

Induction of Torpor: Mimicking Natural Metabolic Suppression for Biomedical Applications

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Numerous cellular processes are halted during torpor, including transcription, translation and ion homeostasis. Hibernators are able to survive long periods of low blood flow and body temperature followed by rewarming and reperfusion without overt signs of organ injury, which makes these animals excellent models for application of natural protective mechanisms to human medicine. This review examines efforts to induce torpor-like states in non-hibernating species using pharmacological compounds. Elucidating the underlying mechanisms of natural and pharmacologically-induced torpor will speed the development of new clinical approaches to treat a variety of trauma and stress states in humans.

Introduction

The ability to hibernate increases survival during periods when food availability is low and energy demands to maintain homeothermy are high. Hibernation is an evolutionarily conserved phenomenon, which has been described in most orders of mammals (Melvin and Andrews, 2009; Heldmaier et al., 2004; Carey et al., 2003a). Hibernating mammals reside most of the time in a state known as torpor, which is characterized by lowered metabolism and reduced body temperature. Entrance into hibernation is characterized by a drop in metabolic rate to 1-5% of euthermic rates and in most species is followed by a drop in body temperature to a few degrees above the ambient temperature (Storey, 1997; van Breukelen and Martin, 2002; Carey et al., 2003a). Hence, typical body temperatures during deep torpor are $\pm 0\text{-}4^{\circ}\text{C}$ (Kenagy et al., 1989), while the lowest body temperature during deep torpor was measured to be -2.9°C in arctic ground squirrels (*Urocitellus parryii*) (Barnes, 1989). The body temperature in daily torpor strongly depends on species and ambient temperature. A typical bout of daily torpor in a Djungarian hamster (*Phodopus sungorus*) is shown in figure 8.1A (Heldmaier et al., 1999). Although hibernators can regularly experience periods with an extremely reduced body temperature followed by rapid rewarming and normalization of physiological parameters, obvious signs of tissue and organ injury in fully rewarmed animals are scarce (Zancanaro et al., 1999; Arendt et al., 2003; Sandovici et al., 2004; Fleck and Carey, 2005; Talaei et al., 2011). The ability to pharmacologically induce a torpor-like state in human trauma patients, following cardiac arrest, organ transplantation, major cardiac and brain surgery or in patients in the intensive care unit (ICU) could provide a valuable tool to limit organ damage (Aslami and Juffermans, 2010). In transplantation medicine, reducing metabolism in donor organs may provide a better preservation method compared to the currently utilized (cold, static) ischemic preservation methods, thus stretching the time window for transplantation of organs to recipients (Maathuis et al., 2007). Although the induction of a torpor-like state sounds promising and several pharmacological compounds are currently subjects of investigation, knowledge about the molecular mechanisms of induction and maintenance of natural torpor and seasonal hibernation is limited. Therefore, the aim of this review is to summarize available literature about the molecular mechanisms of natural hibernation and, particularly, pharmacologically induced suspended animation. Understanding the mechanisms of both might lead to the development of pharmacological strategies to safely induce a torpor-like state in humans.

Tissue and cellular viability during natural torpor and experimental models of stress

Hibernators are able to reduce their metabolism and survive periods of extremely low body temperatures without obvious signs of organ damage (Zancanaro et al., 1999; van Breukelen and Martin, 2002; Arendt et al., 2003; Sandovici et al., 2004; Talaei et al., 2011). Many of the physiological extremes of hibernation would be expected to lead to apoptosis or necrosis in non-hibernating animals. When the shortfall in energy production cannot be compensated for by decreased ATP consumption, cellular homeostasis is disrupted and cell death is induced, leading to organ damage, as observed during hypoxia (Hochachka, 1986; Boutilier, 2001; Aslami and Juffermans, 2010; Storey, 2010). Despite the presence of a pro-apoptotic and oxidative environment during hibernation and evidence of accumulation of DNA damage, apoptosis is suppressed and necrotic tissue injury is minimal.

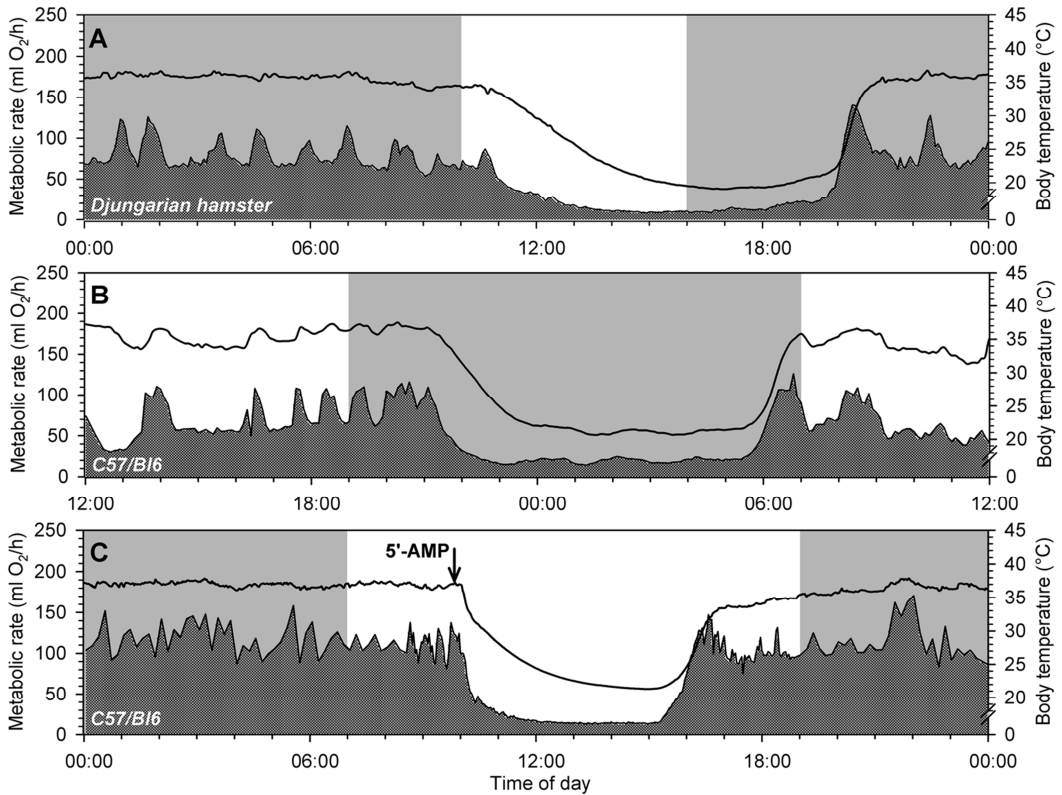


Figure 8.1. Metabolism and body temperature during natural and induced daily torpor. (a) Natural daily torpor induced by short photoperiod in a Djungarian hamster (*Phodopus sungorus*) housed at an ambient temperature of $\pm 15^{\circ}\text{C}$; adapted from: Heldmaier, 1999 (Heldmaier et al., 1999). (b) Torpor induced by lowering temperature from 30°C to 16°C , combined with food deprivation for 22-24h in a C57/Bl6 laboratory mouse; adapted from: Dikic, 2008 (Dikic et al., 2008). (c) Torpor induced by injection of 7.5 mmol/kg 5'-AMP i.p. in a C57/Bl6 laboratory mouse housed at an ambient temperature of $\pm 20^{\circ}\text{C}$; data derived from experiments by A.S. Boerema, H.R. Bouma and A.M. Strijkstra (University of Groningen). Shaded/light area marks lights off/on respectively, MR: Metabolic rate, T_b: body temperature.

In the thirteen-lined ground squirrel, levels of pro-apoptotic p53 protein are four-fold lower throughout hibernation (i.e. torpor and arousal), while levels of anti-apoptotic Bcl-X_L, Akt and pro-apoptotic Bax were increased 12-, 20- and two-fold, respectively, as compared to summer normothermic animals (Fleck and Carey, 2005). Further, caspase-3-activity is reduced during hibernation as compared to summer normothermic animals (Fleck and Carey, 2005). Alterations in the immune system during hibernation may also be involved in preventing exaggeration of the injurious insult, once tissue damage occurs (Bouma et al., 2010a). Downregulation of the number of circulating lymphocytes is governed by Sphingosine-1-phosphate (S1p), a bio-active signaling lipid as shown during torpor in the Syrian hamster (*Mesocricetus auratus*) and Djungarian hamster (*Phodopus sungorus*) (Bouma et al., 2011). The plasma level S1p is decreased during torpor in Djungarian hamsters, Syrian hamsters and thirteen-lined ground squirrels and are restored to normal levels during interbout arousals (Nelson et al., 2010; Bouma et al., 2011). Due to the broad effects of some

bio-active lipids, it is very likely that bio-active lipids such as S1p are involved in regulating other changes during torpor as well that might lead to an increased resistance to hypothermia, ischemia and reperfusion (Melvin and Andrews, 2009; Bouma et al., 2011). Upon arousal, despite the potential for reperfusion injury during torpor/arousal cycles, hibernators do not show signs of massive cell death (Zancanaro et al., 1999; van Breukelen and Martin, 2002; Fleck and Carey, 2005).

Experimental cold ischemia/reperfusion of livers derived from torpid, aroused and summer thirteen-lined ground squirrels and laboratory rats revealed that the hibernation phenotype is associated with an increased resistance to cold ischemia/reperfusion-injury, suggested by a better preserved mitochondrial respiration, bile production, and sinusoidal lining cell viability, lower vascular resistance and Kupffer cell activation *ex vivo* (Lindell et al., 2005). The increased resistance to ischemia/reperfusion induced injury (I/R-injury) was later confirmed *in vivo*, by showing decreased mucosal damage in hibernators following intestinal warm ischemia/reperfusion (Kurtz et al., 2006). Not only the winter phenotype, but possibly the general phenotype of hibernating species is associated with increased tolerance of ischemic stress, as cerebral ischemia induced by cardiac arrest in summer normothermic Arctic ground squirrels lead to less brain injury as compared to rats (Dave et al., 2006). Balancing energy production and consumption as well as upregulation of specific protective pathways appear to play key roles in limiting cell death in these models and hence, tissue injury due to cooling, low oxygen supply, reperfusion, and oxidative stress. However, many of the molecular mechanisms involved in this natural protection are still poorly understood.

Pharmacological induction of torpor-like states

Several pharmacological compounds have been or are currently proposed as inducers of natural torpor or artificial torpor-like states. Here we discuss the evidence for several of these agents, and point out considerations that should be addressed in studies of torpor induction.

Hibernation induction trigger (HIT) and DADLE

In 1969, the existence of a blood-borne “trigger” capable of inducing torpor in summer normothermic ground squirrels was proposed (Dawe and Spurrier, 1969). Summer normothermic ground squirrels were injected with whole blood, red blood cells or serum from hibernating ground squirrels and woodchucks and surprisingly, most of the recipient ground squirrels entered torpor within 60 days after transfusion (Dawe et al., 1970). Later the putative molecule that induced torpor was named hibernation induction trigger (HIT) and described as an 88-kDa peptide showing homology with the human alpha-1-B glycoprotein, a protein with yet undefined function (Horton et al., 1998). HIT has some pharmacodynamic similarities to the synthetic delta opioid peptide DADLE ([D-Ala², D-Leu⁵]-enkephalin) (Oeltgen et al., 1988), as it is a potent releaser of endogenous opioids (Borlongan et al., 2004). However, other studies were not able to reproduce these results, as infusion of a serum dialysate from hibernating ground squirrels to summer normothermic Richardson’s ground squirrels did not lead to any behavioral or physiological changes (nest building, weight gain/loss or torpor) (Abbotts et al., 1979). This finding raises doubts about the hibernation inducing potential of this compound. Although the evidence for opioid-induction of natural torpidity is still inconclusive, opioids might still play a role in torpor by governing specific physiological adaptations. An increased expression of delta-opioid receptors in the brain during torpor supports the idea that opioids are involved in regulating

brain activity and/or tissue protection during hibernation (Otis et al., 2010). Thus, compounds like DADLE might be inducers of potential protective mechanisms that increase resistance to hypothermia, ischemia and reperfusion. Indeed, several studies reported that DADLE enhances survival and preservation time of organs for transplantation (Chien et al., 1991; Oeltgen et al., 1996; Bolling et al., 1997; Inuo et al., 2007; Inuo et al., 2007). Experiments on cultures of cells devoid of opioid receptors showed DADLE to decrease the rate of transcription by 50%. As transmission electron microscopy revealed DADLE to localize both to the cytoplasm and nucleus, it has been suggested that DADLE interferes with transcription and splicing of pre-mRNA through a mechanism that is independent from opioid receptors (Baldelli et al., 2006). Furthermore, it was shown that DADLE treatment induces the formation of nuclear bodies, which are also observed in torpid hibernators (Baldelli et al., 2006; Vecchio et al., 2006). To date, it is unknown whether DADLE can reduce metabolism and the molecular mechanisms which underlie the induction of hibernation by HIT and DADLE thus remain elusive.

Hydrogen sulfide (H₂S)

Hydrogen sulfide (H₂S) is endogenously produced in very low quantities and functions as a signaling molecule in the nervous system and vasculature (Gadalla and Snyder, 2010). H₂S is also a highly potent inhibitor of oxidative phosphorylation complex IV (cytochrome c oxidase) in mitochondria. H₂S competes with O₂ in binding to cytochrome c oxidase, resulting in a decrease of cellular oxygen consumption and induction of reversibly reduced metabolism (Aslami et al., 2009). In addition to the direct effect of H₂S on mitochondrial function, H₂S has been suggested to activate anti-apoptotic signaling pathways and anti-oxidant mechanisms, as reviewed by Calvert et al. in 2010 (Calvert et al., 2010). H₂S is thought to inhibit apoptosis by activating pro-survival kinases of the reperfusion injury salvage kinase (RISK) pathway. Although the downstream targets of the RISK-pathway are not fully understood, research has shown that H₂S treatment increased the expression of HSP70, HSP90 and anti- and pro-apoptotic members of the Bcl-2 family (Calvert et al., 2009). Furthermore, in the same study it was shown that H₂S increases antioxidant mechanisms via Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which upregulates gene expression of several antioxidant proteins. Indeed, HO-1 and thioredoxin 1 (Trx1) were upregulated 24 hours after administration of H₂S (Calvert et al., 2009). The combination of the ability to reduce metabolism by inhibiting complex IV cytochrome c oxidase and anti-apoptotic and anti-oxidant properties, makes H₂S a promising compound to pharmacologically induce suspended animation. In 2005, Blackstone et al. showed that exposure to H₂S induces a state of reduced metabolism and body temperature in mice (concentrations ranging from 20 to 80 ppm). After H₂S treatment, metabolic rate was decreased by 90% and body temperature dropped subsequently to $\pm 2^{\circ}\text{C}$ above ambient temperature, with a minimum body temperature of 15°C. Six hours after the H₂S exposure mice slowly rewarmed and the metabolic rate and body temperatures were restored to baseline values (Blackstone et al., 2005). This experiment was repeated by another group and similar results were obtained (Volpato et al., 2008).

Studies investigating H₂S administration in larger animals, however, describe inconsistent results. Administration of the H₂S-donor Na₂S (2 mg/kg/hr) to anesthetized pigs induced a slight, but significant, decrease in heart rate and body temperature after 8 hours. Furthermore, O₂ consumption and CO₂ production were significantly decreased, indicating a

reduced metabolic rate (Simon et al., 2008). Unfortunately, ATP was administered during the experiment to keep the mean arterial blood pressure (MAP) within the normal range for normothermic, active animals. This makes the results difficult to interpret, since ATP has been shown to induce hypothermia as well (Swoap et al., 2007). Other reports in pigs and sheep did not support an effect of H₂S on the induction of torpor. Administration of 80 ppm H₂S gas to anesthetized pigs had no effect on body temperature (Li et al., 2008), nor did a 30 minute exposure to 60 ppm H₂S significantly reduce metabolism in sedated sheep (measured as body temperature and gas exchange) (Haouzi et al., 2008). It is possible that the larger body mass in sheep and the different regulation of metabolic rate in larger sized mammals prevent a reduction of metabolic rate in response to H₂S (Haouzi et al., 2008; Aslami et al., 2009). Although effects of H₂S on the induction of torpor in larger animals are conflicting, administration of Na₂S prior to aortic occlusion in pigs, attenuates renal injury due to a reduced inflammation, oxidative and nitrosative stress (Simon et al., 2010). Further, H₂S reduces renal ischemia/reperfusion induced damage in mice (apoptosis, necrosis and inflammation) (Bos et al., 2009) and improves post-transplant renal function following preservation of a porcine kidney with NaHS (an injectable H₂S donor) (Hosgood and Nicholson, 2010). Although it is unclear whether H₂S is able to induce torpor in larger animals and potentially humans, H₂S seems a promising pharmacological agent in the field of surgery and transplantation medicine.

5'-adenosine monophosphate

5'-adenosine monophosphate (5'-AMP), is a metabolite of the hydrolysis of ATP and has been proposed to be involved in the induction of torpor in laboratory mice (Zhang et al., 2006). Fasting of mice that are maintained in constant darkness was reported to induce torpor via increased levels of 5'-AMP in plasma, suggesting that 5'-AMP might play a role in the natural induction of torpor. Furthermore, injection of 5'-AMP led to a drop in body temperature as well (Zhang et al., 2006; Lee, 2008) (Figure 8.1C). However, dissimilarities in cooling and heart rate during 5'-AMP induced reduction in body temperature and fasting-induced torpor in mice raises doubts about the role of 5'-AMP in the induction of natural torpor rather than hypothermia (Horman et al., 2005; Swoap et al., 2007). There are two hypotheses to explain the pharmacodynamic effects of 5'-AMP: (a) 5'-AMP is dephosphorylated to adenosine, which activates adenosine receptors and leads to a reduced cardiac output and hence, body temperature and (b) uptake of 5'-AMP activates AMP-activated protein kinase (AMPK) a key enzyme involved in the regulation of metabolism. Administration of adenosine, ADP and ATP all induce a hypothermic response in mice (Swoap et al., 2007), which is consistent with the first hypothesis. Activation of adenosine receptors, seems predominant in the induction of low body temperature by 5'-AMP and adenosine, as the response is significantly blunted following injection of aminophylline (an adenosine-receptor antagonist) (Swoap et al., 2007). The second hypothesis describes the role of intracellular AMPK, an enzyme which increases glucose uptake and fatty acid oxidation and inhibits fatty acid, glycogen and protein synthesis, in the induction of a torpor-like state (Lindsley and Rutter, 2004; Lee, 2008; Melvin and Andrews, 2009). In accordance with the second hypothesis, 5'-AMP is also able to induce a hypothermic response in ecto-5'-nucleotidase-deficient, and mice deficient in expression of one subset of adenosine receptors (A1, A2A, A2B or A3) (Daniels et al., 2010). Although this suggest an important role of AMPK in the induction of the hypothermic response, potential redundancy between different types of adenosine receptors does not allow excluding a role of adenosine receptors. The sensitivity of AMPK to [AMP]:[ATP] ratio makes it a sensor of the cellular energy status. Relatively high levels of AMP lead to

activation of adenylate kinase and restoration of cellular homeostasis by converting 5'-AMP together with ATP to ADP (Lee, 2008). Due to its regulatory role in metabolism and activation of several protective signaling mechanisms similar to ischemic preconditioning, AMPK might be a promising target for preconditioning in transplantation medicine (Bouma et al., 2010b). Activation of AMPK induces an energy-saving state to prevent lactate accumulation, activates several protective pathways resulting in production of the protective factors eNOS and NO and limits cell injury during ischemia (Peralta et al., 2001; Sola et al., 2001). However, activation of AMPK by intracerebroventricular infusion of AICAR (a specific AMPK-activator) in hibernating yellow-bellied marmots (*Marmota flaviventris*) increased food intake and moreover, prevented re-entrance of torpor (Florant et al., 2010). Although it is difficult to discriminate effects induced directly by 5'-AMP and indirectly via its hypothermic effects, the ability of 5'-AMP to activate AMPK and downstream signaling pathways that lead to an increased resistance to ischemia/reperfusion injury make it a potentially promising compound for several clinical applications. However, data regarding the molecular mechanisms underlying 5'-AMP's actions are scarce and its potential clinical use needs further investigation.

Thyroid hormones

3-Iodothyronamine (T1AM), a recently discovered naturally occurring thyroid hormone derivative is a potent agonist of the G protein-coupled trace amine-associated receptor TAAR1 (Chiellini et al., 2007). The physiological role of T1AM is not known but its injection in mice caused dose-dependent reductions of body temperature and heart rate (Scanlan et al., 2004; Chiellini et al., 2007). Furthermore, injection of T1AM in mice and Djungarian hamsters (*Phodopus sungorus*) reduced metabolic rate (Braulke et al., 2008). However, several important differences between the thermal and metabolic effects induced by T1AM and natural torpor exist, which raises questions about the potential of this compound to induce torpor. The metabolic reduction occurs faster and rewarming takes much longer following T1AM treatment than in natural torpor (Braulke et al., 2008). T1AM-induced metabolic depression is accompanied by a rapid inhibition of glycolysis which is less pronounced and delayed in spontaneous torpor. Also, the extent of hypothermia is less pronounced following T1AM treatment than during natural torpor, being $\pm 33^{\circ}\text{C}$ and $\pm 30^{\circ}\text{C}$ following injection of T1AM in hamsters and mice, respectively (Braulke et al., 2008). Most of the T1AM effects are opposite to the effects of other thyroid hormones like T4 and T3, suggesting that thyroid hormones and their aminated derivatives might play an opposing role. Recently, Ju et al. suggested that T1AM can induce multi-day torpor bouts in mice under an experimental protocol that involved provision of a high fat diet for four weeks prior to treatment (Ju et al., 2011). While repeated intraperitoneal injection of T1AM did lower body temperature during consecutive days (Ju et al., 2011), the low number of animals included and particularities of the experimental protocol raise questions about the interpretation. Mice were fasted prior to injection, which is known to induce torpor in mice (Swoap et al., 2006; Dikic et al., 2008) (Figure 8.1B) and required antibiotic treatment to prevent sepsis. Also, repeated administration of T1AM in DMSO adds up to a very high dosage of the solvent. Consequently, the claim that T1AM induces multi-day torpor bouts in mice similar to natural hibernation is questionable. Taken together, additional data are required to assess the potential of T1AM for therapeutic induction of torpor-like states in non-hibernators.

Other compounds of potential interest in torpor induction

Several compounds that affect availability, sensing, production or use of energy fuels have been reported to reduce body temperature and thus induce a state resembling torpor. These include 2-deoxy-D-glucose (2-DG) which acts by disrupting glycolysis (WICK et al., 1957; Dark et al., 1994; Dark et al., 1996; Stamper et al., 1999) and methyl palmoxirate (MP) (Schneider et al., 1993) and mercaptoacetate (MA) (Stamper and Dark, 1997; Stamper et al., 1999; Westman and Geiser, 2004), which limit fatty acid utilization. Intracerebroventricular injection of neuropeptide Y (NPY) leads to the induction of a hypothermic response (Paul et al., 2005), which is similar to injection 2-DG in rats, mice and humans and leads to a mild decrease in body temperature of $\pm 1\text{--}2^\circ\text{C}$ (Freinkel et al., 1972; Shiraishi and Mager, 1980; Shiraishi and Mager, 1980). Intracerebroventricular injection of NPY as well as a specific NPY Y1 agonist in Djungarian hamsters (*Phodopus sungorus*) on the other hand, leads to the induction of pronounced hypothermia for several hours which can be prevented by a specific NPY Y1 receptor antagonist (Paul et al., 2005; Pelz and Dark, 2007; Dark and Pelz, 2008). However, species differences in response to the compounds leave it questionable whether they actually induce natural torpor. Unfortunately, simultaneous measurements of metabolic rate and body temperature after injection of these compounds are not available, which would be required to confirm whether they induce metabolic depression in a manner that mimics natural torpor.

Conclusion

Hibernation is a widely conserved behavior that allows mammals to survive periods of low food supply by reducing metabolism. Specific alterations are believed to conserve energy sources, prevent cell death and limit organ injury during periods with extremely low body temperature and during rewarming. Although unraveling the molecular pathways that are involved in the induction of torpor is of major relevance for human medicine, the molecular mechanisms involved in the induction of torpor are still largely unknown. Comparative studies investigating both natural torpor and pharmacologically-induced torpor are likely to reveal the signaling pathways involved in the induction of torpor and its associated adaptations. Elucidating the regulatory mechanisms of natural hibernation and torpor and their application to non-hibernating species including humans may lead to novel therapeutic approaches in biomedicine, including induction of regulated and reversible metabolic states and pharmacological interventions that limit organ injury following cardiac arrest, major surgery and transplantation.

