammals that may be inherently tolerant to hypothermia. Because of their small size, the surface area to volume ratio of these animals necessitates a high mass-specific metabolic rate to offset heat loss. Indeed, it has been known for over 100 years that metabolic rate scales with body mass, although the value of the exponent that describes this relationship is still under debate [12]. The high mass-specific metabolic rate and low thermal inertia of these small animals makes their body temperature particularly susceptible to any small disturbance in metabolic rate. Thus, application of some exogenous compound may result in hypothermia simply because the compound poisons the metabolic machinery, as opposed to mimicking a torpor pathway. The difference between these two possibilities can be enormous: a drop in body temperature due to a metabolic poison will invoke mechanisms to counter the fall in temperature. That is, when body temperature is forced below some set point, homeostatic mechanisms engage to re-establish a warmer body temperature. Fasting-induced torpor, or seasonal hibernators for that matter, do not defend a warm body temperature (i.e. 37 °C), but rather experience a fall in the set point for body temperature [13], and allow their body temperature to fall without any mitigating physiological feedbacks. Hence, extrapolation of the use of these compounds in hypothermia-tolerant species like the mouse or hamster to the use of these compounds in larger animals, perhaps humans, should be done so with great caution.

2. Neuroendocrinology of fasting-induced torpor

Daily heterotherms that undergo shallow bouts of torpor in response to food deprivation are likely responding to hormonal cues that reflect short term changes in caloric intake. One such hormone appears to be leptin. This peptide hormone, a product of the *ob* gene, is derived primarily from white fat and relays nutritional status from the peripheral fat stores to sites within the hypothalamus [14]. In the hypothalamus, leptin binds its receptor, a product of the *db* gene, to promote satiety and elevate metabolic rate [15]. While leptin was originally thought to reflect long term energy stores, it has become clear that circulating leptin can reflect short term changes in energy intake. In the non-fasting animal, the amount of circulating leptin correlates well with the total amount of WAT [16]. However, fasting causes a fall in circulating leptin on a time scale that is much faster than the disappearance of fat from the animal [17, 18]. Indeed, while fasting causes a near global decline in sympathetic outflow to organs with the net result of a decrease in metabolic rate, the one tissue that receives elevated sympathetic input during food deprivation is WAT [19–21]. Activation of the β_3 adrenergic receptor on WAT in response to elevated sympathetic activity results in a decrease in leptin secretion and an elevation in lipolysis with the liberation of free fatty acids to the blood [22,23]. While the gene for leptin was discovered in 1994 [24], a lack of this circulating “factor” in the *ob/ob* mouse was known previously [25] to be intimately involved with torpor bouts. The *ob/ob* mouse, with its prolific stores of WAT that should allow for an ample supply of stored fuel for metabolic function, nonetheless enters deep bouts of torpor when fasted [25–27] and can enter torpor spontaneously while fed (see Figure 2). Leptin replacement in calorically restricted mice [28], *ob/ob* mice [26] and in the dunnart [29] blunts torpor bouts. Dopamine beta hydroxylase

$-/-$ mice that are unable to sympathetically activate WAT due to the lack of epinephrine and norepinephrine do not lower circulating leptin during a fast and do not enter torpor during a fast [18]. The ability to enter torpor is restored in these mice with the selective activation of the $\beta 3$ adrenergic receptor and subsequent fall in plasma leptin levels [18]. Conversely, the fasted *Fxr* $-/-$ mouse, missing a functional bile acid receptor, exhibits a greater drop in circulating leptin than that found in fasted wild type mice, which correlates with a deeper level of hypothermia during the fast [30]. This depth of torpor is also reversed by physiological replacement of leptin [30]. However, reduced leptin levels or reduced leptin signaling cannot be the sole signal to induce torpor during a fast for the obvious reason that *ob/ob* mice as well as *db/db* mice (missing a functional leptin receptor) are not always in a torpid state. Also, leptin replacement does not blunt bouts of torpor in a genetic model of WAT depletion [26]. Overall, though, it appears that the fall in circulating leptin due to sympathetic activation of WAT during a fast plays a key role in initiation of the torpor bout (see Figure 3).

Satiety and hunger signals, or lack thereof, from the gut seem to be reasonable candidates for the regulation of torpor in daily heterotherms that are so responsive to lack of food intake. The gut influences food intake and metabolism through a suite of factors that include ghrelin, cholecystokinin, oxyntomodulin, glucagon-like peptides, and PP-fold family of proteins, which includes PYY₃₋₃₆ [31]. In addition, vagal afferent activity is influenced by gastrointestinal mechanoreceptors, altering appetite, and may be the primary site of action for some gut-derived hormones [31]. One of these hormones has been reported in detail for effects on torpor. Ghrelin is secreted from the stomach during periods of fasting and is repressed after a meal [32]. Ghrelin stimulates food ingestive behavior, possibly via the vagus nerve [33,34], although ghrelin-producing neurons have been found to reside within the hypothalamus [35]. Administration of ghrelin to a fasted mouse deepens and lengthens a torpor bout, although ghrelin administration in a fed mouse does not influence body temperature [36].

Ghrelin is known to activate neuropeptide Y (NPY)-containing neurons within the arcuate nucleus [37,38]. Activity of NPY-containing neurons is also repressed during feeding, by high circulating leptin levels, and when leptin is administered directly into the 3rd ventricle of the brain [39]. NPY is a powerful orexigen, stimulating hunger when administered centrally. The ingestive behavior of the *Npy* $-/-$ mouse is surprisingly normal, and these mice have a normal body weight [40]. However, deletion of NPY neurons in the adult mouse results in lack of food intake with subsequent starvation and death [41], illustrating the development of other mechanisms to stimulate food intake in the *Npy* $-/-$ mouse. While these *Npy* $-/-$ mice exhibit normal endocrine and feeding responses to fasting, they do exhibit an altered response in body temperature. The *Npy* $-/-$ mice are able to initiate torpor bouts when fasted, but they do not sustain those bouts of torpor [36]. Peripheral administration of ghrelin has no impact on these aborted torpor bouts in *Npy* $-/-$ mice, suggesting that NPY mediates ghrelin's effects on body temperature [36]. Importantly, central injection of either NPY or an NPY Y1 receptor agonist into the cold-adapted Siberian hamster, another daily heterotherm, leads to torpor-like hypothermia [42,43]. The central administration of an NPY Y1 receptor antagonist in these hamsters blunts the depth of natural torpor [44]. These data firmly put NPY action as an important player in the mediation of fasting-induced torpor.

Adjacent to the NPY neurons reside anorexigenic neurons that utilize alpha melanocyte stimulating hormone (α MSH) as a neurotransmitter. α MSH partially mediates the anorexigenic and metabolic effects of leptin through the melanocortin 3/4 receptors of secondary neurons [14,15]. Therefore, this neuron type is a reasonable candidate for mediating the suppression of torpor bouts by leptin. The agouti mouse is a model of adult onset obesity due to the chronic antagonism of the melanocortin receptors within the arcuate nucleus. The juvenile agouti mouse enters torpor bouts normally when fasted. However, the hyperleptinemic adult agouti mouse does not enter torpor [36], likely due to the elevated leptin levels. While the incidence

of torpor in another model of hyperleptinemia (the diet-induced obese mouse) has not been examined, mice that are heavier, either strain-dependent or age-dependent, are less likely to enter torpor after a 24 hour fast (unpublished results from SJS). These data suggest that hyperleptinemia blunts torpor in a pathway that is independent of α MSH signaling.

Importantly, when the region of the hypothalamus containing the NPY and POMC neurons, so called the arcuate nucleus, is ablated, the ability for small mammals to enter torpor is severely impacted. Despite the obesity induced by ablation of the arcuate nucleus by neonatal treatment with monosodium glutamate (MSG), treated mice no longer enter torpor bouts [36,45]; ablation of the arcuate nucleus within suckling aged rats which experience bouts of torpor at such a young age also prevents torpor bouts [46]; and Siberian hamsters treated with MSG also have a blunted torpor response [47]. These data suggest that the arcuate nucleus, and likely the NPY neurons with the arcuate nucleus, play a central role in mediating the torpor response to fasting.

3. Pharmacological induction of hypothermia in small mammals

Adenosine and adenine nucleotides

Adenosine is a logical candidate for involvement with fasting-induced torpor in that its circulating levels are elevated during a fast and it evokes numerous physiological changes consistent with a torpid state. Among the myriad of known functions, adenosine inhibits neural activity, is a potent vasodilator as a particularly efficacious signal for increased blood flow in coronary arteries, and slows heart rate. Administration of adenosine causes profound hypothermia in a dose-dependent fashion in gerbils [48] as well as in mice [45]. Aminophylline, an adenosine A1 receptor antagonist, blocks the hypothermia associated with adenosine in mice [45] and the hypothermia associated with hypoxia in rats [49]. Importantly, adenosine inhibits the hypocretin/orexin neurons within the lateral hypothalamus [50]. These neurons are intimately involved with feeding regulation and energy homeostasis. They receive input from peripheral cues that are known to be involved with torpor regulation, including ghrelin and leptin [51,52]. Targeted deletion of orexin neurons in mice results in abnormal ambulatory response to fasting, although it is unknown whether these mice entered torpor upon fasting [52]. These same neurons play an important role in wakefulness, and inhibition of these neurons by adenosine promotes sleep [50]. Sleep may be related to torpor bouts, particularly in animals that undergo facultative torpor bouts [53].

The singly phosphorylated form of adenosine, adenosine monophosphate (AMP), is also a molecule that signals cellular energy depletion. The enzyme adenylate kinase catalyzes the reaction

Thus, the AMP:ATP ratio varies roughly as the square of ADP:ATP ratio [54], and as such, AMP is poised to play an important sensor for energy stores. AMP influences the activity of numerous enzymes, and of great importance to energy balance is the enzyme AMP-activated protein kinase. This enzyme is expressed in numerous tissues, including the hypothalamus [55]; its activity is impacted by ghrelin and leptin; and it is a major player in the regulation of both sides of the energy balance equation, feeding and metabolism [56,57]. While it appears that this enzyme plays little role in torpor in the ground squirrel [58], an obligate hibernator, it may play some role in fasting-induced torpor. Administration of AMP induces a reversible and dose-dependent hypothermia in mice [45,59], which one group defined as torpor [59]. However, two lines of evidence suggest that the intraperitoneal injection of AMP may be too blunt of a tool to assess whether AMP plays an active role in induction of true torpor. First, AMP-induced hypothermia is prevented using aminophylline [45], an adenosine receptor antagonist, which suggests that AMP must first be dephosphorylated to impact body temperature. Second, other phosphorylated forms of adenosine, namely ATP, induce the same

depth of hypothermia [45]. Perhaps the hypothermia induced by AMP will be therapeutically useful. However, the roles of AMP and AMP-activated kinase in fasting-induced torpor have yet to be conclusively tested.

3-iodothyronamine and H₂S

A derivative of thyroid hormone has recently been discovered and shown to induce hypothermia in the mouse and Djungarian hamster [60,61]. 3-iodothyronamine is generated through decarboxylation and deiodination of thyroxine [62] and has been found in the brains of mice, guinea-pigs, and hamsters [60,61]. Its effects are likely non-genomic, and function through the trace amine associated receptor 1 [63]. Administration of 3-iodothyronamine substantially lowers metabolic rate and body temperature that recover after 6–9 hours. The fall in respiratory quotient to nearly 0.7 with this compound which mimics the increase in lipid oxidation in naturally-occurring torpor bouts, has a recovery period substantially longer than 9 hours [60].

It should be pointed out that both adenosine and 3-iodothyronamine have in common tremendous cardiovascular depression. Both compounds induce a significant fall in heart rate and cardiac output [61,64,65]. Breathing hydrogen sulfide gas should also be included in this group. It was first reported that mice breathing H₂S fall into a hypothermic state, which was given the “sci-fi” type name of “suspended animation” [66]. However, it was very recently shown that breathing H₂S results in a fall in both heart rate and cardiac output of >70% of normal, without impacting stroke volume [67]. It may be that the hypometabolism and hypothermia associated with these compounds (adenosine, 3-iodothyronamine, H₂S) is secondary to the cardiovascular effects (see Figure 4). That is, the drop in metabolic rate with a subsequent drop in body temperature results from a lack of O₂ delivery to peripheral tissues in these hypothermia-tolerant species. Indeed, it has been shown that mice do experience hypothermia when experiencing hypoxia [68]. Most research suggests that hypoxia is not the signal for torpor entry [5], although hypoxia does have a substantial hypometabolic effect [69]. Alternatively, it may be that these compounds somehow act as a major metabolic inhibitor (see Figure 4). For example, H₂S is a known inhibitor of cytochrome c oxidase [70]. The necessary fall in metabolic rate following inhibition of fuel oxidation will have a great impact on a small mammal, lowering its body temperature, which in turn lowers heart rate and cardiac output simply due to the temperature-dependence of the cardiovascular system. Volpato et al. [67] tested these possibilities by administering H₂S to mice at an ambient temperature warm enough (35 °C) to prevent a fall in body temperature. They found that both metabolic rate and heart rate still fell even though body temperature did not, demonstrating direct cardiovascular effects of H₂S. It remains to be determined whether the metabolic effects of H₂S are independent of the cardiovascular effects.

4. Conclusion

To sum, hypometabolism and hypothermia is a readily-used tool by small mammals to cope with acute food shortages. These animals clearly have adaptations within multiple organ systems to cope with bouts of hypothermia on a daily basis. Those hormonal and metabolic signals that induce a hypometabolic state in response to food deprivation are likely many of the same signals that govern both sides of the energy balance equation (caloric intake and caloric expenditure) in all mammals, including humans. A valid question to ask is whether fasting-induced torpor or compound-induced hypothermia can be extrapolated from mice to humans. To state the obvious, mice are small with a large surface area to volume ratio. A portion of the metabolic suppression during torpor in a small animal is a result of their small size ... as metabolism falls, body temperature falls when heat loss outpaces heat generation from metabolism. Then, as body temperature falls, metabolic rate falls due to the thermal dependence

of biochemical reactions. So, even if “torpor-inducing” genes or “hypothermia-tolerant” genes exist within the human genome, one has to consider the thermal inertia and low surface area to volume ratio in a 75,000 gram human relative to a 25 gram mouse. As model organisms like mice are small, great care should be taken to determine whether metabolic inhibition either through inhibition of fuel oxidation or cardiovascular depressant effects results in a natural bout of torpor or achieves hypothermia simply because the animal cannot maintain a high enough metabolic rate to offset heat loss.

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Figure 1. Metabolism falls before body temperature in the fasted mouse

The core body temperature (blue line) and oxygen consumption (pink line) of an *ob/ob* mouse were measured simultaneously over a 24 hour fasting period. The fast was initiated at time “0”, which was also the beginning of the 12 hour dark cycle. Body temperature was recorded using an abdominal temperature telemeter while oxygen consumption was calculated from the oxygen content leaving the mouse’s metabolic cage. Note the fall in oxygen consumption precedes the fall in body temperature during entrance into this bout of torpor.

Figure 2. Spontaneous torpor bouts in leptin-deficient mice

Six different *ob/ob* mice were implanted with temperature telemeters, allowed to recover for 10 days from the surgery, and housed individually at 20 °C. These mice were on a 12:12 light schedule, with the 12 hour dark cycle (D) commencing at the beginning of each day. These mice had free access to food and water during the duration of the 5 days of data collection. Each mouse entered a torpor bout at least once over the five days. On every day, at least two mice entered a spontaneous bout of torpor. Note the timing of entrance into torpor was typically near the end of the dark cycle, which was consistent across animals and across the different days.

Figure 3. Hypothetical mechanism for the induction of torpor in daily heterotherms

Periods of fasting in mammals are associated with elevated sympathetic discharge to white adipose tissue. This causes a rapid decline in circulating leptin. It is hypothesized that the combination of low leptin and elevated ghrelin from the gut during a fast increase the activity of NPY neurons within the arcuate nucleus of the hypothalamus such to initiate the large fall in metabolic rate. It is unclear whether the increase in lipolysis and resultant release of free fatty acids to the blood also participate in initiation of torpor or serve only as a fuel for oxidation.

Figure 4. Potential pathways that an exogenous compound might lead to hypothermia

In the “metabolic-driven pathway”, the compound may activate / mimic the natural torpor-inducing pathway or may act simply as a metabolic poison. In either case, one would expect a fall in metabolic rate and therefore heat production, resulting in hypothermia in a small mammal. In the “cardiovascular-driven pathway”, the compound has its primary effect on the heart and/or circulatory system. This impairs the ability of the cardiovascular system to deliver oxygen, resulting in a fall in metabolic rate, followed by hypothermia. Unless the fasting-induced torpor pathway is activated or mimicked, the animal will likely engage homeostatic mechanisms to defend against the fall in body temperature.

Table				
Animal	Typical depth of torpor	Typical length of torpor	Classification	Reference
Mouse <i>Mus musculus</i> <i>Peromyscus leucopus</i>	20 °C	6–10 hours	Daily heterotherm	1,2
Hamster <i>Phodopus sungorus</i>	20 °C	10 hours	Daily heterotherm	5
Golden-mantled ground squirrel <i>Spermophilus lateralis</i>	5 °C	14 days	Hibernator	3
Marmot <i>Marmota marmota</i>	10 °C	5–6 days	Hibernator	4
Rat <i>Rattus rattus</i>	Does not enter torpor	N/A	Homeotherm	6